PETER PÉCZELY

Avian Reproduction: Structure, Function and Molecular Regulation

PETER PÉCZELY

Avian Reproduction: Structure, Function and Molecular Regulation

AGROINFORM
2017

Issue of this Volume was supported by the Hungarian Academy of Sciences

Scientific consultants of the hungarian edition:
Judit Barna, PhD
Krisztina Kovács, DSc
János Török, DSc

Translated:
Judit Szabó, PhD

Proofreading and editing the english version:
Nicholas Paisley

Published by Agroinform Publishing and Printing House Ltd.

Managing Editor:
István Bolyki

Making up:
Anna Sándor

ISBN 978-615-5666-15-5

Printed by:
Agroinform Publishing and Printing House Ltd.
1149 Budapest, Angol u. 34.
Leader: Stekler Mária
www.agroinform.hu
2017/24

Contents

Preface

The English version of the book „Madár Szaporodásbiológia", published in Hungarian in 2013 is printed in two individual volumes. The first volume treats reproduction as part of the annual cycle, introducing differences that originate from sedentary and migratory life styles and ecological characteristics of the breeding site. In this volume we introduce the determining role of photoperiod in avian reproduction.

This, second volume focuses on the morphological and functional characteristics of avian reproductive organs. It introduces in detail molecular biological aspects of sex determination and differentiation. In addition, it deals with the physiology of the ovary, ovum, and egg development and the complex calcium metabolism of female birds. In regard to male birds, we discuss testis development and spermatogenesis and different types of sperm. Detailed analyses of the hormonal aspects of gonadal function in birds and the neuroendocrine processes of its regulation form an important part of the volume. These two volumes form an integral unit, which I considered when constructing the chapters.

Peter Péczely

1. Sex determination and gonadal differentiation

1.1. The genom and sex chromosomes

The avian chromosome assembly is similar to the reptilian, i.e., it consists of chromosomes of two or three different size classes. The largest are about 5-12 μm and the medium size is 1.5-3 μm long (these two types are called macrochromosomes), while the smallest, the so-called microchromosomes are about 0.5 μm long. Microchromosomes probably form when bigger chromosomes break down, and about 15 of them are assumed to be pair-forming elements. Most microchromosomes are acrocentric, and only four or five could be subacrocentric or submetacentric. The recombination rate of microchromosomes is greater, they replicate earlier and have a higher level of methylation (Axelsson et al. 2005). The best-known chromosome assembly belongs to the domestic fowl, which has 39 pairs. Out of these, eight macrochromosomes could be considered autosomes. The pair of sex chromosomes is easy to distinguish: Z is the size of a macrochromosome and is metacentric, while W is of medium size, is submetacentric and strongly hereochromatic. Additionally, the genom consists of 30 microchromosomes. The large-, and medium-size macrochromosomes consist of long (q) and short (p) arms, connected by a centromere. Microchromosomes 15-18, and most likely 17 carry the nucleolus organising region (NOR), which corresponds to the satellite element of the mammalian chromosome (Fechheimer, 1990). Most studied bird species have been found to have a similar chromosome number and distribution, with the exception of Falconiformes, for instance in European kestrel (*Falco tinnunculus*) 2n=52. In this species, fusions and breaks are often observed between macro- and microchromosomes and between microchromosomes (Nishida et al., 2008).

Differential staining of chromosomes shows a high level of conservatism among avian macrochromosomes, with few differences among them. Considering the first nine autosomes of domestic fowl, there is complete accordance with the respective chromosomes of Japanese quail and domestic goose. Chromosome 4 of three pheasant species, the red-shouldered blackbird (*Agelaius phoeniceus*) and rhea show complete accordance with chromosome 4 in fowl, and an additional microchromosome pair (Guttenbach et al., 2003). Staining reveals minor differences among fowl, chaffinch (*Fringilla coelebs*) and redwing (*Turdus iliacus*), i.e. chromosome 1 in fowl corresponds to chromosomes 3 and 4 in chaffinch and chromosomes 2 and 5 in redwing (Derjusheva et al.,

2004). Only parrots *(Psittaciformes)* seem to show a greater macrochromosome realignment compared to domestic fowl. This is mostly true for smaller-bodied species: considering chromosome 6, 7 and 8 of chicken, elements of two or three of these show up mixed on one chromosome in lovebirds (*Agapornis* sp.), budgerigar *(Melopsittacus undulatus)* and cockatiel *(Nymphicus hollandicus)* (Nanda et al. 2007).

The avian cell nucleus contains about 3 pg of DNA, about half of the amount of that in human or mouse. This size of chicken genome is 1.2 Gbp, 40% of that of the mouse or human genome. The source of this difference probably originates from the smaller size of the introns and the shorter distance between genes in birds. The size of chromosome 1 is 150-200 Mpb, and microchromosomes are 5-20 Mbp. Gene mapping EST projects estimate the number of genes in chicken 35,000, which is in agreement with the amount found in vertebrates (Boardman et al., 2002). According to fluorescence in situ (FISH) studies, 65% of chicken genome binds to macrochromosomes, however, these only carry 25% of the genes, and about 75% of the genes are bound to microchromosomes, which make up about 30% of the genome (McQueen et al., 1998).

Sex chromosomes

During the evolution of the animal (and plant) kingdom sex chromosomes have developed multiple times and independently from each other and they shows parallel characteristics (Ohno, 1967). In birds, sex chromosomes are heterogametic (heterozygotic) in females, which carry a ZW chromosome pair. Males are homogametic, having a ZZ chromosome pair. Therefore avian sex chromosome arrangements differ from the male heterogamecy characteristic of mammals, the XY (male) and XX (female) system. The similarity between the two types is that Z, similarly to X, is large and contains many somatic genes, and W, similarly to Y, has lost most of its somatic genes by a deletion around the centromere, and that arrived to its final structure by the development of DNA sequences (Ssp1, Xho1 and EcoR1) that are repeated multiple times (amplification) and the presence of a large amount of heterochromatin. In spite of the similarities between X and Z and between Y and W chromosomes, they probably developed independently from each other from two different autosomes of a common reptilian ancestor, which had temperature-dependent sex determination. The similar characteristics of Z and X chromosomes (low gene density and the amplification of the genes determining the testes) suggest that – even though Z and X carry mostly genes that determine male and the female sex, respectively, they are a result of convergent evolution (Graves et al., 2001, Bellott et al., 2010). The first step of this process was likely a homomorphic autosome pair, and initially selection resulted in a bond between sexually antagonistic alleles and the development of a locus responsible for one sex on

each of the chromosomes. Next, recombination between the two "proto-sex chromosomes" largely decreased, which resulted in relatively few – corresponding – gametologous genes.

The arrangement of these gametologous genes reflects the temporal aspect of chromosome specialisation, and suggests that the development of sex chromosomes occurred various distinct ocassions. For instance, on the human X chromosome four evolutionary strata were identified, among which the oldest is found on the distant part of the long (q) arm, while the youngest is found on the short (p) arm. This process has likely taken place 240-320 million years ago. Handley et al., (2004) studied five known (gametologous) gene pairs on the avian Z and W chromosomes, and using intron sequence analysis they identified two distinct zones (strata) on the Z chromosome. The stratum located on the distal part of Zq likely developed about 102-170 million years ago, therefore before the development of Neoaves. Its development is possibly determined by an inverse mutation of W, which stops its homology with Zq. This zone contains three gametologous genes. The second zone located on Zp likely developed about 58-85 million years ago, possibly at different times during the evolution of different groups of birds. This younger stratum contains two gametologous genes. According to Nam and Ellegren (2008), the development of strata in Z and W chromosomes occurred in three stages. The first, oldest stratum developed 132-150, the second 71-99 and the last 47-57 million years ago. These changes are most obvious in the 1-11Mb section of Chromosome Z, where they affect the location of six genes, and are less important in the case of two genes located in the 42-50 interval and for the four genes in the 16-53 interval.

The developed sex chromosomes at times could have partially fused with autosomes, which made sequence evolution and gene degeneration possible. These temporarily fused chromosomes are **neo-sex chromosomes**, which were studied in warblers. In these, a fusion was observed between the first part of autosome 4a (about 10 Mb) and the Z or W sex chromosome. For instance, this autosomal element contains androgen receptor genes, therefore the fusion enhances the potential of the sex chromosome. The resulting neo-sex chromosomes are characterised by a smaller diversity of neo-Z genes and smaller sequence divergence between Z and W, which reflect a relatively more recent evolutionary age (37-47 million years), compared to the more ancient development (about 150 million years) of the original sex chromosomes (Pala et al., 2012a, b).

Comparing reptilian and avian chromosomes and sex-determination systems shows that even though some reptiles (snakes, lizards and some turtles) have ZZ/ZW sex chromosomes, these, and the location of their determining genes show certain differences from those of birds. Comparative FISH studies of the genes of the Z chromosome in domestic fowl and Chinese soft-shell

turtle (*Pelodiscus sinensis*), Japanese rat snake (*Elaphe climacophora*) and Siamese crocodile (*Crocodylus siamensis*) show that chicken Z chromosome corresponds to turtle 6q, snake 2- and crocodile chromosome 3 (Kawai et al., 2007).

More ancient *Ratites* (e.g., ostrich (*Struthio camelus*), cassowary (*Casuarius* spp.) and kiwi (*Apteryx* spp.)), which separated early during the phylogenetic evolution of birds, possess sex chromosomes that are similar to those in boas and pythons. On the other hand, in emu (*Dromaeus novaehollandiae*) and rhea (*Rhea* spp.) species the different bands can be found on chromosome 5 of females, reflecting the beginning of genetic rearrangement. Comparative staining studies of macrochromosome 1-9 and Z chromosome in domestic chicken, emu, cassowary, ostrich, greater (*Rhea americana*) and lesser (Darvin's) rhea (*R. pennata*) and a tinamou species show that probes prepared from certain chicken chromosomes hybridised with only one chromosome pair in each of the six paleognathous species studied. An exception was chromosome 4 in chicken, which in addition to giving a good fit with the fourth biggest chromosome of the paleognathous species, also paired with one of the microchromosomes. The chicken Z probe hybridised with both sex chromosomes of the studied paleognathous species. This study underlines the great agreement between Ratite and Carinata species, i.e., the great degree of stability of the avian karyotype during 80 million years of evolution. On the other hand, FISH marking of four genes in two rhea species shows a small rearrangement in the proximal end of Wq, which shows the beginning of a chromosome specialisation (amplification) in W, i.e., the accumulation of sex-determining genes (Shetty et al., 1999; Nishida-Umehara et al., 2007).

The Z chromosome in Carinatae is large and carries 7-10% of the full genome, containing over 700 genes, carrying elements that mostly code somatic characteristics. In contrast, the smaller, strongly heretochromatic W chromosome carries only 1.5% of the full genome, with 45 genes identified until now (Schmid et al., 2000; Clinton and Haines, 2001; Smith et al., 2007, Mank, and Ellegren, 2009).

Among the genes of the **Z chromosome**, the following have been identified using FISH technique: ZOV-3 gene, found on the p arm, responsible for initiating the development of steroidogenic cells; a growth-hormone receptor gene; a prolactin-receptor gene and two interferon-1 genes. DMRT1 gene plays a pivotal role in testis determination. This gene expresses in the germinal ridge during the early developmental stage and later, in ZZ individuals it gets activated in mass. The tyrosine kinase gene, found on the q arm, the chromohelicase-DNA-binding 1 (CHD-Z) gene and the qk-1 gene all play important roles in determining spermatogenesis, while the vitellogenin gene is one of the regulating elements of vitellogenesis in ZW (female) individuals (Kunita el al., 1997; Nanda et al., 1998; Suzuki et al., 1999, Fridolfsson and Ellegren, 2000, Shan et al., 2000). A distinctive, histidine-triad-nucleotide

binding protein-coding gene, also known as chicken-protein-kinase-inhibitor (X-ch pkci) gene, has a possible role in sex determination. This gene is also found on the W chromosome (W-ch-pcki) and shows affinity for heterodimer forming (Ceplitis and Ellegren, 2004, Moriyama et al., 2006, Smith et al., 2009). Recently, c-HEMGN has been identified on the q arm of the Z chromosome, which expresses in haemopoetic tissues and is the avian equivalent of the mammalian hemogen gene. This gene expresses in the pre-Sertoli cells later in the process of sex determination i.e., at the beginning of gonadal differentiation. This process follows the expression of DMRT1 and is proceed by the expression of SOX-9. This presumably makes it a member of the DMRT1-c-HEMGN-SOX-9 cascade, and gives it a very probable role in primary sex determination in male birds (Nakata et al., 2013).

Among the 14 Z-specific genes identified earlier in zebra finch (*Taeniopygia guttata*), the following have been found on the chicken Z chromosome: ATP5A1 and UBE2R2 genes on p, and NR2F1, PAM, HSD17B4, NIPBL, ACO1, HINT1, SMAD2, SPIN and NTRK2 genes on q. These genes however, are found at very different locations compareds to zebra finch, suggesting different characteristics in chromosome development in the two species (Itoh et al., 2006).

Among the known genes of the **W chromosome**, five could play a role in sex determination: CHD1, ATP5A1, UBAP2, SPIN and the ASW gene, which differs from Z HINT1 in the AMP-binding region, are also found on Z (Pace and Brenner, 2003). W-pkci (HINT-W) gene can be important in sex determination, which is 65% equal to ch-pkci, found on the Z chromosome. It is, however, missing the histidine triad, characteristic of the Z-pkci product, but has a section rich in leucine and arginine (Ceplitis and Ellegren, 2004). Recently, three genes have been found on the W chromosome that do not have an equivalent on Z. Among these are FET1 (female-expressed transcript 1), which in chicken is expressed up to Days 4.5-6.5 of embryonic development, but only in the left gonadal colony. Its role in sex determination is not obvious, as some data suggest that FET1 codes an avian retrovirus (Reed and Sinclair, 2002; Smith et al., 2007). Similarly bound to W chromosome are the genes 2d-2D9 and 2d-2F9, which express from Day 2 of incubation (before gonad development). The protein coded by 2d-2D9 is not known, while the protein expressed by 2d-2F9 is an enzyme belonging to the ATP-ase superfamily (Yamada et al., 2004). ABTW (avian brain W-linked transcript) gene seems to have a special role, as it expresses in the brain around Day 4 of embryonic development and shows a dominance in females (Scholz et al., 2006). (Figure 1).

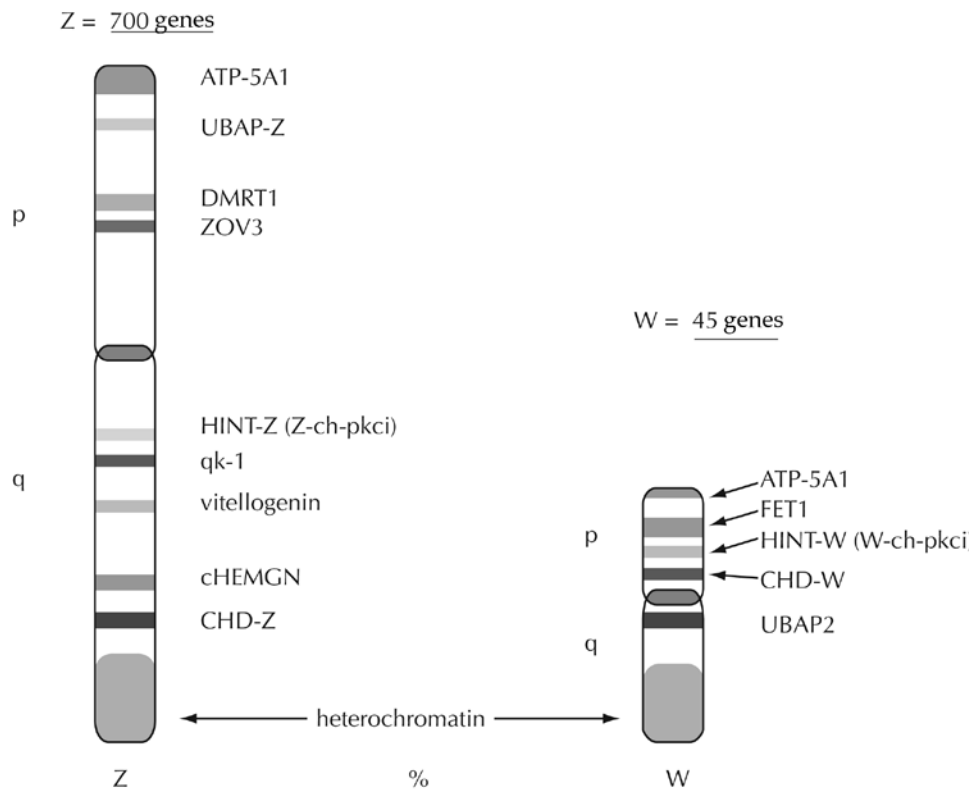

Figure 1. Avian Z and W chromosomes

Autosomes involved in sex determination and regulating gonadal differentiation

Many autosomal genes play a role in avian sex determination, similarly to other vertebrates. However, compared to other animals, less is known about the localisation and functional relationships of these genes in birds.

SOX9 gene is located on a **microchromosome pair**, but its exact location is not known (Kuroiwa et al., 2002). In chicken embryo, it expresses mostly in male individuals, from Day 5 of incubation. The expression of SOX-9 is increased by the product of prostaglandin D(2)/lipocalin-type prostaglandin D synthetase, which gets activated simultaneously with SOX-9 and is a part (or a determining element) of the testis-development inducing system found in every vertebrate that autoregulates the functioning of SOX9 gene (independently from the mammalian SRY) (Moniot et al., 2008). The transcription factor of cHEMGN gene that gets activated before its expression can be another upstream regulation element (Nakata el al. 2013).

In chicken, the FOXL2 gene is located on **Chromosome 9**, WT1 (Wilms tumor 1) gene on **Chromosome 5**, NROB1 (nuclear receptor subfamily 0) gene on **1q**, WNT4 (wingless-type MMTV integration site family, member 4) on **Chromosome 21**, WNT1 (wingless-type MMTV integration site family, member 1) on **Chromosome 1**, GATA4 (GATA binding protein 4) on **Chromosome 3**, steroidogenic factor 1 or NR5A1, also called SF-1 gene (NR5A1 nuclear receptor subfamily 5, group A, member 1) on **Chromosome 17** and as the location of the STARD1 gene that expresses StAR protein is known in humans (**8p11.2**), a similar location is assumed for the homolog gene in chicken (Gene database, 2012).

1.2. Sex determination and the indifferent period of embryonic development of the gonads

Sex determination in birds differs from mammals in at least two basic points. Nevertheless, the exact mechanisms are yet to be known. First, there is no avian equivalent of SRY, found on the mammalian Y chromosome. SRY expresses early, produces a transcription factor and is active for a short time. It activates a gene-cascade system and is responsible for the determination and differentiation of the heterozygotic sex (which in birds is the female). Second, many studies suggest that in birds the equivalent of the mammalian X-inactivation mechanism (the presence of the X-IST gene) is also lacking (in males the inhibition of the expression of one of the Z chromosome-linked genes) and/or the there is no dubling of the expression of Z genes, i.e., dosage compensation (Mank and Ellegren, 2009). In a possible dosage compensation these mechanisms would be much less efficient genetically compared to mammals, because these processes would not expand to the whole of chromosome Z, only to certain genes of the short (p) arm (Itoh et al., 2007, McQueen and Clinton, 2009). In this case, in homozygote ZZ individuals "double copies" could form compared to ZW females, i.e., there could be Z-linked quantitative differences, which alone could form the base of a dose-dependent (quantitative) sex-determining mechanism.

In birds there is a realistic chance of this in the case of DMRT1 (i.e., avian TDF, direct testis-determining function), given that this gene expresses in both sexes, but the amount of protein product in females is very small (Cutting et al., 2012).

Recently, several publications have referred to dosage compensation in birds. Upregulation of sex-determining genes was found in the Z chromosome in female (ZW) carrion crow (*Corvus corone*), zebra finch and chicken, however, it does not mean dosage compensation of all genes (Wolf and Bryk, 2011). Julien et al., (2012) arrived to a similar conclusion in chicken: they assumed partial

upregulation of genes in the Z chromosome in heterogametic (female) individuals. According to others, the expression of somatic-acting Z genes increases in female avian embryos, which could result in Z dosage compensation (Naurin et al., 2010). Another assumption is that masculinising genes on the Z chromosome of males get upregulated, which does not occur in ZW females.

The discovery of the ASW gene calle the attention of "transient intersex forms" in birds, which also suggest some kind of dosage compensation. The ASW gene only expresses in females that possess the W chromosome, and its effect even manifests in triploid chickens (3A:ZZW) initially, against the effect of double Z.

Triploid chickens have an intersex aspect at hatching, they possess a right testicle and a left ovo-testis, and phenotypically they seem female. Later, their ovary tissue degenerates (Thorne and Sheldon, 1993). Similar intersex ZZW chicks have been described in great reed warbler (*Acrocephalus arundinaceus*), in which, similar to chickens, W-linked sex characteristics could not manifest (Arit et al., 2004).

Sex determination is a process that occurs when a haploid Z/W oocyte and a haploid Z/Z sperm unite, which in birds results in ZZ male and ZW female zygotes. After karyogamy, initiator, sex determining gene expression begins, which by activating gene cascade(s), results in **gonadal differentiation** i.e., the somatic manifestation of sex. Currently, two genes are assumed to have **initiator sex determination** function in birds.

One of them is **DMRT1** (dublesex and Mab-3 related transcription factor 1), located on the Z chromosome, which can determine male sex, as a testis determining factor (TDF). In the case of emu, DMRT1 is only found on the forming Z chromosome, on all species it is absent on W, so in birds it is a general, Z-linked sex determining factor. This gene expresses a transcription factor that is able to bind to DNA. It shows close similarity to a DMY gene identified in some Osteichthyes species that expresses a protein, which is a transcriptic factor initiating a cascade mechanism. At the same time, it is dissimilar to mammalian SRY. DMRT1 is known in all vertebrates, including mammals. In humans its malfunctioning causes sex reversion, while in mice its null-mutant causes testicular disfunction. It is known to express in the urogenital ridge well before gonadal differentiation, especially in male animals. Its expression in the germinal ridge and the Mullerian duct of the chicken embryo initiates on Day 3.5-4.5 of incubation, which considering the latter structure, is much more intense in males (Smith, 2007, Cutting et al., 2012).

Recent studies of DMRT1 have revealed a more detailed role of this gene in avian sex determination. According to Zhao et al., (2007), DMRT1 can produce six transcription factors. During the embryonic stage, DMRT1-b is produced in the gonads of both sex, but in a greater amount in males compared to females, starting from the 31st developmental stage (gonad differentiation

time in chicken). Since then in the ovarian primordium DMRT1-c expresses to a greater degree, and DMRT1-f can only be found in the testicular primordium. On the other hand, in mature animals the expression of DMRT1 (combined determination) can only be detected in males.

The other, presumably initiator sex determinant gene is **HINTW** (histidine triad nucleotide binding protein, W-linked), found on the W chromosome. Intensive expression of HINTW also starts very early. In chicken embryo, it can be detected from Day 4.5, i.e., already 1.5-2 days before gonadal differentiation. Earlier, this gene has been identified with the protein kinase inhibitor (**PKCI**) gene, found on both W and Z, and one of its homologues. Z-linked PKCI-Z (**HINTZ**) lacks the catalytic domain, so it can act as a dominant negative isoform against PKCI-W (**HINTW**) present in females, and the resulting heterodimer (HINTW/HINTZ) blocks the activities of HINTZ in the developing gonad. Such interaction between W and Z can lead to the formation of the ovary in a ZW embryo, therefore HINTW can be considered an ovary-determining gene (Pace and Brenner, 2003; Moriyama et al., 2006, Smith et al., 2009). HINTW expression achieved under experimental conditions (after using RCASBP avian retrovirus) triggered the formation of ovaries and normal, bilateral testes in ZW and ZZ embryos, respectively, which suggests that HINTW is not an initator, or at least it is not the sole initiator sex-determining gene (Smith et al., 2009). The sex determining role of HINT genes in Ratites seems even less plausible (Hori et al., 2000).

W and Z chromosomes can interact in a unique way during sex differentiation in birds. In chicken, a **heavily methylated section** (**MHM**) was found on the two Z chromosomes in males, and the same section it is less developed in females (on the single Z chromosome). The difference can be the consequence of a demethylation factor expressed by the W chromosome in females. This less methylated Z results in RNA transcription, which in females involves most of Z, pushing the genes of Z into "silence" state, including DMRT1, responsibe for male sexual characteristics (Teranishi et al., 2001). If this hypothesis proves correct, it means that birds possess a unique sex-determining phenomenon, unknown in other vertebrates.

Another unusual possibility has been raised by Bisoni et al., (2005), who described a different type of W-Z functional relationship. According to their study, a protein expressed by W causes **hyperacetylation** near the MHM region of Z, causing increased expression of Z-linked genes. This can lead to a dosage compensation in the single Z chromosome of the female embryo. This phenomenon can be the genetic base of female sex determination and/or dosage compensation affecting genes of the single Z chromosome and autosomal genes of the female embryo.

Typically, in very early-stage chicken embryo, at the HH4 (Hamburger-Hamilton) gastrulation stage of embryonic development (Day 1), well before

the beginning of sexual differentiation, certain genes show sex-dependent expression that later will be determining sexual dimorphism of the gonads. At this time, PKCIW (HINTW) is intensively expressed in female embryos, while this protein can not be detected in ZZ males. Another, female-specific gene is a Z chromosome-linked thio-redoxin-1-like (TXNL1) gene, which expresses 11 times more in females compared to males, and its synthesis is possibly downstream regulated by the PKCIW protein. At the same time, intensive expression of PELOTA, FANCG, HSD17B4, PGTER4 and STARD4 genes can be detected in male embryos, at about twice the rate of that in females (Zhang et al., 2010).

Following the mammalian model, the function of initiator genes that could be considered sex determining would have two characteristics: 1. their expression begins right before morphological differentiation; 2. they produce transcription factors that rapidly, in a cascade-like way initiate tissue-differentiation regulating genes. In birds, the expression of genes that seem to be sex-determining (initiator), only partially meets these mammalian criteria. As a matter of fact, in chicken embryo, DMRT1 and HINTW are both activated on Day 4.5 of development, one and half or two days before tissue differentiation of the gonads (Day 6.0-6.5), and the expression of SOX9 or CYP19A1 genes, which play an important part in it (Day 6.0). This contradiction seems to be resolved by the fact that both DMRT1 and HINTW genes show a later wave (maximum) in activity. The early expression period of these two sex-determining gene presumably means that a transcription factor isoform is produced by autoregulation that later leads to the formation of a downstream-effective isoform and triggers the sex-determining cascade (Hori et al., 2000; Zhao et al., 2007).

The longer lag between the working intervals of the initiator sex-determining genes and the cascade genes that regulate gonad differentiation can be bridged – theoretically – by intermedier factors, which can be considered early members of the **sex-determining cascade**. These initiator genes express relatively early and are located between the genes responsible for sex-specific differentiation. One possible example is the autosomal FOXL2 (forkhead transcription factor 2), which only expresses in the germinal ridge of the female from Day 5.0 of incubation and its product activates the CYP19A1 gene, i.e., the expression of aromatase enzyme (Hudson et al., 2005a). ZOV-3 gene, found on the Z chromosome, can act as an additional intermediate factor, contributing to the formation of steroid-producing cells and facilitates the genes SF1 (steroidogenic factor) and directly AMH (anti-Müllerian hormone, MIS) stimulated by SF-1 enzyme (Hudson et al., 2005a).

AMH, a tissue hormone of the TGF-β (transforming growth factor-β) glycoprotein group, plays an important role in the sexual differentiation of amniote vertebrates. It is generally secreted in males by primordial-Sertoli cells and later by Sertoli cells. In chicken, AMH expresses during two periods: early expression initiates on Day 4.5-5.0 in both sexes, and it gets stronger on Day

5.5-6.0 in males. This later period in males coincides with the increased expression of DMRT1 and the beginning of the expression of SOX9. In male embryos on Day 7 of incubation the expression of AMH-SOX9 keeps increasing in a cascade, and these processes occure about a day before the formation of the testis bundle. Later, AMH is produced nearly exclusively in the deeper bundles and the older bundles gradually lose their AMH expression capacity. The production of AMH is not exclusive to males, it is also expressed in certain degrees in female birds (Vaillant et al., 2001). Its early production in female embryos can be responsible for the degeneration of the right Mullerian duct. It is possible that further differentiation of the left ovary and the left Mullerian duct – in spite of the effect of AMH – is assured by the higher estrogen-binding ability (higher number of receptors) of the left-side areas (McLaughlin et al., 1983; Oréal et al., 2002).

The expression of the genes that regulate the growth and differentiation of the gonadal primordia should occur before the initiation of morphological differentiation, which means before Day 6 of the incubation in chicken embryo. At this time, the expression of two genes has indeed been detected. The expression of SOX9 (SRY-like high mobility group-box containing gene 9) initiates in chicken testicular primordium on Day 6 (in quail on Day 5, while in duck on Day 7 of incubation), perhaps as a result of the stimulation from DMRT1 that expresses probably a bit earlier or at the same time.

It can be assumed that the stimulative effect of DMRT1 is enhanced by the transmissive role of other (not yet identified) male-specific gene(s). The role of SOX9 is well known in mammals, where its expression is triggered by the protein product of the SRY gene, which makes it the second member of the male sex-determining cascade mechanism, and its transmission factor activates AMH, the third member of the cascade. SOX9 regulates the differentiation of the Sertoli cells of the testis, and the development of the seminiferous bundles and tubuli. In birds, as SOX cannot activate AMH, as it expresses later, therefore it cannot function as part of a sex-determining cascade, unlike in the mammalian system (Oréal et al., 1998; Oréal et al., 2002; Takada et al., 2006). At the same time, the expression of SOX9 coincides with the development of the testicular bundles and the Sertoli cells (Kent et al., 1996).

In the mammalian ovarian primordium RSPO1 (R-spondin homolog 1) gene expresses initially, and its protein product, along with transmission factor of the gene Wnt4, stimulate the production of B-catenin. This latter is the upstream regulator of the FOXL2 gene. FOXL2 protein is an important activator of CYP19A1 gene. In chicken ovarian primordium on Day 5.0 of incubation FOXL2 protein triggers the expression of CYP19A1 (aromatase) gene (Smith et al., 1997; Nakabayashi et al., 1998; Smith et al., 2005). The upstream regulation of FOXL2 in birds has been assumed to be similar to that in mammals,

i.e., through B-catenin as well as RSPO1 and WNT4 genes, which stimulate B-catenin simultaneously (Cutting et al., 2012).

CYP19A1 (aromatase), the rate limiting enzyme of estrogen synthesis, has a crucial role in the regulation of ovarian development in birds (Smith and Sinclair, 2004). Estrogen plays a proliferative role by binding to α-estrogen receptors (ERα) that develop in the germinal ridge. Before sexual differentiation, estrogen receptors express in both sexes, which suggests estrogen plays an early regulative role in avian embryos. It can be first detected in urogenital tissue of chickens on Day 3.5 – however, at this time only in females, and from Day 4.5 estrogen receptor expresses in the developing gonads of both sexes (Smith et al., 1997). In males, the amount of ERα begins to decrease on Day 6.5, while in females it significantly inreases around the cortical areas of the ovarian primordium (Smith et al., 1997). The important role of estrogen in the sexual differentiation of birds is suggested by the observation that if fadrozole is administered to experimentally inhibit aromatase on Day 3.5 of incubation, the left gonadal primordium will not develop into an ovary in genetically female embryos and the right gonad will develop into a testis.

During gonadal differentiation further genes will express, which are known as part of the mammalian sex-determining cascade: STARD1 (StAR protein), SF1 (steroidogenic factor-1, or: Ad4BP), DAX1 (dosage sensitive sex reversal, adrenal hypoplasia congenita, X-linked 1), WNT4 (wingless, int-related, 4) and AMH (anti-Mullerian hormone). Feng et al., (2007) detected the expression of seven genes in chicken embryonic gonads between Days 4-12 of incubation. There was sexual dimorphism in the expression of cLhx9, cGATA4, cVnnl, cPptl and cBrd3, while cEki2 and cFog2 expressed in both sexes with the same intensity.

These genes regulate gonadal tissue differentiation, sexual steroid biosynthesis, and the development of the Mullerian duct. Unlike in mammals, where SRY regulates male sexual development, none of these genes possess a primary sex-determining function. At the same time, they can probably get activated by the transcription factors of HINT (PKCI) and/or DMRT1 genes, and therefore they could be second or third elements of the avian sex-determining cascade.

During sexual differentiaton, the intensification of the expression of sex-steroid synthesising enzymes (the second "expression wave" of their genes) coincides nearly completely with tissue differentiation. On Day 6.5-7 of incubation in chicken, the activity of P-450 scc., 3-β-HSD and P-450c17 increases in both sexes equally, suggesting transformation of cholesterol to androstenedione. On the other hand, the genes of 17-β-HSD and P-450 aromatase enzyme only express in the medullar cells of the bilateral ovaries of female embryos from Day 6.0-6.5, which corresponds to the sexual dimorphism in the conversion of androstenedione to estrogen, which is present from the beginning.

Nevertheless, some studies suggest that steroids do not play a part in early gonadal differentiation. It has been found that neither the lack of aromatase nor the presence of testosterone play a role in testis development. The observation that in female birds on Day 14 the genes for enzymes necessary for estrogen synthesis keep expressing in the already strongly regressed right ovarian primordium suggests that tissue differentiation and steroid synthesis occur independently from each other (Nakabayashi et al., 1998; Vaillant et al., 2001; Smith, 2007).

The asymmetry between right and left gonads that develops during gonadal differentiation and is characteristic of birds, can be partially explained by the fact that in chicken during gonadal differentiation the estrogen receptor gene expresses only in the cortical part of the left germinal gland. The expression continued to decrease in the developing left testis on Day 10-12 and ceased on Day 14, while in the left ovarian primordium it maintained its levels during the study period (Day 7-14) (Nakabayashi et al., 1998).

Several genes responsible for gonadal differentiation express in both sexes (e.g., DMRT1 and AMH), but the amount of their products differs between the testis and the ovary. The expression of these genes seems to be largely regulated post-transcription by **micro-RNA (miRNA).** miRNA genes are located in intragene and intergene positions on the chromosomes, and are activated autonomously or along with the transctiption of the host gene. miRNA transported to the cytoplasm gets embedded in the RISC (RNA-induced silencing complex) that forms by the translation of transfer RNA and activates it. Next, RISC causes translation repression, and through this increases or decrases polypeptide synthesis (Brennecke et al., 2005). The sexually dimorphic expression of miRNA has been found not only in mammals, but also in birds, so it can be assumed (even though not directly proven) that they regulate genes responsible for sexual differentiation in a similar way. Their amount significantly increases in chicken gonad at the beginning of tissue differentiation (Day 6.5), more so in the testis than in the ovary. They seem to operate through the modulation of the SOX9 – AMH - TGF-β signal mechanism (Cutting et al., 2012).

Early gonadal tissue differentiation

The paired gonadal primordium (genital ridge, plica genitalis) can first be detected as a thickening cell layer, ventro-medial to the pronephros at 72 hours of incubation in chicken, at 90–96 hours in duck and at 50–55 hours in Japanese quail. The coeloepithel gradually proliferates to several layers thick, and transforms into a squamous or columnal peritoneal epithelium that covers the mesenchyme that lies underneath. In this phase, the intensively proliferating cells show laminin-1 activity, suggesting their ability to rearrange (Sekido and Lovel-Badge, 2007). The gradually bulging gonadal primordium is separated

by the pronephros by a lateral bridge, and by the dorsal root of the mesentery by a medial ridge (Figure 2).

Coinciding with this process – but only in male embryos – certain cells of the pronephros turn amoeboid cells and colonize the epithelium of the germinal rige. These cells contribute to the forming of the interstitial cells among the forming testis bundles. It is not known whether these cells turn into Leydig cells, myoid cells or vascular endothelium. The role of PDGF (platelet-derived growth factor) in the regulation of cell migration is probable (Smith et al., 2005).

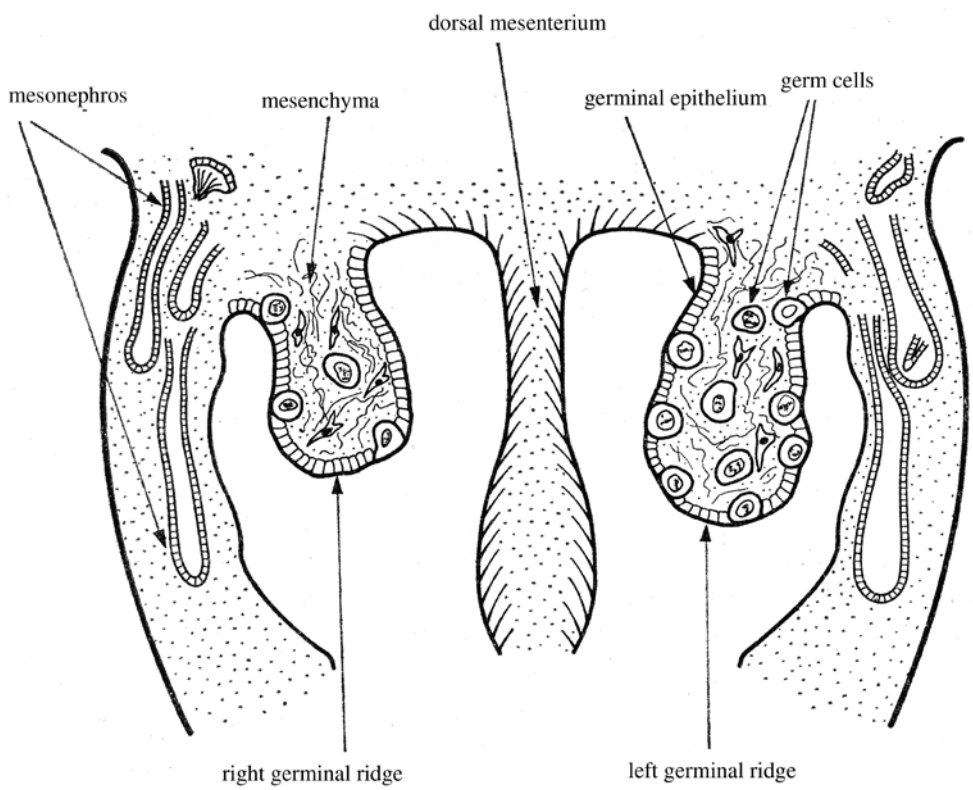

Figure 2. Germinal ridges in the 96-hours old chicken embryo, modified after Shahin (1973)

On Day 4 of incubation a part of the germinal ridge sinks into the deeper-laying mesenchyme. Primary, primitive germinal ridges (bundles) are formed, surrounded by basal membrane, with a cortex forming on the surface. In chicken embryo, the gonadal primordium looks morphologically similar in genetically male and female individuals on Day 3.5-6.5, therefore, this stage is called the indifferent developmental stage. Histological characteristics of gonadal differentiation initiate somewhat later, on Day 6.5-7 (Shahin, 1973).

In the indifferent (biopotential) phase several specific gene expression occur. The fibroblast growth factor regulating gene plays a role in the initiation of tissue proliferation, which leads to the thickening of the mesenchymal stroma that develops under the peritoneal epithelium (Yoshioka et al., 2005). The regulating effects of the GET (gonad expressed transcript) gene on tissue development are of similarly wide range. This gene expresses in many parts of the urogenital system and in chicken it is first detected on Day 2.5 in mesonephric glomeruli. On Day 4.5 it expresses in the cortical region of the developing gonads in both sexes. This gene continues to be active in adults, but it is only detectable in the ovary. It plays a role in the development of the Wolffian and Mullerian ducts, as well as the external genitalia (Hudson et al., 2005b). Other genes play a part in the proliferation of the germinal epithelium that originates from the coeloepithel and the formation of steroid-producing enzymes. These genes, such as ZOV-3, WT-1, GATA-4, LHX-9 and the steroidogenic factor (SF-1) appear in chicken embryo on Day 3.5 (Brennan and Capel, 2004; Smith and Sinclair, 2004). The genes SF-1 and ZOV-3 also express in the cells of the mesonephric glomerules, suggesting these play a role in the morphogenesis of the germinal ridge genitalia (Hudson et al., 2005b).

Presumably, sexual steroids play an important role in the regulation of early gonadal tissue development in birds (similar to reptiles, amphibians and fish). This is suggested by the first expression wave of their enzymes, still in the indifferent phase, when 3-β-hydroxysteroid-dehydrogenase and 17-β-hydroxysteroid dehydrogenase appear early in the mesonephros and germinal ridge. An exception is P-450-aromase, which can only be detected on Day 6.0 of incubation, right before gonadal differentiation initiates.

In mammals, on the other hand, steroidogenic enzymes synthesis only starts later, when the differentiation of gonads already shows definite sexual differences, syntethising mostly testosterone in males and estrogens in females. Therefore, in mammals steroids play a role only in the later, differential development of gonadal structure (Lun et al., 1998, Wolf, 1999, Villalpando et al., 2000).

Histologically characteristic sexual differentiation of the germinal ridge begins on Day 5.5-6.5 of incubation in chicken (Romanoff, 1960), Day 5 in quail and Day 7 in duck. In the species studied, testis and ovary differentiation begins at the same time, which is a fundamental difference compared to mammals, where the priority of SRY expression results in earlier development of the testes. With the beginning of differentiation in male avian embryos the primary germinal epithelial bundles form a meshwork by branching and anastomosing into secondary and tertiary arms, while the cortical germinal epithelium atrophies and becomes a single-layered, thin epithelium. In female embryos, on the other hand, the surface cords thicken into a compact cortical layer, while the

medullar epithelium breaks ups and lacunae are formed between the cords in the deeper layers.

In males, the gonadal primordium on the left and right sides develop with nearly equal intensity. Nevertheless, in females the development is stongly asymmetrical, the right ovarian primordium grows only until Day 10-12 of incubation, when its growth slows down, the right cortex degenerates, and the medullar bundles disintegrate and later atrophy. Later, the rudimentary right ovary becomes a few cell-layer thin, flat disc. In the left ovary, however, the cortical material keeps thickening, even though the medullar cords disintegrate, decrease in volume and lacunae form among them. The right, rudimentary ovary has a masculinising potential – if during development the left ovarian primordium gets injured, the right ovary develops into a testis, and the embryo becomes masculinised. This developmental abnormality can be explained by insufficient or complete lack of estrogen production by the left ovarian primordium or the inhibition of aromatase enzyme expression in the primordial gonads (Vaillant et al., 2001). The opposite process does not occur, i.e., treating a male embryo with estrogen only causes termporary feminisation, while ovotestis develop on both sides, these atrophy when the α-estrogen receptors that were earlier present in the male gonad and gonadal track disappear (Nakabayashi et al., 1998). (Figure 3.)

Considering ovarian development, exemptions are members of *Apterigyformes, Falconiformes* and *Accipitridae,* in which rudimentary ovaries remain on both sides and become gonads. On the other hand, in *Strigidae* the right ovary completely disappears (Romanoff, 1960).

Figure 3. Gonad differentiation, the effects of major genes

AMH: anti-Mullerian hormone gene, **CYP19A1**: member of the cytochrom P450 family, 19 subfamily, 1. polypeptide gene, a monooxigenase element of steroid biosynthesis, **DAX-1**: dosage-sensitive sex-reversal, adrenal hypoplasia critical region on chromosome X, gene 1, a determining element in the hypothalamus-hypophysis system, endocrine functioning of the gonads and the adrenal cortex, **DMRT-1**: DSX/Mab-3 Related Transcription Factor-1, Dublesex and Mab-3 Related Transcription Factor, basic sex-determining/differenciating gene, **EαR**: estrogene-α receptor gene, **FANCG**: Human Fanconi anemia complementation group G gene, chromosome stability regulating factor, **FOXL-2**: Forkhead box L-2 gene, determining element of ovarian differentiation, **HINTW**: WPKCI-8 histidine triad nucleotid binding protein W, determining gene in ovarian differentiation, **HSD17β4**: 17β-hydroxysteroid dehydrogenase-gene, which regulates the expression of two enzymes: peroxysomal β-oxidation of fatty acids and catalyses the formation of 3-ketoacyl-CoA, **miRNA**: micro-RNA genes: their expression shows sexual dimorphism: – miRNA – mi-transfer RNA translation – RISC (RNA-induced silencing complex) activation controlled by miRNA, **PELOTA**: PELO, Pelota Homolog, DOM34, CGI-17: meiosis regulating gene, **PGTER4**: gene resulting in the expression of the prostaglandin-E2,EP-4 receptor, **PGR2**: PTGR2, GPR142-gene, its product is the G-protein-linked receptor 142: member of the rhodopsin group, prostaglandin reductase-2, which converts 15-ketoprostaglandin E2 into 13-14-dihydroprostaglandin E2 and also stimulates a peroxisome-proliferator-activated receptor, **P450c17**:

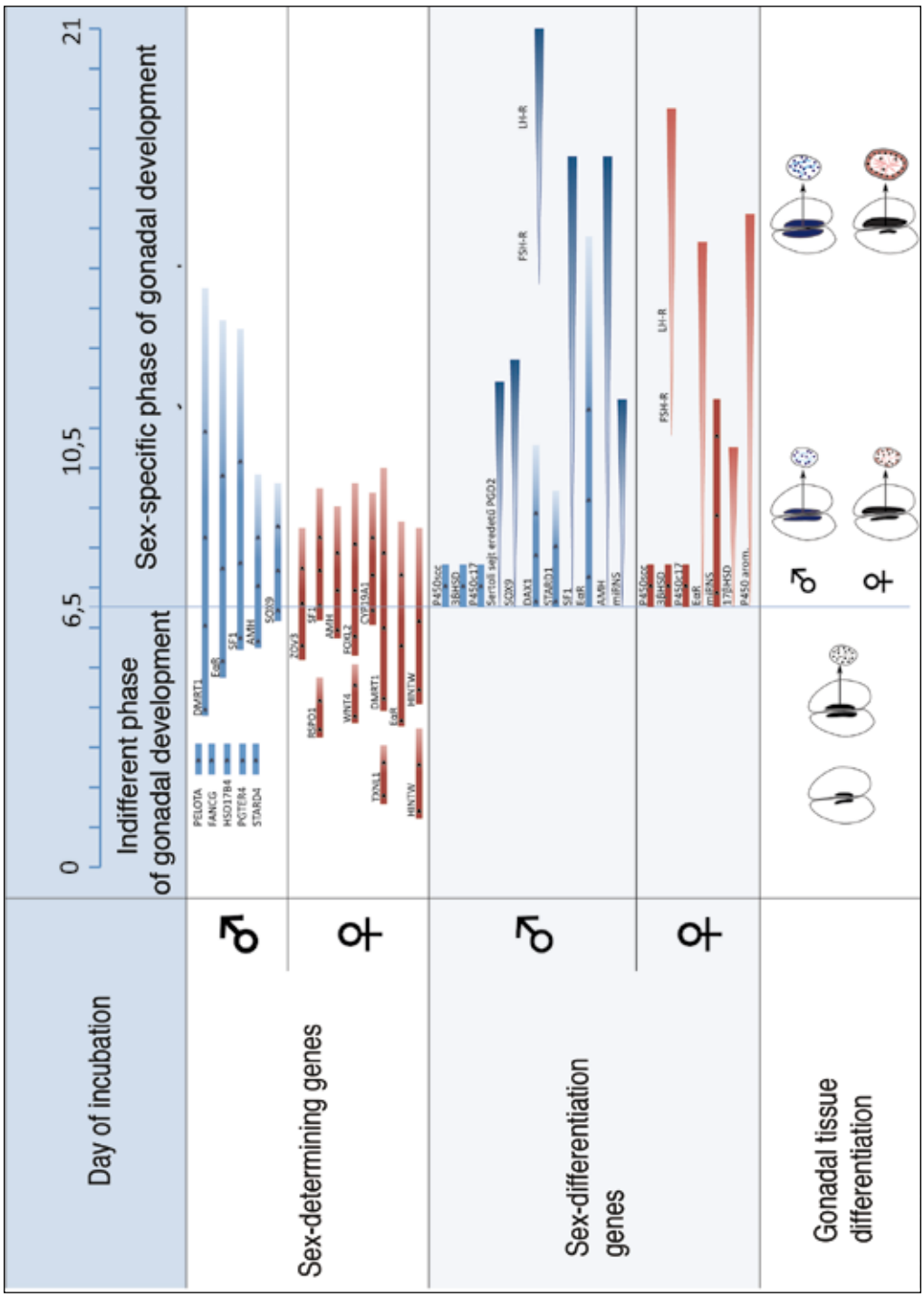

Figure 3. Gonad differentiation, the effects of major genes

cytochrome P450-17-hydroxylase gene, its product is the P450c17-enzyme, which has two effects: it causes 17 hydroxylation and 17,20 liase activity, **P450scc**: a gene resulting in P450 side-chain clevage enzyme expression, which is a factor initiating steroid genesis, **P450arom**: Cyp19a, Cyp 19b, cytochrome P450 aromatase gene: its product converts aromatisable androgens into estradiol, **RSPO1** gene: its product is a member of the R-spondin group, which is an activator of the β-catenin signal cascade, causing cell proliferation, **SF-1**: steroidogenic factor-1, its product regulates genes that regulate the endocrine functions of the hypothalamo-hypophyseal system, the adrenal cortex and the gonads, with an important role in the regulation of steroidogenesis, **SOX9**: sex-determining region-y gene, its product is the main regulator of the AMH, **STARD-1**: STAR, steroidogenic acute regulatory gene, its product regulates pregnenolone – progesteron conversion, **STARD-4**: START domain containing-4, sterol regulated gene: its product is a steroidogenic acute regulating protein (STAR)-linked type 4 lipid transfer-protein, **TXNL1**: Thioredoxin-Like 1 gén: its product is the disulphid oxydoreductase electro transporter, **WNT4** gene: Wingless type MMTV integration site family member-4, its product is a testis determining factor, **ZOV3** gene: Basigin, CD147, neuroplastin, a member of the basigin group, its multifunctional product is a transmembrane protein that regulates cell adhesion, **3βHSD**: 3-β-hydroxysteroid dehydrogenase gene, its enzyme product determines all steroid hormone synthesis, especially estrogens, **17βHSD**: 17-β-hydroxysteroid dehydrogenase gene, its enzyme product catalyses androstenedione – testosterone conversion by reduction (hydrogenation)

The development of primordial germ cells

During early development of the germinal ridge (in chicken Day 3.0-3.5 of incubation) larger basophil cells appear along the blood vessels, in greater amount on the left side. There are two hyphotheses about the origin of these primordial germ cells. According to one, they originate from the hypoblast (or endophyll area) from the deeper, δ-ooplasm, near the nucleus of Pander (Callebaut, 2005). A different, earlier hypothesis is confirmed by Smith (2007), which suggests that primordial germ cells originate from from the surface, β-ooplasm and from the originating epiblast (Eyal-Giladi et al., 1981; Tsunekawa et al., 2000).

The appearance of primordial germ cells can be followed from the stage when the germinal disk is in the 300-blastomere cell phase. The expression of the vasa gene (and the production of the vasa protein), characteristic of primordial germ cells, can be detected in the 6-8 cells located in the central part of the germinal disc, so these cells can be considered primordial germ cell predecessors. Later, with the thinning of the area pellucida, the central region becomes well separated and this area extends towards the edges with the development of the primitive streak, where its cells sink towards the deeper layers. These displaced cells form the hypoblast layer of the germinal disc, which during gastrulation spreads out and moves forward, forming the extra-embryonic endoderm of the germinal crescent. By the end of gastrulation, when the embryo is in the 10 somite stage, about 200 larger cells separate out near its cranial end. These cells are typical primordial germ cells, characterised by a large, excentric nucleus, high glycogen and lipid content, high alkaline phosphatase activity

and the development of several lectin-binding sites on the surface (Tsunekawa et al., 2000; Ginsburg, 1997; Callebaut, 2005; Armengol et al., 2007).

After about 40 hours of incubation, primordial germ cells start to enter the blood stream. Vascular transport of primordial germ cells into the germ ridge is basically different from amphibians and mammals, where primordial germs cells reach the germ ridge by extravascular migration using amoeboid movements. In chicken embryo, the supracranial region gets rapidly engulfed by capillaries. These capillaries grow around and engulf the developing gonocytes, and these gonocytes can also actively peneterate blood vessels.

In this process, molecules originating from the extracellular matrix (laminin, fibronectin, chondroitin-sulphate and tenascin-C) and citokins can also play a role (Nakamura et al., 2007). After 54 hours of incubation the amount of gonocytes gradually decreases at the cranial end of the embryo, while their number increases near the germinal ridge, along the dorsal mesentery. These cells leave the blood vessels and by amoeboid movement penetrate into the peritoneal epithelium, where they become rounded (Eyal-Giladi et al., 1981). This process is likely to be regulated by a chemokine, SDF-1 (stromal cell-derived factor-1) (Strebler et al., 2004).

Some studies suggest that there are sexual differences in the quantity and size of primordial germ cells before Day 6.5 (Zaccanti et al., 1990). However, according to the accepted view, the appearance of primordial germ cells does not influence the development of the primordial gonad, as their amount does not have a major effect on the induction of histological or developmental processes (Romanoff, 1960, Ebensperger et al., 1988; Hudson et al., 2005b).

In contrast, it seems like in birds the sexual characteristics of the primordial gonad are able to influence the differentiation of developing gonocytes. It has been shown in ZZ/ZW chicken embryo chimeras that, unlike in mammals, ZZ (male) gametes transferred into a female (ZW) gonad turn into oocytes and the other way around, female (ZW) gametes turn into spermatocytes in a male (ZZ) gonad. This phenomenon suggests that the genes necessary for oogenesis and spermatogenesis are not sex chromosome-, but autosome linked (Naito et al., 1999).

1.3. Ovarian differentiation

From Day 6.0-6.5, the development of differentiating gonads in a chicken embryo results in more and more sex-specific morphological changes. Sexual differentiation is linked to hormonal changes in the gonadal tissue, as well as to hormones originating from the hypothalamo-hypophyseal system. The first sign of differentiation is the initiation of aromatase and 17β-HSD enzyme expression on Day 6.0, in the medullar area of the ovarian primordium. Among

the avian female sex-determining genes, the role of SF1 is apparent in this process, as its product links to the promoter region of the aromatase gene, acting as a transctription factor. At this stage however, there is also SF1 expression in the male embryo, therefore additional factor(s) are necessary for the specific sex development of females. Another gene that can play an important part in the specific differentiation of the ovary is FOXL2 (forkhead box-transcription factor, class L2). The mRNA of FOXL2 only expresses in the female embryo on Day 5.5, right before aromatase, in the medullar region of the gonadal primordium. The promoter region of aromatase also has FOXL2 binding sites, which suggests a certain functional (cascade) relationship. The resulting estrogen is known to increase the expression of FOXL2 via positive feedback, and therefore affects the differentiation of the gonadal primordium into ovary.

Central regulation also plays an important role in the specific sex differentiation of females, i.e., it strengthens aromatase expression. The first sign of the development of a central regulation in chicken embryo is the appearance of gonadotrop receptors on incubation Day 4, both in the ovary and the testis. The expression of LH receptor and FSH receptor remains low on Day 4-6 in the testis, while from Day 6 it increases in the developing ovary. After incubation Day 12 the ovary contains nine times more FSH receptors and seven times more LH receptors compared to the testis tissue (Akazome et al., 2002). In the case of the ovary, the regulating effect of adenohypohysis on the proliferation of primordial germ cells and somatic cells can already be detected from Day 9, which is likely to act via FSH. The regulating role of the hypophysis in the development of testis appears later in the life of the embryo, from Day 14 of incubation (Méndez et al., 2003; 2005). Therefore, hormonal effects affecting ovarian differentiation are more pronounced compared to that of the testis, and the ovary begins to differentiate earlier. These observations suggest important differences between birds and mammals.

Typically, in the differentiating ovarian cells of the deeper-laying germinal bundle that have sunken into the mesenchymal connective tissue, show fatty and luteal degeneration and these structures rapidly disappear. At the same time, the surface area of the germinal epithelium thickens and gradually forms a lobulated cortex. An important change occurs in the distribution of primordial germ cells, their numbers decrease in the deeper-laying mesenchymal stroma and in the medullar material rich in blood vessels, while most of them are located in the cortical tissue, which is first pliated then becomes lobulated.

On Day 8-10 of incubation, primordial germ cells begin to differentiate into smaller oogonia by intensive mitotic division. Around the oogonia, the cuboidal epithelium cells that have differentiated from the germinal epithelium start to conglomerate. These cells will form the follicular or granulosa layer of the future follicles, connected by tight junctions in their membrane. The developing granulosa (ovarian follicle) cells can be divided into three groups: Type A

cells will remain in both ovaries until hatching, and have phagocytic activity. The organelles (tubular mitochondria and smooth endoplasmatic reticulum) of type B and C cells suggest steroid (estrogen) production. The reduction in the number of B cells is typical of the degenerating right ovary (Avila et al. 1989 a,b). Calbindin-D2K, a calcium-binding protein appears from incubation Day 8, mostly in the follicular cells of the left ovary. This protein seems to play an active role in the regulation of the early oogonium-oocyte development (Inpanbutr and Taylor, 1993).

The differentiation of the somatic elements of the ovarian primordium is followed significantly later, from incubation Day 15.5, by the transformation of oogonia to primary oocytes, which is accompanied by the altered divisional capability of the primordial oocytes. When the cell gets into primer oocyte stage, it stops miotic division and moves into the first prophase of meiosis, and its division stays in the diplotene phase. In the embryonic ovary certain stages of the meiotic prophase of the primer oocyte can be observed. In the leptotene phase, the DNA double helix splits in the thin despiralising chromonemal fibres that form the chromatida, while intensive transcription occurs. In the zygotene phase, synapsis forming and recombination initiates between the autosome homologues, and transctiptive processes slow down to a great degree. The synaptic coupling of Z and W chromosomes is not yet initiated. The W chromosome is completely covered by three histone elements, while the Z chromosome is only covered at the heterochromatic region. At the beginning, the pachytene autosomes form synapses in their entirety, while the increased spiralisation of chromonemes leads to their shortening. The histon-coat gradually disappears from the Z and W chromosomes, they attach to each other by their homologous end, and in the later stage of pachyten – unlike in mammals – synapses extends to their whole length, and becomes heterologous. At this time, they have substantial transcription activity. However, some parts of Z and W do not form a heterologous synapse at the beginning of prophase and do not enter the diplotene phase, but disintegrate. In the diploten phase the shortened and aligned autosome pairs separate from each other lengthwise and the synapses disappear. Synapses also stop forming between Z and W chromosomes (Guioli et al., 2012).

The meiotic divison of the heterologous (XY) mammalian chromosome assembly is characterised by meiotic sex chromosome inactivation (MSCI), during which in the first prophase a significant part of X and Y chromosomes do not form synapses. In the nucleus of the sperm synaptonemal complex only forms in the pseudoautosomal, apical X and Y region, in other areas genes do not express. The genetic importance of this phenomenon is not yet known. According to Schoenmakers et al., (2009), the situation is similar in chicken, where the ZW pair temporarily does not form synapses between early pachyten and early diploten, which would result from heterochromatin translocation

from W to Z. At the same time, the γ-histone coat that covers the body of XY in mammals, does not form in this region. Recent studies on embryonic and early postembryonic studies on chicken suggest the lack of meiotic sex chromosome inactivation (MSCI), typical of mammals, as Z and W chromosomes form synapses along their total length temporarily, between the pachytene and the early diplotene phases (Guioli et al., 2012).

Temporal inactivation of the non-synapsis-forming chromatin material of sex chromosomes has also been shown in male birds. A specific chromosome (peculiar germline restricted chromosome – GRC) has been found in both female (two copies) and male (one copy) zebra finch. In females, the two copies form a synapsis and recombine during prophase. In contrast, in males GRC temporarily gets inactivated during the early leptotene, stays mostly inactive during the first prophase and does not undergo meiotic DNA duplication. After metaphase, a micronucleus is formed. This process that occurs in spermatocytes can be considered a temporal inactivation that preceeds synapsis formation. This process of inactivation is different from the temporal meiotic inactivation linked to synapsis-forming chromatin that occurs is mammals (Schoenmakers et al., 2010).

During embryonic development, the oogonium-oocyte transformation and the arrest of oocyte in prophase I (diplotene) of meiosis signify basic sexual dimorphism in the development of germ cells, characteristic of all vertebrates. In the male embryo – presumably – the pre-Sertoli cells of the developing testis bundles produce a mitosis-inhibiting substance, which is absent in the developing ovary. The fact that primordial germ cells (independently of the sex of the individual) continue to develop by meiosis in all tissues except for the testis, and also under *in vitro* conditions, leads to two assumptions: 1. primordial germ cells are "tuned to meiosis" that occurs spontaneously or 2. there is an inductive effect that originates specifically from the ovary.

This second theory seems plausible, as formation of retinoic acid has been detected in the urogenital system during the critical developmental stage of gonocyte development by studies first in mammalian and later in chicken embryo (Smith et al., 2008). In mammals, the expression of Raldh2 (retinal aldehyd-dehydrogenase 2) enzyme initiates in the mesonephros right before the oogonium-oocyte transformation and before the beginning of meiotic division. This enzyme is responsible for retinoic acid synthesis. In chicken, its expression begins in the cortex of the left ovary on Day 12.5 of incubation, preceeding the meiotic division of primary oocytes that form from the oogonia on Day 15.5. The resulting retinoic acid initiates meiosis in oocytes via meiotic markers (Stra8 – protein stimulated by retinoic acid gene 8 and Scp3 – synaptonemal complex 3).

In mouse embryo, the sexual dimorphism of primordial germ cell development is (also) regulated by the enzyme (Cyp26b1) responsibe for the metabolism

of retinoic acid. The Cyp26b1 gene expression i.e., retinoic acid degradation becomes very strong in male mammalian embryos right before the end of the mitotic division of spermatogonia. Therefore, the lack of retinoic acid in males inhibits the process of meiosis after mitotic division of primordial germ cells. In contrast, in female embryos the expression of inhibiting Cyp26b1 gene ceases right before the beginning of meiosis, therefore retinoic acid synthesis can continue intensively, and its meiosis-initiating effect can take place.

In chicken embryo, the synthesis of Raldh2 and Cyp26b1 co-occures in an intergrated way. On Day 10.5 of incubation, the expression of the Cyp26b1-stimulating degrading enzyme strongly decreases in the developing (mostly left) ovary, making the accumulation of retinoic acid and meiotic markers possible. In the testes, however, Raldh2 and Cyp26b1 are syntethised at the same rate, which inhibits retinoic acid accumulation and the conversion of mitotic into meiotic division. In the female embryo, the processes in the right ovary are similar to those in the testis, and as a result, no or few oocytes develop meiotically (Smith et al., 2008).

Gonadotrop hormons are likely to have an early effect in the oogonium-primer oocyte transformation that occurs in the embryo. In ovo LH treatment of chicken embryo decreases oogonium proliferation and increases the amount of oocytes and primordial folliculi (González-Morán, 2007).

The development of oocytes i.e., the molecular mechanism of arresting and restarting meiotic division twice, has not been studied in birds, but it has been the topic of intensive studies in mammals (especially mice), consequently the process is now well known. It can be assumed that the basic steps of the process are similar in birds. Therefore, it is useful to review the whole process of meiosis based on the mammalian model.

During embryonic development of mammals, in the diplotene stage of the first prophase of meiosis, after receiving impulses from the granulosa, GPR3 receptors in the oocyte activate the G8 protein of the G protein family, which in turn stimulates adenylcyclase. By getting continuously activated, adenylcyclase produces cAMP, while concurrently a significant amount of cAMP also enters the oocyte by diffusion from the granulosa. The increased amount of cAMP in the oocyte activates protein kinase A (PKA). When the amount of PKA reaches a critical threshold, it phosphorylates the CDK1/cyclinB protein complex (meiosis promoting factor, MPF), which blocks the meiosis from getting into metaphase (Stop 1). Having a low level of the degradation enzyme, oocyte cAMP phosphodiesterase (PDE3A), is also crucial in maintaining a high level of cAMP. The arrest of meiosis 1. lasts for a long time into post-embryonic development, and only gets released during puberty, when the increased flow of LH causes the amount of cAMP to decrease in the oocyte. LH initiates the first restart of meiosis by activating INSL3 (Leydig insulin-like 3 polypeptid growth factor) in the theca cells and when this growth factor diffuses into the oocyte it

stimulates LGR8 (Leu rich repeat containing G-protein coupled) receptor. This receptor binds a protein wih GTP-ase activity, which binds to the G8 protein to increase GTP-GDP transformation on its subunit a. This process eventually inactivates the G8 protein that plays a key role in cAMP synthesis, and therefore decreases the cAMP content of the oocyte. Alternatively, in the granulosa cells LH stimulus triggers the synthesis of epiregulin, an epidermis growth factor, which increases the activity of cGMP phosphodiesterase, and therefore cGMP production. Consequently, the degradation of cAMP significantly increases, its level sharply drops in the oocyte, which leads to the termination of meiotic stop 1. Meiosis-activating sterol (MAS), which gets synthetised in the granulosa cells as a result of the effect of LH, can have a synergistic effect, as it plays a role in the restart of meiosis 1. when diffused into the oocyte. The effect of this steroid is proven in fish and amphibians, but its role is currently disputed in mammals.

The development (division) of oocytes triggered by LH proceeds until the secondary oocyte stage, and it stops again after ovulation, in the second metaphase of meiosis. This Stop 2. is triggered by the effect of a cytostatic factor (CSF), which inhibits the degradation of MPF, and is therefore necessary for the continuation of division. APC/C (anaphase promoting complex/cyclosome) that acts at the beginning of anaphase gets inhibited, and the division cannot proceed into anaphase. CSF is not a single compound, but the combined inhibitory effect of many factors. On the one hand, it is a protein kinase cascade, also called SAC (spindle assembly checkpoint) protein: cMOS/MEK/MAPK/p90rsk chain (MAPK = mitogen-activated protein kinase, MEK = extracellular signal regulated kinase-kinase, i.e., MAPK-kinase, MOS = mitogen-activated protein kinase-kinase-kinase, i.e., a kinase that activates the whole line of MAP kinases, p90rsk = 90 kD ribosomal protein S6 kinase). The other ingredient of CSF is Plk – Emi2 (Emi2 = early mitotic inhibitor 1-related protein 1, Plk = polo-like kinase). Emi2 is the substrate of Pl(k)-kinase. This kinase plays an important role as the stabiliser of metaphase.

Stop 2. of meiosis gets resumed by fertilisation, i.e. by the penetration of a sperm, when the division continues. The large amount of Ca imported into the oocyte by the sperm activate calmodulin-dependent protein kinase II (CalkII), which directly phosphorylates Emi2. Plk also plays a part in the phosphorylating degradation of Emi2. This process ends the inhibitory effect of CSF, the cyclinB element of MPF gets degraded, which lifts the inhibiting effect on APC/C, and therefore the process of division enters (II.) anaphase (Richard, 2007, Madqwick and Jones, 2007, Sun et al., 2009, Tsafriri and Motola, 2007).

In chicken embryo, concurrently with the formation of primer oocytes, small (20-80 μm) primordial follicles develop in the cortex. The primer oocytes that lack single-layered granulosa layer become apoptotic. The number of primordial follicles and therefore the primary oocytes in domestic chicken embryo is

28,000 on Day 9 of incubation, their number reaches a maximum on Day 17 (680,000) and by the time of hatching it decreases to about 480,000. From this large amount only about 2-500 oocytes undergo ovulation during the lifetime of a hen.

From Day 14 of incubation until hatching, tunica albuginea (connective tissue tunic) is formed on the surface of the ovary, which is relatively rich in cells and is of mesenchymal origin and it tightly coats the the single-layer surface peritoneal epithelium. This tunica extends dorsally to cover the mesovarium, the peritoneal fold connecting the ovaries with the vertebral column (Figures 4. – 5.)

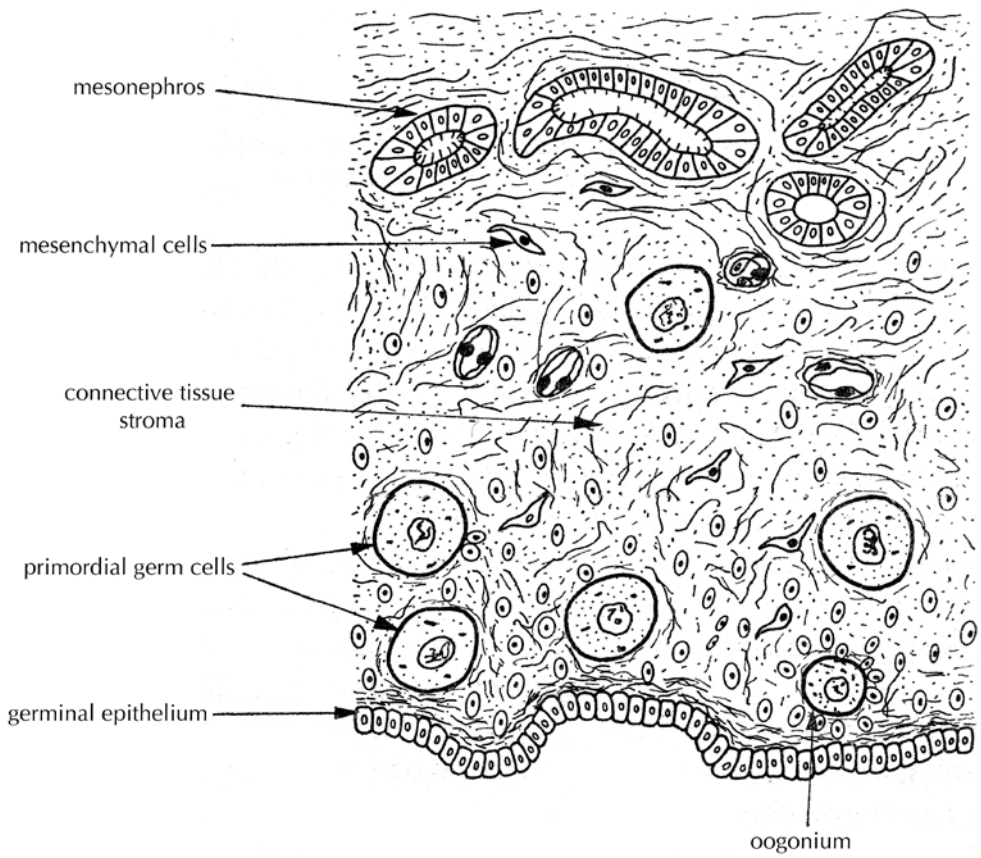

Figure 4. The structure of the ovary in a 10 day old chicken embryo, modified after Shahin (1973)

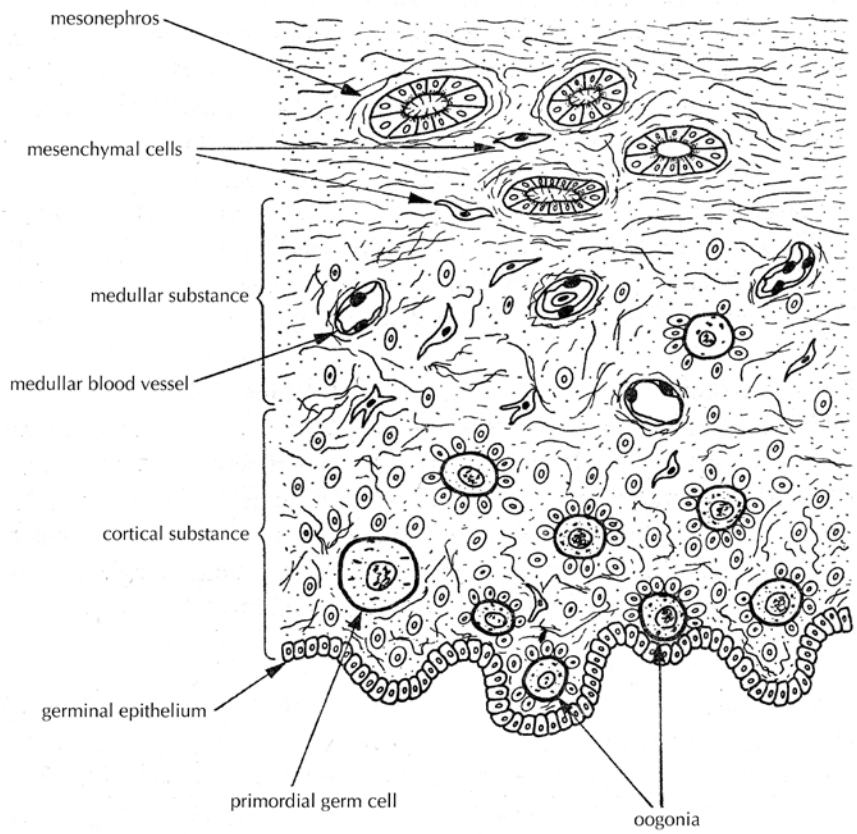

Figure 5. The structure of the ovary in a 16 day old chicken embryo, modified after Shahin (1973)

1.4. The differentiation of the testis

The differentiation of the developing testicular primordium starts somewhat later compared to the embryonic ovary. The sex chords descend from the germinal epithelium on Days 7–14 of incubation, thicken and gradually lose their connection with the surface cortical epithelium. These proliferating sex chords will later differentiate into the seminiferous tubules (tubuli seminipheri contorti). The thinning germinal epithelium that stays on the surface and the connective tissue underneath will become the tunica albuginea of the testis (Shahin, 1973).

On Day 10-13 of embryogenesis, several primordial germ cells colonise the germinal bundles – mostly of the left gonad – and undergo intensive mitotic division to form pro-spermatogonia. These are aligned along the basal membrane on the periphery of the germinal bundles. Their development is regulated by unknown factors that originate in the germinal bundle as well as by

early androgen production (Thurston and Korn, 2000). Pro-spermatogonia (spermatogonial stem cells) are characterised by densely packed mitochondria around the nucleus and they have a darker, ribosome-rich cytoplasm. Stem cell-like pro-spermatogonia remain present post-embryonically, and in an active testis they are called Type A spermatogonia. On Day 13-21 of incubation the amount of pro-spermatogonia gradually increases and concurrenlty the number of primordial germ cells that remain centrally in the germinal bundles decreases. Elongated peritoneal epithelium cells – originating from the germinal epithelium – appear among them, from which later wider-based Sertoli cells differentiate. Sertoli cells later play a basic role in the tissue differentiation of the embryonic testis. The cascade-like function of SOX9, SF1 and AMH genes that express in these cells has a critical role in this process. A basic regulator of the differentiation of Sertoli cells is the anti-Mullerian protein, which is produced in substantial amounts. The intestive proliferation of Sertoli cells starts at hatching and lasts until Week 8. Then they abruptly stop dividing. This process coincides with the occurrence of the first spermatocytes (Bozkurt et al., 2007).

In the Sertoli cells, later appear a lipocalin-type prostaglandin D synthetase and prostaglandin D2 production increases. This tissue hormone maintains and increases SOX9 gene expression in the Sertoli cells by autocrine signalling, which in turn determines morpho-functional differentiation of Sertoli cells and functional restitution in certain spermatogenic waves of the active testis (Vaillant et al., 2001; Moniot et al., 2008).

During the embryonic differentiation of germinal bundles, the mesenchymal connective tissue cells among the bundles transform into three distinct types of interstitial cells. One has increased cytoplasmic material and substantial smooth endoplasmic reticulum and tubular mitochondria, which suggest increased steroid (testosterone) production. These cells will turn into Leydig cells by a transformation mostly regulated by insuline, produced by the embryo. Insuline is an important factor in cell proliferation, as well as a stimulator of the formation of gonadotrope receptors, therefore indirectly increasing androgen synthesis (Bobes et al., 2001; Velázquez et al., 2006). The second type of differentiated cell types is a smooth muscle cell-like peritubular myoid cell. The third type forms a vascular cell precursor. A known regulator of cell migration and differentiation is a growth factor, PDGF (platelet-derived growth factor).

On Day 19-21 of embryonic development, histolytic processes initiate in the central region of the thickening germinal bundles, and a lumen starts to form in the glomuliferous tubules. During these days, the stroma material among of the germinal bundles and the amount of interstitial cells greatly increases.

In the last day of incubation and during the first weeks of prostembryonic development, the development of spermatogonia slows down at the base of seminiferous tubules, and many of them perish (get selected out). The Fas-ligand

– Fas-receptor apoptosis-regulating system of the differentiating Sertoli cells and spermatogonia play a determining role in this process (Thurston and Korn, 2000). Among others, the synthesis of prohibitin, a 30 kDa protein initiates in proto-spermatogonia and in Type A spermatogonia. Prohibitin inhibits DNA synthesis, and therefore mitotic division. It can block the germcell-forming ability of the testis and the Type A-Type B transformation of spermatogonia in the early (embryonic-postembryonic) phase of spermatogenesis, therefore it serves as a factor to protect against premature initiation of meiosis (Han et al., 2009). Blocking the division of spermatogenic cells in this period – according to mammalian data – helps the methylation of different methylation regions and therefore the reorganisation of heterochromatine material – and consequently the epigenetic modification of mostly paternal genes (Kato et al., 2007, Yoshioka et al., 2009).

The survival of cells that passed the stop in the proto-spermatogonium phase and did not get selected against is assured by growth factors (KL, LIF, BMP-4, SDF-1 and bFGF) and stimulating substances (N-acetyl-L-cystine, forskolin and retinoic acid) that originate in the Sertoli cells. The metabolism of spermatogenic cells occurs through the connection with the Sertoli cells by tight junctions, special surface membrane structures and adhesion-providing compounds (integrins, oligosaccharides, E-cadherine and tyrosine kinase receptor c-kit) (Bergmann and Schindelmeiser, 1987; De Felici, 2000; Farini et al., 2005). Membrane specialisations present in the embryonic and early postembryonic stages do not yet form a blood-testis barrier, it will only appear among the Sertoli cells later, during puberty, when the haploid secondary spermatocytes are produced (Bergmann and Schindelmeiser, 1987). (Figure 6.)

1.5 Embryonic development of the Wolffian duct, the epididymis and the defferent duct

The Woffian duct, that later forms the epididymal duct and the defferent duct, develops in the nephrogonotome mesoderm at 30-35 hours of incubation. After intensive posterior extention it reaches the cloaca, into which it opens, by the end of Day 3. Proliferative and cell migration processes both contribute to its development. During development, the apical end of the extending tube keeps its mesenchymal characteristics, while the structure behind it gradually transforms into epithelial cells. The growth of the Wolffian duct does not seem to be influenced by locally present fibronectin, vitronectin, b1-integrin receptor or NCAM polysialic acid, as earlier presumed. On the other hand, during a later stage of development, the tissue-stimulating effects of PSA adhesive molecules has been proven (Bellairs et al., 1995).

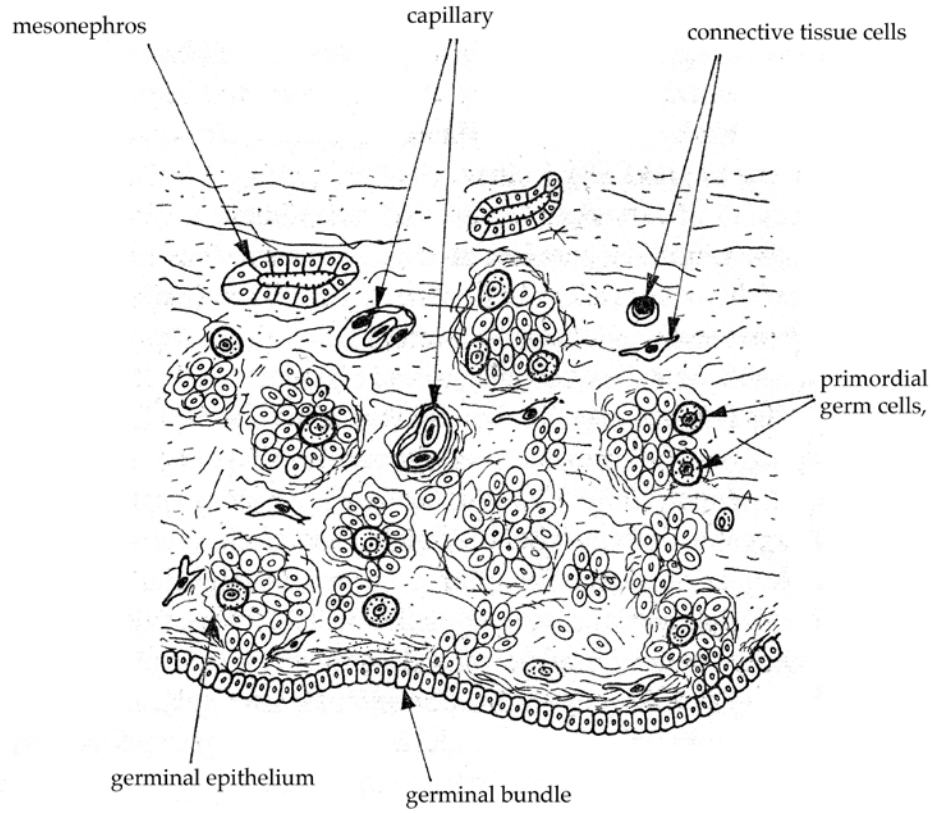

Figure 6. The structure of the testis in a Day 10 chicken embryo modified after Shahin (1973)

The development of the testicular primordium is stongly connected to that of the mesonephros, as its dorso-medial surface and the pars sexualis of the mesonephros has the same mesenchymal origin. During the last few days of incubation in this area develop the rete Halleri that connect to the straight seminiferous canalicules, the efferent ducts that develop from the pars sexualis, and the conjugant ducts, which are part of the epididymis. This later open into the ductus epididymis, that develops from the proximal part of the Wolffian duct. This thicker tube continues in the deferent duct that develops from the distal part of the Wolffian duct and has a greater diameter. A tissue connection that had formed earlier (during mesonephros differentiation, on incubation Day 3-4) between the contortous seminiferous ductules and the proximal section of the Wolffian tube extends to cover the lacuna system of the rete on the hilus of the testis (Shahin, 1973; Bellairs et al., 1995; Smith, 2007).

In female birds, the Wolffian ducts rapidly degenerate at the end of the embryonic life and completely disappear by the end of Week 2 after hatching. The anterior section of the Wolffian tube fuses with the pars sexualis of the

mesonephros and as blindely ending tubules (epoophoron) they grow into the posterior part of the ovary. The degenerative processes are caused by the low testosterone concentration characteristic of females.

The copulatory organ develops on the proctodeal part of the cloaca from the genital (or cloacal) tubercle and can be recognised from incubation Day 10. The appearance of this structure is initially identical in male and female birds, it is in the indifferent phase. During the first developmental stage (Day 10-12) it shows stong estrogen dependence: low estrogen levels cause male-type development, while treatment with estrogen leads to female-type genital tubercule. Later the differentiation of the genital tubercule becomes androgen dependent. Experimental studies in chicken and duck embryo showed that in males only high androgen levels trigger phallos development, while in females the higher estrogen concentration can inhibit the differentiaton towards male-type genitalia.

1.6. Embryonic development of the Mullerian duct and the oviduct

In chicken embryo, the Mullerian duct appears on Day 4-5 of incubation as an eptithelial thickening of the coeloma in the anterior part of the mesonephros, lateral to the Wolffian duct. On this, a pit-like depression forms that is the initial stage of the infundibulum that will develop later. From this funnel, cells start to grow intensively in the posterior direction, and from this initially solid epithelial column will develop the internal single-layer lining of the Mullerian duct. The canal of the Mullerian duct gradually develops during the next few days, in the anterior-to-posterior direction, and its development continues until the first few days of postembryonic life. During its development it maintains a close contact with the Wolffian duct, even though the cells of the later do not take part in the formation of the Mullerian duct. The Wolffian duct possibly induces the Mullerian duct by cell-cell contact, acting via tissue hormones. The Mullerian duct consists of three parts, all of which have differentiated from the coeloma epithelium: the layer of the internal-lining epithelium, the epithelial tube is surrounded by a layer of mesechyma cells that have detached from the epithelium by delamination, and the layer of coeloma cells around the whole structure.

On Day 12-13 of incubation sections of the Mullerian duct can be distinguished according to their future role: the future infundibulum, the magnum, which is thicker than the other sections, and the preliminary uterus. The vagina, which opens into the urodeum of the cloaca only develops during the first few days after hatching.

On Day 8 of incubation in females the right, in male both Mullerian ducts begin to degenerate, starting from the anterior end. The first phase of this

process is the apoptotic degeneration of the epithelial lining and then the duct turns into a mesenchymal ribbon by epithelium-mesechymal transformation. These processes are supported by b-catenin, lymphoid growth factor-1 and matrix metalloprotease. The regression is regulated by the anti-Mullerian hormone (AMH), which in birds is produced by the gonadal primordium of both sexes (in mammals it is only produced by the Sertoli cells of the testis). The production of AMH is much more intensive in males, however, it stops earlier. In females, the AMH production of the left ovary is the most intensive around Day 14 of incubation, when the right ovary and Mullerian duct are strongly degenerating.

The fact that the Mullerian duct remains in female embryos despite the effect of AMH can probably be explained by the higher nuclear estrogen receptor concentration, their estrogen-binding ability and therefore the higher expression of special proteins. Another regulating mechanism can arise from the fact that in chicken the promoter region of the AMH gene has an estrogen-binding ability, so the synthesis of the hormone can be moderated by the estrogen hormone of the female embryo. The minimal estrogen production in males does not inhibit the strong AMH upregulation. AMH inhibits aromatase production, decreases the production of estrogen, and therefore helps testis development and at the same time leads to the atrophy of the Mullerian ducts on both sides (Smith, 2007; Jacob and Bakst, 2007).

1.7. The embryonic development of sexual dimorphism of the brain

Sexual dimorphism in the behaviour of sexually mature birds can be deducted to the male-, or female-type development during the ontogenesis of the brain. For a long time somatic tissues (including the central nervous system) of the developing embryo were believed to be sexually neutral until the gonads start sex-specific hormone production during early embryonic development. In chicken embryo this occurs around Day 5-6 of incubation. Thus, the sexual dimorphism of the brain is created by the sexual steroids of the gonads, which act by binding to early-expressed steroid receptors in specific regions of the brain. According to an alternative theory, this happens because of the neurosteroids that form de novo, in the brain, from cholesterol. Detailed studies conducted on zebra finch show that even though the amount of steroids initially produced by the gonads is equal in male and female embryos, the development of neural-tracts of the song-control systems of the brain shows sexual dimorphism, and control centres typically only develop in males. The early development of sexual dimorphism of the brain suggests that other (steroidal or non-steroid dependent) regulating mechanisms can be at play in neural

structures preceeding the sex-specific development of the gonads (Agate et al., 2003).

Scholz et al., (2006) conducted a detailed study to find that in the brain of a 4-day old female chicken HINTW, a Z-linked MHM (male hypermethylated gene, which has been modified by female-type methylational and acetylational epigenetic modification) and ABTW (avian brain W-linked transcript) express in a greater amount compared to males. On Day 4 of incubation in chicken – even though the genes of P450scc, P450c17, 3-β-HSD, 17-β-HSD and P450-aromatase express in the gonads of both sexes – no sexual dimorphism is detectable yet in the expression genes for steroidogenic enzymes. Therefore, sex-dependent gene activation in the brain is not the results of a steroidal effect. Sexual dimorphism in steroid production occurs later, after Day 5 of incubation. The steroid-independence of the sexual dimorphism of certain genes responsible for brain differentiation is supported by the results that in-ovo ethinylestradiol treatment of a Day 4 chicken embryo does not affect the sexual dimorphism of the later occurring gene expression in the brain in either sex.

In regard to the sexual dimorphism of genes expressing in chicken brain, the same authors suggest that the expression of Z-chromosome-linked genes is more intensive in males compared to females. This suggests a dosage-compensating mechanism, but does not mean the inactivation of genes on the Z chromosome. Studies conducted on Day 4-10 showed that out of 133 identified genes 19 express in females and 114 in males. Among the female-type genes three are located on W, one on Z, 11 on the autosomes, and the localisation of four could not be identified. Among the 114 male-type genes, 61 are located on chromosome Z, 29 on the autosomes, and 29 could not be located.

WPKCI-8, SPINW and ABWT genes show early, female-type expression in the brain on Day 4 of incubation. Since WPKCI-8 also intensively expresses in the gonad on Day 4.5, its sex-determing role in females is very probable. The equivalent of this gene on chromosome Z is HINT, but it only expresses to a small degree. Both of them produce a very similar dimer nucleotide hydrolase (PKC inhibitor), which combine. However, the product of WPKCI-8 does not contain a histidine triad, and because of this, most of the HINT/WPKCI heterodimer degrades. According to these data, the mechanism that determines sexual characteristics of the female brain is the low level of this heterodimer, which later continuously inhibits both the expression of the HINT gene, as well as the effect of WPKCI-8 protein by negative feedback.

From Day 4 of incubation, the male embryonic brain is characterised by the activation of three Z chromosome-linked genes that are important for sexual differentiation. Two of them, CCNH and CDK7 code the cyclin-dependent kinase activating kinase (CAK), while the third codes BTF2-P44, which expresses TFIIH (transctription factor II H). Forming an integrated complex, CAK and TFIIH regulate the transactivation of the α-estrogen receptor, as well

as mitotic and meiotic divisions. Additionally, SPINZ, which originally codes neural structures is later also detected in the testis. There is strong expression of nine further structure-protein coding Z-linked genes in the male embryonic brain.

Besides the early expression of steroid-independent protein-coding genes, the organisational role of protein-coding special genes that activate later in the embryonic brain and act along with steroids, primarily estrogen, can also be determining in the development of sexual dimorphism. Examples are the so-called ribosomal proteins L17 and L37, which in interaction with the synthethised estradiol regulate the development of dimorphism in the brain. In male zebra finch ribosomal protein expression is more intensive and neurogenesis is stronger in the areas that will later be responsible for song control, such as Area X of the brain, the archistriatum (RA) and hyperstriatum ventrale (HVc) (Tang and Wade, 2009).

The early regulating effect of estrogens is indicated by the appearance of estrogen receptors in the developing brain of the embryo. Both α- and β-estrogen receptor genes were shown to be active in early developmental stage brain of Japanese quail. Later, on Day 9 of incubation sexual dimorphism appeared, as in the male embryo only β-estrogen receptor mRNA was detected in the medial preoptic nucleus and in the bed nucleus of the stria terminalis – in the regions that regulate copulation behaviour in adult male quail. Therefore, estrogens responsible for brain differentiation mostly act through β-receptors (Brunström et al., 2009). At the same time, α-estrogen receptors play a crucial role in the differentiation of the oviduct and the degeration of the Mullerian duct in males (Mattson et al., 2008).

In stage 30 of the embryonic development of zebra finch, α- and β-estrogen receptor mRNA and aromatasa mRNA were detected in the diencephalon and in the dorsal mesencephalon, and somewhat later, in stage 34 in the archistriatum too.There was however, no sexual differentiation considering the appearance of estrogen receptors and aromatase in the song-control centers (Perlman and Arnold, 2003). Nevertheless, more recent studies found that estradiol treatment of 6-10 day old female zebra finch increased the amount of neurons in Area X, the archistriatum (RA) and hyperstriatum ventrale (HVc) of the brain, and masculinised these areas. At the same time, the amount of sex-related ribosomal proteins (RPL17, RPL37) also increased in the neurons of the area. In males this treatment had the opposite effect, it decreased the amount of neurons in the vocal centres (Tang and Wade, 2009).

Neurosteroidogenesis initiates early in the embryo, which is important in the case of steroids, especially estrogens, which regulate the structural development of the brain, neuronal growth and differentiation. In chicken embryonic brain, the expression of the SF-1 gene that regulates steroidogenic enzyme synthesis can already be detected on Day 5.5 in the ventromedial nucleus

of the hyphothalamus, the centre responsible for sexual behaviour and is stronger in males (Endo et al., 2007). In Japanese quail the presence of several enzymes important for steroid biosynthesis (P450scc, 3β-HSD, P450/17a-lyase/ and 17β-HSD), as well as the synthesis of pregnenolon, progesterone, 3β, 5β-tetrahydroprogesterone, androstenedione, testosterone and estradiol from cholesterol have been detected from Day 7 of incubation. The level of progesterone and 3-β-HSD mRNA show distinct sexual dimorphism, the latter being higher in the prosencephalon of females compared to males on Day 7. In males, the level of 3-β-HSD mRNA fluctuates: it is higher on Day 9 and 15 than on Day 7 and 11. The level of aromatase is age dependent, but does not show sexual dimorphism (Tsutsui et al., 2006, Aste et al., 2008).

De novo neurogenesis in the lateral side of the lateral ventricules (ventricular proliferative zone) and the neuron migration initiating from here have an important role in the development of the song control centre of the brain. In male zebra finch, during and right before neuronal proliferation androgen neurosteroid biosynthesis can be detected at the lateral edge of the lateral ventricules (ventricular proliferative zone) around hatching and in the following five days (London and Schlinger, 2007). Also, at this time the magnocellular nucleus of the anterior neostriatum is formed, with plays a role in learning processes, including male song recognition. The magnocellular nucleus develops in both sexes during embryonic development, but only shows sexual dimorphism after sexual maturation, when singing develops. At this time, in males the number of dendrite projections found on the neurons decreases, but the synaptic contact surface, the cellular body and the size of the nucleus increase (Nixdorf-Bergweiler, 2001).

Besides their direct structural organisational effect on the brain, estrogens affect the expression of other, non-steroid producing elements of the neuroendocrine system. There is sexual dimorphism in the distribution and characteristics of the arginine-vasotocin (AVT) parvocellular neurons in Japanese quail and songbirds. In males, AVT cells are found in the medial stria terminalis and in the medial preoptic nucleus. These areas lack AVT cells in females. AVT bundles project into the lateral septum (where GnRH cells occur), the intercollicular nucleus (vocalising centre) and the locus coereleus (gondadotrop-regulating noradrenerg centre). In these areas some bundles terminate on aromatase-, GnRH-, and estrogen receptor-expressing neurons. Critically low levels of embryonic estrogen are crucial for the formation of AVT. If males are treated with estrogen in the embryonic stage, they do not perform copulation behaviour when they become sexually mature, and the extent of sexually dimorphic AVT areas in their brain is greatly decreased. Experimental inhibition of estrogen synthesis in the embryo increases male copulation behaviour when the female is treated with testosterone as an adult, and it leads to the apprearence of a large number of AVT neurons in different areas of the brain. In

quail, castration decreases, while testosterone-treatment of the castrated individuals increases the amount of AVT neurons in the medial stria terminalis and the medial preoptic centre. These changes suggest that along the direct effects of estrogens and androgens, the modification of embryonic expression levels of the AVT system can also influence the development of male sex-specific behaviour (Panzica et al., 2001).

References 1.

1. Agate RJ, Grisham W, Wade J, Mann S, Wingfield J, Schaen C, Palotie A, Arnold AP (2003): Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch PNAS 100, 4873-4878.
2. Akazome Y, Abe T, Mori T (2002): Differentiation of chicken gonad as an endocrine organ: expression of LH receptor, FSH receptor, cytochrome P450c17 and aromatase genes Reproduction, 123, 721-728.
3. Amengol C, Carretero A, Nacher V, Ruberte J, Navarro M (2007): Carbohydrate characterization of quail primordial germ cells during migration and gonadal differentation J.Anat., 210, 98-111.
4. Arit D, Bensch S, Hansson B, Hasselquist D, Westerdahl H (2004): Obsevation of a ZZW female in a natural population: implications for avian sex determination Proc. Biol. Sci., 271, Suppl.4, S249-251.
5. Aste N, Watanabe Y, Shimada K, Saito N (2008): Sex- and age-related variation in neurosteroidogenic enzyme mRNA levels during quail embryonic development Brain Res., 1201, 15-22.
6. Avila RE, Samar ME, de Fabro SP (1989 a.): Structural, ultrastructural and cytochemical study of interstitial cells of the chick ovary during embryo development Rev.Fac.Cien.Med.Univ.Nac. Cordoba, 47, 9-12.
7. Avila RE, Samar ME, de Fabro SP (1989 b.): Granulosa cells of the chick ovary: evolution studied during embryo development Rev. Fac. Cien. Med. Univ. Nac. Cordoba, 47, 5-8.
8. Axelsson E, Webster MT, Smith NGC, Burt DW, Ellegren H (2005): Comparison of the chicken and turkey genomes reveals a higher rate of nucleoide divergence on microchromosomes than macrochromosomes Genom Research, 15, 120-125.
9. Bellairs R, Lear P, Yamada KM, Rutishauser U, Lash JW (1995): Posterior extension of the chick nephric (Wolffian) duct: the role of fibronectin and NCAM polysialic acid Dev.Dyn., 202, 333-342.
10. Bellott DW, Skaletsky H, Pyntikova T, Mardis ER, Graves T, Kremitzki C, Brown LG, Rozen S, Warren WC, Wilson RK, Page DC (2010): Convergent evolution of chicken Z and human X chromosomes by expansion and gene acquisition Nature, 466, 612-616.
11. Bergmann M, Schindelmeiser J (1987): Development of the blood-testis barrier in the domestic fowl (Gallus domesticus) Int.J.Androl., 10, 481-488.
12. Bisoni L, Batlle-Morera L, Bird AP, Suzuki M, McQueen HA (2005): Female-specific hyperacetylation of histone H4 in the chicken Z chromosome Chromosome Res., 13, 205-214.
13. Boardman PE, Sanz-Ezquerro J, Overton IM, Bosch E, Fong WT, Tickle C, Brown WR, Wilson SA, Hubbard SJ (2002): A coprehensive collection of chicken cDNAs Curr.Biol., 19, 1965-1969.
14. Bobes RJ, Casreo JI, Miranda C., Romano MC (2001): Insulin modifies the proliferation and function of chicken testis cells Poultry Sci., 80, 637-642.
15. Bozkurt HH, Aktas A, Ulkay MB, Firat UB (2007): Sertoli cell proliferation during the posthatching period in domestic fowl J.Vet.Sci., 8, 219-222.
16. Brennecke J, Stark A, Russel RB, Cohen SM (2005): Principles of microRNA-target recognition PLoS Biol.,3, e85.
17. Brennan J, Capel B (2004): One tissue, two fates: molecular genetic events that underlie testis versus ovary development Nat.Rev.Genet., 5, 509-521.
18. Brunström B, Axelson J, Mattsson A, Halldin K (2009): Effects of estrogens on sex differentiation in Japanese quail and chicken Gen.Comp.Endocr., 163, 97-103.
19. Callebaut M (2005): Origin, fate, and function of the components of the avian germ disc region and early blastoderm: role of ooplasmic determinants Dev. Dyn., 233, 194-216.
20. Ceplitis H, Ellegren H (2004): Adaptive molecular evolution of HINTW, a female specific gene in birds Mol.Biol.Evol., 21, 249-254.

21. Clinton M, Haines LC (2001): An overview of factors influencing sex determination and gonadal development in birds EXS 91, 97-115.
22. Cutting AD, Bannister SC, Doran TJ, Sinclair AH, Tizard MV, Smith CA (2012): the potential role of microRNAs in regulating gonadal sex differentiation in the chicken embryo Chromosome Res., 20, 201-213.
23. De Felici M (2000): Regulation of primordial germ cell development in the mouse Int.J.Dev.Biol., 44, 575-580.
24. Derjusheva S, Kurganova A, Habermann F, Gaginskaya E (2004): High chromosome conservation detected by comparative chromosome painting in chicken, pigeon and passerine birds Chromosome Res., 12, 715-723.
25. Ebensperger C, Drews U, Wolf U (1988): An in vitro model of gonad differentiation in the chicken. Estradiol-induced sex-inversion results in the occurrence of serological H-Y antigen Differentiation, 37, 192-197.
26. Endo D, Murakami S, Akazome Y, Park MK (2007): Sex difference in Ad4BP/SF-1 mRNA expression in the chick-embryo brain before tgonadal sexual differentiation Zoolog.Sci., 24, 877-882.
27. Eyal-Giladi H, Ginsburg M, Farbarov A (1981): Avian primordial germ cells are of epiblastic origin J.Embriol.Exp.Morphol., 65, 139-147.
28. Farini D, Scaldaferri ML, Iona S, La Sala G, De Felici M (2005): Growth factors sustain primordial germ cell survival, proliferation and entering into meiosis in the absence of somatic cells Dev. Biol., 285, 49-56.
29. Fechheimer NS (1990): The domestic chicken (Gallus domesticus) as an organism for the study of chromosomal aberrations. In: Farm Animals and Biomedical research, Eds. Pliska and Stranzinger, P.Parey, Hamburg, 43-54.
30. Feng Y, Zhang S, Peng X, Yuan J, Zhan H, Gong Y (2007): Expression analysis of genes putatively involved in chicken gonadal development Acta Biol.Hung., 58, 163-172.
31. Fridolfsson AK, Ellegren H (2000): Molecular evolution of the avian CHD1 genes on the Z and W sex chromosomes Genetics 155, 1903-1912.
32. Gene Database, 2012
33. Ginsburg M (1997): Primordial germ cell development in avians Poult.Sci., 76, 91-95.
34. Gonzalez-Morán MG (2007): Effects of luteinizing hormone treatment on oogenesis in ovarian germ cells of the chick (Gallus domesticus) Domest.Anim.Endocr., 33, 154-166.
35. Graves JA (2001): From brain determination to testis determination: evolution of the mammalian sex-determing gene Reprod.Fertil.Dev., 13, 665-672.
36. Guioli S, Lovell-Badge R, Turner JM (2012): Error-prone ZW pairing and no evidence for meiotic sex chromosome inactivation in the chicken germ line PLoS Genet., 8, e1002560.
37. Guttenbach M, Nanda I, Feichtinger W, Masabanda JS, Griffin DK, Schmid M (2003): Comparative chromosome painting of chicken autosomal paints 1 – 9 in nine different bird species Cytogenet. Genome Res., 103, 173-184.
38. Han BK, Jung JG, Nam J, Moon JK, Kim JN, Lee SI, Kim JK, Kim H, Han JY (2009): Identification of the major proteins produced by cultured germline stem cell in chicken J.Androl., 30, 690-702.
39. Handley LJ, Ceplitis H, Ellegren H (2004): Evolutionary strata on the chicken Z chromosome: implications for sex chromosome evolution Genetics, 167, 367-376.
40. Hori T, Asakawa S, Itoh Y, Shimizu N, Mizuno S (2000): Wpkci, encoding an altered form of PKCI, is conserved widely on the avian W chromosome and expressed in early female embryos: implication of its role in female sex determination Mol.Biology Cell 11, 3645-3660.
41. Hudson QJ, Smith CA, Sinclair AH (2005a): Aromatase inhibition reduces expression of FOXL2 in the embryonic chicken ovary Dev.Dynamics, 233, 1052-1055.
42. Hudson QJ, Smith CA, Sinclair AH (2005b): Conserved expression of a novel gene during gonadal development Dev.Dyn., 233, 1083-1090.
43. Inpanbutr N, Taylor AN (1993): Expression of calbindin-D28k in developing and growing ovaries of chicken embryos Am.J.Vet.Res., 54, 514-519.
44. Itoh Y, Kampf K, Arnold AP (2006): Comparison of the chicken and zebrafinch Z chromosomes shows evolutionary rearrangements Chromosome Res., 14, 805-815.

45. Itoh Y, Melamed E, Yang X, Kampf K, Wang S, Yehya N, Van Nas A, Replogle K, Band MR, Clayton DF, Schadt EE, Lusis AJ, Arnold AP (2007): Dosage compensation is less effective in birds than in mammals J.Biol., 6, 2.
46. Jacob M, Bakst MR (2007): Developmental anatomy of the female reproductive tract. In: Reproductive biology and phylogeny of birds (ed. B.G.M. Jamieson): Science Publishers Enfield, Yersey, Plymuth, I.4. 149-179.
47. Julien P, Brawand D, Soumillon M, Necsulea A, Liechti A, Schütz F, Daish T, Grützner F, Kaessmann H (2012): Mechanisms and evolutionary patterns of mammalian and avian dosage compensation PLoS Biol., 10 (5) e1001328.
48. Kato Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, Okano M, Li E, Nozaki M, Sasaki H (2007): Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse Hum.Mol.Genet., 16, 2272-2280.
49. Kawai A, Nishida-Umehara C, Ishijima J, Tsuda Y, Ota H, Matsuda Y (2007): Different origins of bird and reptile sex chromosomes inferred from comparative mapping of chicken Z-linked genes Cytogenet.Genome Res., 117, 92-102.
50. Kent J, Wheatley SC, Andrews JE, Sinclair AH, Koopman P (1996): A male-specific role for SOX9 in vertebrate sex determination Development, 122, 2813-2822.
51. Kunita R, Nakabayashi O, Kikuchi T, Mizuno S (1997): Predominant expression of a Z-chromosome linked immunoglobulin superfamily gene, ZOV3, in steroidogenetic cells of ovarian follicles and in embryonic gonads of chickens Differentiation, 62, 63-70.
52. Kuroiwa A, Uchikawa M, Kamachi Y, Kondoh H, Nishida-Umehara C, Masabanda J, Griffin DK, Matsuda Y (2002): Chromosome assignment of eight SOX family genes in chicken Cytogenet. Genome Res., 98, 189-193.
53. London SE, Schlinger BA (2007): Steroidogenic enzymes along the ventricular proliferative zone in the developing songbird brain J.Comp.Neurol., 502, 507-521.
54. Lun S, Smith P, Lundy T, O'Connell A, Hudson N, McNatty KP (1998): Steroid contents of and steroidogenesis in vitro by the developing gonad and mesonephros around sexual differentiation in fetal sheep J.Reprod.Fertil., 114, 131-139.
55. MacLaughlin DT, Hutson JM, Danahoe PK (1983): Specific estradiol binding in embryonic Mullerian ducts: a potential modulator of regression in the male and female chick Endocrinology, 113, 141-145.
56. Madquick S, Jones KT (2007): How arrest at metaphase II: MPF stabilisation plus APC/C inhibition equals Cytostatic Factor Cell Div., 26, 2:4.
57. Mank JE, Ellegren H (2009): All dosage compensation is local: gene-by-gene regulation of sex-biased gene expression in the chicken Z chromosome Heredity, 102, 312-320.
58. Mattsson A, Mura E, Brunström B, Panzica G, Halldin K (2008): Selective activation of estrogen receptor alpha in Japanese quail embryos affect reproductive organ differentiation but not the male sexual behavior or the parvocellular vasotocin system Gen.Comp.Endocr., 159, 150-157.
59. McQueen HA, Clinton M (2009): Avian sex chromosomes: dosage compensation matters Chromosome Res., 17, 687-697.
60. McQueen HA, Siriaco G, Bird AP (1998): Chicken microchromosomes are hyperacetylated, early replicating, and gene rich Genome Res., 8, 621-630.
61. Méndez C, Carrasco E, Pedernera R (2005): Adenohypophysis regulates cell proliferation in the gonads of the developing chick embryo J.Exp.Zool.A, Comp.Exp.Biol., 303, 179-185.
62. Méndez MC, Ramírez M, Varela AR, Chávez B, Pedernera E (2003): Follicle stimulating hormone increases cell proliferation in the ovary and the testis of the chick embryo Gen.Comp.Endocr., 133, 181-188.
63. Moniot B, Boizet-Bonhoure B, Poulat F (2008): Male specific expression of lipocalin-type prostaglandin D synthetase (cPTGDS): during chicken gonadal differentiation: relationship with cSOX9 Sex Dev., 2, 96-103.
64. Moniot B, Boizet-Bonhoure B, Poulat F (2008): Male specific expression of lipocalin-type prostaglandin D synthetase (cPTGDS) during chicken gonadal differentiation: relationship with cSOX9 Sex Dev., 2, 96-103.
65. Moriyama S, Ogihara J, Kato J, Hori T, Mizuno S (2006): PKCI-W forms a heterodimer with PKCI-Z and inhibits thr biological activities of PKCI-Z in vitro, supporting the predicted role of PKCI-W in sex determination in birds 139, 91-97.

66. Naito M, Matsubara Y, Harumi T, Tagami T, Kagami H, Sakurai M, Kuwana T (1999): Differentiation of donor primordial germ cells into functional gametes in the gonads of mixed germ cells isolated from embryonic blood J.Reprod.Fert., 117, 291-298.
67. Nakabayashi O, Kikuchi H, Kikuchi T, Mizuno S (1998): Differential expression of genes for aromatase and estrogen receptor during the gonadal development in chicken embryos J.Mol. Endocr., 20, 193-202.
68. Nakamura Y, Yamamoto Y, Usui F, Mushika T, Ono T, Setioko AR, Takeda K, Nirasawa K, Kagami H, Tagami T (2007): Migration and proliferation of primordial germ cells in the early chicken embryo Poult.Sci., 86, 2182-2193.
69. Nakata T, Ishiguro M, Aduma N, Izumi H, Kuroiwa A (2013): Chicken hemogen homolog is involved in the chicken-specific sex-determining mechanism Proc.Natl.Acad.Sci.USA, 110, 3417-3422.
70. Nam K, Ellegren H (2008): The chicken (Gallus gallus) Z chromosome contains at least three nonlinear evolutionary strata Genetics, 180, 1131-1136.
71. Nanda I, Karl E, Griffin DK, Schartl M, Schmid M (2007): Chromosome repattering in three representative parrots (Psittaciformes) inferred from comparative chromosome painting Cytogenet. Genome Res., 117, 43-53.
72. Nanda I, Sick C, Münster U, Kaspers B, Scharti M, Staeheli P, Schmid M (1998): Sex chromosome linkage of chicken and duck type I interferon genes: further evidence of evolutionary conservation of the Z chromosome in birds Chromosoma, 107, 204-210.
73. Naurin S, Hansson B, Bensch S, Hasselquist D (2010): Why does dosage compensation differ between XY and ZW taxa? Trends Genet., 26, 15-20.
74. Nishida C, Ishijima J, Kosaka A, Tanabe H, Habermann FA, Griffin DK, Matsuda Y (2008): Characterization of chromosome structures of Falconinae (Falconiformes, Aves) by chromosome painting and delineation of chromosome rearrangements during their differentiation Chromosome Res., 16, 171-181.
75. Nishida-Umehara C, Tsuda Y, Ishijima J, Ando J, Fujiwara A, Matsuda Y, Griffin DK (2007): The molecular basis of chromosome orthologies and sex chromosomal differentiation in paleognathous birds Chromosome Res., 15, 721-734.
76. Nixdorf-Bergweiller BE (2001): Lateral magnocellular nucleus of the anterior neostriatum (LMAN) in the zebra finch: neuronal connectivity and the emergence of sex differences in cell morphology Microsc.Res.Tech., 54, 335-353.
77. Ohno S (1967): Sex Chromosomes and Sex-Linked Genes Springer, Berlin 1-140.
78. olf JB, Bryk J (2011): General lack of global dosage compensation in ZZ/ZW systems? Broadening the perspective with RNA-seq. BMC Genomics, 12: 91.
79. Oréal E, Mazaud S, Picard JY, Magre S, Carré-Eusébe D (2002): Different patterns of anti-Müllerian hormone expression, as related to DMRT1, SF-1, WT1, GATA-4, Wnt-4, and Lhx9 expression, in the differentiating gonads Dev.Dyn., 225, 221-232.
80. Oreal E, Pieau C, Mattei MG, Josso N, Picard JY, Carré-Eusébe D, Magre S (1998): Early expression of AMH in chicken embryonic gonads precedes testicular SOX9 expression Dev.Dyn., 212, 522-532.
81. Pace HC, Brenner C (2003): Feminizing chick: a model for avian sex determination based on titration of Hint enzyme activity and the predicted structure of an Asw-Hin theterodimer Genome Biol., 4 R18
82. Pala I, Naurin S, Stervander M, Hasselquist D, Bensch S, Hansson B (2012 b): Evidence of a neo-sex chromosome in birds Heredity (Edinb.), 108, 264-272.
83. Pala L, Hasselquist D, Bensch S, Hansson B (2012 a): Patterns of molecular evolution of an avian neo-sex chromosome Mol.Biol.Evol., Aug.25. (Epub.)
84. Panzica GC, Aste N, Castagna C, Vigletti-Panzica C, Balthazart J (2001): Steroid-induced plasticity in the sexually dimorphic vasotocinergic innervation of the avian brain: behavioural implications Brain Res.Rev., 37, 178-200.
85. Perlman WR, Arnold AP (2003): Expression of estrogen receptor and aromatase mRNAs in embryonic and posthach zebra finch brain J.Neurobiol., 55, 204-219.
86. Reed KJ, Sinclair AH (2002): FET-1: a novel W-linked, female specific gene up-regulated in the embryonic chicken ovary Gene Expr. Patterns 2, 83-86.

87. Richard FJ (2007): Regulation of meiotic maturation J.Anim.Sci., 85, 13 Suppl.E4-6.
88. Romanoff AL (1960): The avian embryo: structural and functional development MacMillan, New York
89. Schoenmakers S, Wassenaar E, Hoogerbrugge JW, Laven JS, Grootegoed JA, Baarends WM (2009): Female meiotic sex chromosome inactivation in chicken PLoS Genet., 5, e1000466.
90. Schoenmakers S, Wassenaar E, Laven JS, Grootegoed JA, Baarends WM (2010): Meiotic silencing and fragmentation of the male germline restricted chromosome in zebra finch Chromosoma, 119, 311-324.
91. Scholz B, Kultima K, Mattson A, Axelsson J, Brunström B, Halldin K, Stigson M, Dencker L (2006): Sex-dependent gene expression in early brain development of chicken embryos BMC Neuroscience, 7: 12
92. Sekido R, Lovell-Badge R (2007): Mechanisms of gonadal morphogenesis are not conserved between chick and mouse Dev.Biol., 302, 132-142.
93. Shahin MA (1973): Thesis, Cairo
94. Shan Z, Nanda I, Wang Y, Schmid M, Vortkamp A, Haaf T (2000): Sex specific expression of an evolulutionairly conserved male regulatory gene, DMRT1, in birds Cytogenet.Cell Genet., 89, 252-257.
95. Shetty S, Griffin DK, Graves JA (1999): Comparative painting reveals strong chromosome homology over 80 million years of bird evolution Chromosome Res., 7, 289-295.
96. Smid M, Nanda I, Guttenbach M, Steinlein C, Hoehn M, Schartl M, Haaf T, Weigend S, Fries R, Buerstedde JM, Wimmers K, Burt DW, Smith J, A'Hara S, Law A, Griffin DK, Bumstead N, Kaufman J, Thomson PA, Burke T, Groenen MA, Crooijmans RP, Vignal A, Fillon V, Morisson M, Pitel F, Tixier-Boichard M, Ladjali-Mohammedi K, Hillel J, Maki-Tanila A, Cheng HH, Delany ME, Burnside J, Mizuno S (2000): First report on chicken genes and chromosomes 2000 Cytogenet. Cell Genet., 90, 169-218.
97. Smith CA (2007): Molecular genetics of avian sex determination and gonadal differentiation. In: Reproductive biology and philogeny of birds II. (ed. BGM Jamieson) Science Publishers Enfield, Yersey, Plymuth, 479-506.
98. Smith CA, Andrews JE, Sinclair AH (1997): Gonadal sex differentiation in chicken embryos: expression of estrogen receptor and aromatase genes J.Steroid.Biochem., Mol.Biol., 60, 295-302.
99. Smith CA, McClive PJ, Hudson Q, Sinclair AH (2005): Male-specific cell migration into the developing gonad is a conserved process involving PDGF signalling Dev.Biol., 284, 337-350.
100. Smith CA, Roeszler KN, Bowles J, Koopman P, Sinclair AH (2008): Onset of meiosis in the chicken embryo: evidence of a role for retinoic acid BMC Dev.Biol., 17, 8:85.
101. Smith CA, Roeszler KN, Hudson QJ, Sinclair AH (2007): Avian sex determination: what, when and where? Cytogenet.Genome Res., 117, 165-173.
102. Smith CA, Roeszler KN, Sinclair H. (2009): Genetic evidence against a role for W-linked histine triad nucleotide binding protein (HINTW) in avian sex determination Int.J.Dev.Biol, 53, 59-67.
103. Smith CA, Sinclair AH (2004): Sex determination: insights from the chicken Bioessays, 26, 120-132.
104. Stebler J, Spieler D, Slanchev K, Molyneaux KA, Richter U, Cojocaru V, Tarabykin V, Wylie C, Kessel M, Raz E (2004): Primordial germ cell migration in the chick and mouse embryo: the role of the chemokine SDF-1/CXCL 12 Dev.Biol., 272, 351-361.
105. Sun QY, Miao Yl, Schatten H (2009): Towards a new understanding on the regulation of mammalian oocyte meiosis resumption Cell Cycle, 8, 2741-2747.
106. Suzuki T, Kansaku N, Kurosaki T, Shimada K, Zadworny D, Koide M, Mano T, Namikawa T, Matsuda Y (1999): Comparative FISH mapping on Z chromosomes of chicken and Japanese quail Cytogenet Cell Genet., 87, 22-26.
107. Takada S, Wada T, Kaneda R, Choi YL, Yamashita Y, Mano H (2006): Evidence for activation of Amh gene expression by steroidogenetic factor-1 Mech.Dev., 123, 472-480.
108. Tang YP, Wade J (2009): Effects of estradiol on in-corporation of new cells in the developing zebra finch song system: potential relationship to expression of ribosomal proteins L17 and L37 Dev. Neurobiol., 69, 462-475.
109. Teranishi M, Shimada Y, Hori T, Nakabayashi O, Kikuchi T, Macleod T, Pym R, Sheldon B, Solovei I, Macgregor H, Mizuno S (2001): Transcipts of the MHM region on the chicken Z chromosome

accumulate as non-coding RNA in the nucleus of female cells adjacent to the DMRT1 locus Chromosome Res., 9, 147-165.
110. Thorne MH, Sheldon BL (1993): Triploid intersex and chimeric chickens useful models for studies avian sex determination. In: KC Reed and YA Graves (eds) Sex chromosomes and sex determining genes Harwood Academie, Chur, Switzerland, 201-208.
111. Thurston RJ, Kom N (2000): Spermiogenesis in commercial poultry species: anatomy and control Poultry Sci., 79, 1650-1668.
112. Tsafriri A, Motola S (2007): Are steroids dispensable for meiotic resumption in mammals? Trends Endocr. Metab., 18, 321-327.
113. Tsunekawa N, Naito M, Sakai Y, Nishida T, Noce T (2000): Isolation of chicken vasa homolog gene and tracing the origin of primordial germ cells Development, 127, 2741-2750.
114. Tsutsui K, Matsunaga M, Miyabara H, Ukena H (2006): Neurosteroid biosynthesis in the quail brain: a review J.Exp.Zool. A Comp.Exp.Biol., 305, 733-742.
115. Vaillant S, Dorizzi M, Pieau C, Richard-Mercier N (2001): Sex reversal and aromatase in chicken J.Exp.Zool., 290, 727-740.
116. Velázquez PN, Peralta I, Bobes RJ, Romano MC (2006): Insulin stimulates proliferation but not 17-beta-estradiol production in cultured chick embrio ovarian cells Poultry sci., 85, 100-105.
117. Villalpando I, Sánchez-Bringas G, Sánchez-Vargas I, Pedernera E, Villafán-Monroy H (2000): The P450 aromatase (P450 arom): gene is asymmetrically expressed in a critical period for gonadal sexual differentiation in the chick Gen.Comp.Endocr., 117, 325-334.
118. Wolf U (1999): Reorganization of the sex determining pathway with the evolution of placentetation Hum.Genet., 105, 288-292.
119. Wolf U, Bryk J (2011): General lack of global dosage compensation in ZZ/ZW systems? Broadening the perspective with RNA-seq BMC Genomics, 1, 12–91.
120. Yamada D, Koyama Y, Komatsubara M, Urabe M, Mori M, Hashimoto Y, Nii R, Kobayashi M, Nakamoto A, Ogihar J, Kato J, Mizuno S (2004): Comprehensive search for chicken W chromosome-linked genes expressed in early female embryos from the female minus-male subtracted cDNA macroaaray Chromosome Res., 12, 741-754.
121. Yoshioka H, Ishimaru Y, Sugiyama N, Tsunekawa N, Noce T, Kasahara M, Morohashi K (2005): Mesonephric FGF signaling is associated with the development of sexually indifferent gonadal primordium in chicken embryos, Dev.Biol., 280, 150-161.
122. Yoshioka H, McCarrey JR, Yamazaki Y (2009): Dynamic nuclear organization of constitutive heterochromatin during fetal male germ cell development in mice Biol.Reprod., 80, 804-812.
123. Zaccanti F, Vallisneri M, Quaglia A (1990): Early aspects of sex differentation in the gonads of chick embryos Differentiation, 43, 71-80.
124. Zhang SO, Mathur S, Hattern G, Tassy O, Pourquié O (2010): Sex-dimorphic gene expression and ineffective dosage compensation of Z-linked genes in gastrulating chicken embryos BMC, Genomicws 11,1-13.
125. Zhao Y, Lu H, Yu H, Cheng H, Zhou R (2007): Multiple alternative splicing in gonads of chicken DMRT1 Dev.Genes Evol., 217, 119-126.

2. Female reproductive organs

2.1. The ovary

2.1.1. Postembryonic development of the ovary

It is the last third of embryonic development and the first few days following hatching, when asymmetry typical of avian ovarian development becomes macroscopically visible. During this period the right ovary gradually regresses, and in chicken only remains as rudimentary connective tissue. This asymmetry favouring the left gonadal colony is of course much earlier detectable looking at its histology, or by studying the enzyme synthesis of steroid hormones and the invasion rate of primordial germ cells. Nevertheless, there are a few exceptions when both ovaries remain and become functionally active in mature females. Such species are found among *Apterygiformes*, some members of *Falconidae a*nd *Accipitridae,* and in *Cathartidae*. There is a certain asymmetry in these species as well, as the left ovary is always larger. Besides the above-mentioned groups, as part of individual variation, sometimes the right ovary is present in *Laridae, Columbidae* and *Passeridae* (Péczely, 1987, Johnson et al., 2007).

During postembryonic development following hatching, the oogonium-oocyte transformation stops. From the large amount of primary oocytes that are formed by this time, only a small percentage will continue to develop and reach ovulation, and most of them will get degraded by continuous, complicatied processes.

Gilbert (1971) distinguishes three periods of postembryonic oocyte development.

1. The first period is characterised by the slow incorporation of neutral fats. This early deposition of white yolk results in the gradual, slow growth of the oocyte. The length of this period, depending on the lifespan of the species, can last months or years. Primary oocytes, 20-50 μm in diameter, soon get covered in a single cell layer, formed by the gradually differentiating granulosa that sticks to them. The resulting 0.08-0.1 μm-size ball is the primordial follicle. All oocytes of the hatchling are in this stage, but some oocytes remain in this first stage in all later stages of the female bird's life. These, and the primary follicles supply the replacement for the continuously maturing follicles.

2. The second period – while still characterised by slow yolk deposition – is shorter than the first (in domestic chicken it lasts for approximately two months). In addition to neutral fats, more and more protein gets deposited, resulting in a vacuolated structure, changing the white yolk in a quantitative way. Both the granulosa surrounding the oocyte and the connective tissue theca gradually become thicker, and 1-2 mm large primary, and larger, 2-4 mm sized secondary white follicles develop, which are visible macroscopically.

3. The third period is the puberty or maturation, which lasts for 1-2 weeks, bringing rapid changes. During this phase, a large amount of lipoprotein coloured by lutein, i.e., the yellow yolk gets deposited, forming yellow follicles. The follicle that surrounds the oocyte undergoes rapid growth and its structure fundamentally changes. First, it becomes multilayered, then the granulosa in the large yellow follicles becomes single-layered again, and then theca is formed, with two differentiated layers. This structure continues to bulge out from the surface of the ovary, while connected to the stroma of the cortex by a stalk rich in blood vessels and nerve fibres. Among the follicles showing hierarchical growth, the biggest are the preovulatory follicles, which will ovulate first (Johnson et al., 2007).

2.1.1.1. The prepubertal period

During embryogenesis a small, thin ovarian primordium is formed, that looks slightly granulated macroscopically. In the first few days after hatching regression of the right ovary finishes, and it remains as a few cell-layer deep structure, consisting of rudimentary germinal epithelium and connective tissue. In the postembrionic period the oogonium-oocyte transformation ceases, and after hatching no new primary oocytes are produced. The first period after hatching is characterised by the formation of primordial follicles.

Primordial follicles consist of primary oocytes covered in single-layered cuboidal epithelium (granulosa), borded by vitelline membrane that covers the oolemma. The oocytes that lack this granulosa coat undergo apoptotic degradation. Tight junctions are formed between the granulosa cells, and begins the formation of sole-like widening basal limbs that furrow the oolemma. These protrusions contribute to the development of the vitelline membrane, the nutrition of the primordial oocyte, by yolk and the import of maternal information (maternal RNA, hormones and carotenoids) into the cell during follicle development. The primordial follicles survive for months or years remaining the same size as their development is arrested, and they serve as backup for follicles that will mature later.

In the growing ovary of young birds several larger **primary follicles** develop from these primordial follicles. The primordial follicle-primary follicle development is regulated by intraovarian paracrine factors, such as the facilitating

effect of the TGF-β-induced bone morphogenic proteins (BMP-4, BMP-7) and the negative effect of the anti-Mullerian hormone (Knight and Glister, 2006). The appearance of primary follicles indicates the beginning of the slow phase in follicular development, when cells and connective tissue fibres separate out from the stromal mesenchyma that surrounds the primordial follicles, and this, around the granulosa cells starting to form a gradually thickening theca layer. This connective tissue differentiation is possibly triggered by the inductive effect of the granulosa, as stem cell factor production can be detected. A typical basal membrane (lamina) is formed between the thickening theca and the granulosa layers. This lamina becomes multilayered, consisting of thin connective tissue fibres and ground substance. FSH and LH receptors develop in both the granulosa and the theca cells, steroidogenic enzymes are synthesised, and the production of increasing amount of androstenedione, dehydroepiandrosterone, testosterone, 17β-estradiol and progesterone can be detected.

In the growing primary follicles the (still) primary oocyte is increasing in size, which is primarily the result of the slow incorporation of neutral fats. Primordial and primary follicles do not emerge from the surface of the ovary, as they are embedded in the periferial and deeper stroma matter (Carlson et al., 1996; Asem et al., 2000; Robinson et al., 1988; Gilbert, 1971; Johnson et al., 2007).

In the juvenile and early prepubertal period, the mass of the ovary keeps increasing, and the growing primary follicles gradually move up into the cortical layer, bringing blood vessels along with them. The gradually buldging wall of the developing **small white follicles** becomes thicker, as the outer surface of the theca gets new layers of single-layer germinal epithelium and thin stromal connective tissue (that carries the blood vessels). The cortex becomes lobulated. The proliferation of the granulosa layer continues inside the follicles and the multilayered structure thickens. The theca layer also thickens. The size of the primary oocyte reaches 0.1-0.3 mm diameter, the Balbiani body becomes visible in the cytoplasm, which consists of the Golgi area around the paired centrioles and a mitochondrial cloud. In oocytes of larger than 0.3 mm, the Balbiany body is no longer visible. More lipoproteins, phospholipids and other lipids get deposited in the plasma of the oocyte, forming the so-called white yolk. The size of the massively forming first generation small white follicles is usually 1-2 mm. During this developmental stage several, up to 20%, of primary follicles undergo atresia. According to in vitro studies, these small white follicles mostly produce testosterone, and to a smaller degree also estrogen, that originates mostly in the theca layer (Forgó et al., 1988a, 1988b).

In a later phase of prepuberty (which in chicken is during the second month), the growth rate of some of the small white follicles increases, which can be deduced to more intensive incorporation of proteins and lipoproteins into the primary oocyte. At this stage, the diameter of the small white follicles

is 2-4 mm, they represent an intermediate stage to the medium-large category, and they are called secondary small white follicles.

The granulosa cells of the growing small white follicles become taller, turning into columnar epithelium. On both sides of the apical cytoplasm of this columnar epithelium dense plates develop, bordered by ribosome-like structures, which are the primordial transosomes. Later, in mature transosomes, which consist of a bent, 100-150 A-thick dense layer, ribosomes are only found on the inside, convex side in a single layer. These structures are located along adjoining the lateral membrane of the granulosa cells, and in the wide-based apical projections that push deeply in the oolemma. Transosomes are special desmosome (tight junction) structures, but they never fuse with the bordering membranes. Where they occur, complex membrane interdigitations are formed, with radial tonofibrillums on both sides (Forgó et al., 1988a, 1988b, Kovács et al., 1992). The primary component of transosomes is occludin, which is synthesised in the granulosa cells as a result of the combined effect of FSH and activin-A. The specific tight junctions temporarily prevent the incorporation of larger VLDL and vitellogenin particles into the oocyte, blocking the way to the VLDL receptors (Schuster et al., 2004). The slow uptake of smaller (lipid and lipoprotein) components of the yolk occurs partially through the transosomes (by a process still unknown) and at this time, to a smaller degree through paracellular transport. The theca layer separates into theca externa and theca interna. The first has a looser structure, retains more of connective tissue characteristics and contains glandular-type cell-nests, while the latter contains visibly modified, more compact cells that are elements of the increasing androgen and estrogen biosynthesis.

During the last prepubertal phase (in chicken at 4-5 months of age) the ovary becomes more lobulated, its mass significantly increases, and its blood vessels branch out extensively. In the granulosa cells the expression of activin-A decreases and get replaced by the production of inhibin-A and follistatin. The simultaneously decreasing activin-A and increasing inhibin-A and follistatin concentrations decrease the sensitivity of granulosa cells to FSH, which leads to the degeneration of the transosoma-forming occludin. Subsequently, granulosa cells separate from each other and the paracellular transport channels that connect them extend. The elements of the receptor type that is responsibe for the uptake of the two components (vitellogenin and VLDL) of yellow yolk – syntethised in the oocyte and incorporated into the oolemma – become available, and the increased paracellular transport will result in greater uptake of white and later more yellow yolk (Schneider, 1996, Schuster et al., 2004).

These processes do not affect all developing follicles, some enter into a second follicular developmental stage (Gilbert, 1971), forming a group of **medium, and large white, prehierarchical follicles** of 4-8 mm (Johnson et al., 2007), while most stay in the 1-2 mm size category. Therefore, righ before the onset

of puberty, generations of medium and large white follicles appear along the multitudes of small white follicles.

The theca and granulosa layers of pre-hierarchical follicles (medium and large white follicles) show basic morphological and functional changes. As a result of autocrine and paracrine effects of EGF and growth-differentiating factor-9 (GDF-9), their granulosa layer is largely proliferated, keeps thickening and becomes multilayered (Tilly et al., 1992). The number and size of transosomes decreases, but the amount and width of the paracellular channels increases.

The cholesterol transport protein that was absent earlier in the granulosa begins to express. Simultaneously, a greater amount of FSH receptors appear, and these cells respond to in vitro FSH treatment with increased cAMP production. Under in vitro conditions, cAMP triggers the expression of StAR, P450scc, 3beta-hydroxysteroid-dehydrogenase (3β-HSD) and P450-17α-OH, and the secretion of pregnenolone, progesterone and androgen (Li and Johnson, 1993). Therefore, even though the granulosa is able to produce progesterone and androgen, there is no steroid synthesis in vivo, because the plasma FSH level is still too low (Johnson et al., 2002). Triggered by increased FSH and LH levels, during yellow follicle (F5-F1) development 3β-HSD expresses in proportion to the growth of the follicles, and the granulosa layer becomes able to syntethise progesterone. Nevertheless, this layer still does not secrete neither androgens not estrogens in vivo (Porter et al., 1989, 1991).

The vascularisation and innervation of theca interna becomes richer, the amount of smooth endoplasmic reticulum, tubular mitochondria and lipid vacuoles increase in its cells, showing morpholical signs of substantial steroid production. Large amounts of LH receptors appear in the membrane of the theca cells. The increased cAMP production initiates the expression of cytochrome P450 side-chain cleavage (P450scc) enzyme and steroidogenic acute regulatory protein (StAR), which leads to increased pregnenolone synthesis. Cytochrome P450-17α-hydroxylase (P450-17α-OH) converts pregnenolone into dehydroepiandrosterone, which in turn gets converted into androstenedione by 3β -hydroxysteroid-dehydrogenase (3β-HSD). Alternatively, 3β-HSD converts pregnenolone into progesterone, which also gets converted into androstenedione by P450-17α-OH. The resulting substantial amount of androstenedion gets converted into testosterone by 17β-hydroxysteroid-dehydrogenase (17β-HSD). The theca interna cells therefore supply progesterone and testosterone into the blood stream, but they have no aromatase expression, and consequently cannot synthetise estrogen (Porter et al., 1989, 1991).

Theca externa cells have more FSH and LH receptors – and exclusively among the follicular layers – express the cytochrome P450-aromatase enzyme, and in vitro they syntethise mostly 17β-estradiol along with androgens and progesterone. Steroid synthesis is primarily regulated by LH (Forgó et al., 1988a, 1988b; Porter et al., 1989, 1991, Nitta et al., 1991; Kowalski et al., 1991).

Coincubating isolated theca interna and theca externa cells with ovine LH results in higher estrogen production compared to the amount produced by theca externa cells alone, which suggests that the majority of the androgen substrate originates from the theca interna. Progesterone, on the other hand, predominantly gets secreted by the granulosa layer. Some of it enters the circulation as final product, while some serves as a precursor for androgen biosynthesis in the theca interna cells. According to the three-cell model for follicular steroidogenesis, progesterone is produced by the granulosa, androgens in the theca interna and estrogens in the theca externa (Porter et al., 1989, 1991, Kato et al., 1995).

Follicular selection occurs on prehierarchical follicles, through which a smaller amount of white follicles pass into the preovulatory phase of rapid growth and maturation, while others get retained as small white follicles, staying in the prehierarchical stage.

Several ovarian paracrine factors participate in the process, initiating rapid growth. Some examples include gherlin and calcitonin, which get expressed in the theca and granulosa of the ovary and affect proliferation, apoptosis and hormone secretory activity both directly (through a specific receptor, GHS-R1a) and indirectly, through the PKA-MAPK cascade (Sirotkin et al., 2006, Sirotkin and Grossmann, 2008, Krzysik-Walker et al., 2007). Another example is leptin, which has a wide effect spectrum, as it can inhibit apoptotic and proliferative processes and it can also affect steroid secretion. Leptin receptors have been detected in chicken ovary, however, their numbers do not increase during maturation. Increase in lepin receptor numbers has been observed only in the intestine (Ohkubo et al., 2000). Leptin acts (mostly in the granulosa) by using MAPK and PKA as intercellular mediators (Sirotkin and Grossmann, 2007).

The differentiation of granulosa determines if the follicle enters the **preovulatory system**. To become preovulatory, the granulosa cells need to form large numbers of gonadotrop (FSH and LH) receptors and the cells need to develop their steroidogenic ability. TGFβ and activin play a crucial role in this process. TGFβ, after binding to type I. and II. receptors, acting through activin phosphorylates Smad2 (transcription activating protein, which after forming a heteromer complex in the cytoplasm enters the nucleus, where it causes gene expression by attaching to certain DNA-binding proteins) to increase FSH receptor synthesis. During this process follistatin, an activin-binding protein, modulates the effects of activin (but not the effects of TGFβ). Hormone binding by FSH receptors triggers the formation of cAMP and protein expression of the LH receptor. The LH effect results in P450 scc-StAR synthesis, and the differentiated granulosa cells starts to produce progesterone (Johnson et al., 2007).

Mitogen-activated protein kinase (MAPK) stimulated by epidermal growth factor receptor ligands (EGF-R-L) probably play a role in maintaining the **prehierarchical stage** (i.e., by inhibiting the growth of certain follicles). It

has been shown that certain factors of the EGF group (EGF, TGFα and betacellulin), acting through MAPK, extracellular-regulated kinase (Erk) and protein kinase-C (PKC), can inhibit the TGFβ-activin-induced phosphorylation process, which is necessary for the development and functioning of Smad2, and therefore essential for rapid follicular development. Therefore, in spite of the increasing FSH level, the number of FSH receptors remains the same, which results in the arrest of follicular development and the differentiation of granulosa cells. This theory seems to be supported by the fact that experimental inhibition of MAPK and Erk (the driving forces behind the inhibitory system) increases StAR expression, progesterone and FSH synthesis and also initiates the development of LH receptors (Woods and Johnson, 2005, 2007, Johnson and Woods, 2009). GnIH receptors expressing in the theca and granulosa layer of certain prehierarchical follicles leads to the decrease of vitality of these prehierarchical follicles and therefore decreases the number of preovulatory follicles. There is a substantial amount of these receptors in prepubertal chicken ovary, however, their expression activity substantially decreases following maturation, probably as a result of increased production of estradiol and progesterone. The GnIH that reaches systemal circulation therefore, through its specific receptors, can limit ovarian follicular maturation during a critical period (Maddineni et al., 2008).

2.1.1.2. Puberty (maturation)

Puberty is the quickest and most intensive period of follicular and oocyte maturation (third follicular developmental stage of Gilbert) that usually initiates 1-2 weeks before laying the first egg. In the brown kiwi (*Apteryx mantelli*), which lays only one egg, it lasts longer, 15-17 days. By the end of this period, an active ovary has developed and the female becomes sexually active. This period is accompanied by typical behavioural and physiological changes, i.e., a breeding pair is formed and their relationship is strengthened. During this period, the birds occupy a nesting territory, build a nest and mate. These processes result from and at the same time are prerequisites of the functioning of an active ovary.

The period of puberty is characterised by a very rapid growth of **preovulatory follicles**, during which, for instance in domestic chicken, a 4-5 mm large, 50-100 mg white follicle becomes a 35 mm large, 18-20 g large yellow follicle awaiting ovulation. During this rapid growth, the primary oocyte, as well as the granulosa and theca layers of the follicular wall undergo substantial changes. Typically, the follicular stalk, which is visible first in the large white follicle generation, extends, the rapidly growing yellow follicles noticeably rise from the surface of the ovary, and become structures hanging from a stalk, therefore the whole of the ovary turns into a structure similar to a bunch of grapes.

Two populations of **yellow follicles** can be distinguished macroscopically based on their different rate of growth. **Small yellow follicles** form a slowly, nearly simultanously growing group, with little size difference among individual follicles. Members of the other group are a few, rapidly growing follicles, with a substantial size difference among **medium-large** (F5-F4-F3) and **large** (F2-F1) **yellow follicles**.

In the active ovary, there is always only one present of the largest, F1 follicles, awaiting ovulation. This has the longest stalk and a Y stigma is visible through its thin wall, where the follicular wall will rupture during ovulation. With the beginning of egg laying, yellow follicles gradually progress in the hierarchical order. The speed of maturation is species specific, follicles usually spending 24-36 hours in stage F1 until ovulation (Figure 6.).

The driving force behind follicular growth is the incorporation of **yolk** into the oocyte. During this process, the increasing amount of deuteroplasm shifts the ooplasm and the nucleus into an eccentric position, forming the germinal disc (or blastoderm) under the oolemma.

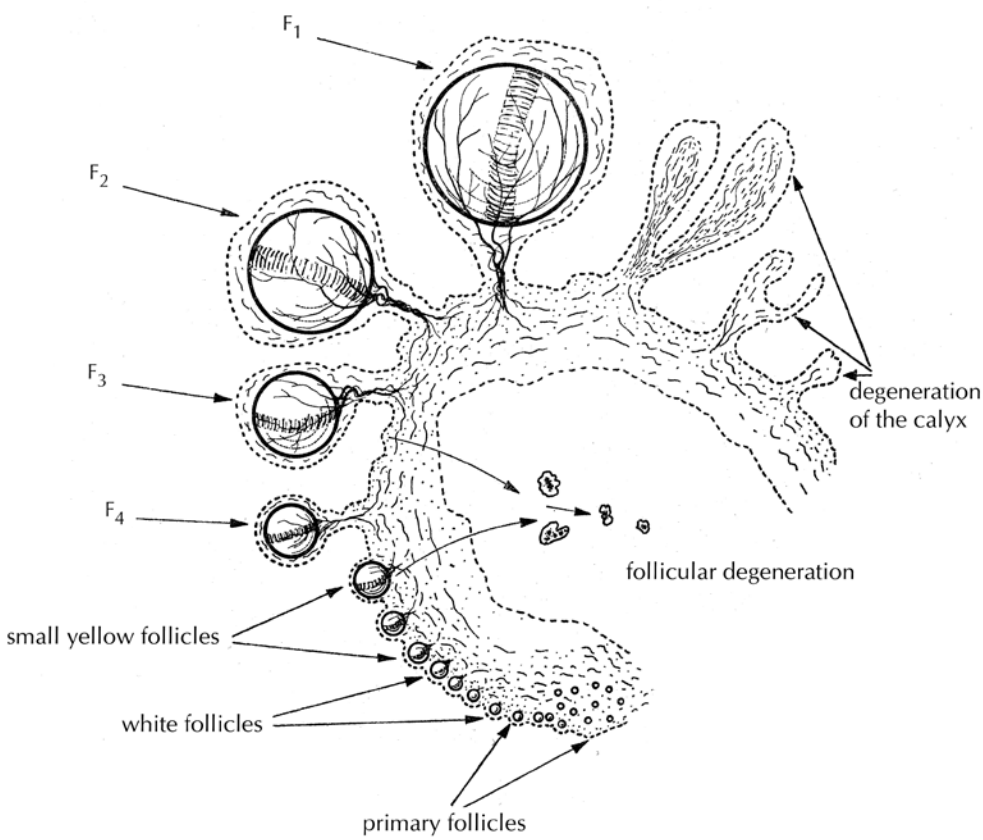

Figure 7. Schematic structure of the avian ovary, modified after Péczely (1987)

During the first few days of puberty still only white yolk gets deposited in the oocyte, and the big white follicles continue growing. Soon begins the incorporation of carotenoid-soaked yellow yolk, leading to the formation of small yellow follicles. After this, white and yellow yolk get deposited into the ooplasm alternated, their daily pattern resulting in a layered, onion-like structure. The mature oocyte consists of approximately 95% yellow yolk and 5% white yolk. Yolk gets deposited in granules of varying sizes, often bordered by membrane. Inside the granules, there are subgranules or paracrystals. The larger white and smaller yellow yolk-granules are suspended in aqueous liquid. The protein content of the granules is larger, while the yolk material contains less protein, a great amount of lipid globules and its water content is substantial (Gilbert, 1971).

Yolk contains 50% water, 33% lipids, 16% proteins, as well as carotenoids, vitamins, hormones (mostly steroids), maternal RNA, glucose and mineral salts.

One of the most important component of the **protein fraction** is **vitellogenin** (phosvitin, phosphovitelline, VTG), a 36-40 kDa phosphoglycolipoprotein, consisting of a larger and a smaller component that get synthetised in the liver. Its phosphorous content is approximately 10%, present in the form of phosphoserine. It has a great affinity to bind mostly calcium, but also to a smaller degree magnesium and iron ions. Iron ions get transported to the liver by transferrin-phosvitin iron transport system, stimulated by estrogens, and from the liver iron gets transported bound to phosvitin into the yolk of the oocyte (Lopez-Berjes et al., 1981).

The other important protein fraction is made of **α, β and γ livertins**. These protein fractions are similar to serum albumin, a2-glucoprotein and gamma globulin in several physico-chemical properties (Gilbert, 1971, Sturkie, 1976).

Avidin, which is a biotin-binding protein, as well as arginine-glycine amidotransferase, plasma retinol-binding protein (RBP) and riboflavin-binding protein (RfBP) are present in lower concentrations in the yolk, and are also expressed in the liver, modulated by estrogen (Vieira et al., 1995, Vieira et al., 1996, Zhu and Evans, 2001). They enter the oocyte using a broad-binding affinity membrane receptor of the VLDL receptor family, which besides VTG and VLDL, also binds these proteins and their ligands (Schneider, 1996).

The **lipoprotein** content of the yolk is similar to that of the serum, and it can be divided into three fractions. Above 1063 g/cm^3 density is the high-density lipoprotein (HDL) fraction, and below are low-, (LDL) and very low-density lipoproteins (VLDL). Approximately 10% of the total amount of lipoproteins belongs to the HDL fraction, which has a relatively higher, over 30% protein content. LDL and VLDL of the remaining 90% contain lysine on their N-terminal and tyrosine on their C-terminal. Most of the cholesterol content of the yolk is attached to VLDL. HDL is a complex group of compounds, its best known

member is lipovitellin, which is similar to plasma a2-globulin (Gilbert, 1971; Board and Hornsey, 1978).

Specific lipoproteins of the yolk are apo-VLDL-I, apo-VLDL-II and apolipoprotein-D (29 kDa), syntethised by the intestine and in smaller amount by the liver, and transfers directly in the blood of the portal vein (differently from mammals, where the lipoprotein secreted by the intestine enters the lypmphatic circulation). Apolipoprotein-D gets taken up by clathrin-coated vesicules in the growing oocytes. During embryogenesis it (also) plays an important role activating lecithin-cholesterol acyltransferase, and later becomes one of the regulators of food intake and body mass increase (Lin and Chan, 1981 Vieira et al., 1995, Steinmetz et al., 1998, Tso et al., 2001). The synthesis of Apo-VLDL-II is triggered by estrogen, and it acts as a specific inhibitor of the lipolysis of triglyceride-rich lipoproteins (Schneider et al., 1990).

Carotenoids (lutein, zeaxanthin, criptoxantin and carotene) provide the characteristic colour of yellow yolk. Vitamin A and its derivatives belong to this group, and are transferred into the yolk by the retinol-binding protein (RBP), located in the plasma. The synthesis of this transport protein (also) occurs in the liver, however, interestingly, the expression of its mRNA is not stimulated by estrogen, but, uniquely, inhibited by it (Vieira et al., 1995).

Riboflavin-binding protein (ribNP, RCP) is synthesised in the liver, stimulated by estrogen, and then gets transferred into the plasma. In the plasma it binds to vitellogenin, which also transports lipids, phosphates and various inorganic ions. VTG and ribBP bind together, as a specific ligand, to the multifunctional 95 kDa-lipoprotein receptor of the oocyte, which is also expressed by a gene of the LDL group. The binding process of this complex ligand is calcium and phosphate dependent (Mac Lachlan et al., 1994). Ionic forms of inorganic salts (Na, K, Ca, Mg, Fe, Zn, S, P and Cl) bind to the lipoprotein fraction in a water-soluble form (Péczely, 1987).

Maternal RNA is localised in the germinal disc and (mostly) in the surrounding cytoplasm (Malewska and Olszanska, 1999). Most of the detectable (maternal) transcripts found in the non-fertilised germinal disc are pro-, and antiapoptotic proteins, which protect the developing embryo from environmental stressors, such as temperature changes after laying (Muscarella et al., 1998).

Among the **hormones** deposited into the yolk, tyroide hormone and steroids are best known. Triiodothyronine (T3) and thyroxine (T4) are early regulators of embryonic differentiation, and also direct the initiating cartilage formation (Wilson and McNabb, 1997). The steroid content of the yolk shows a concentric pattern. In dark-eyed junco (*Junco hyemalis*) and red-winged blackbird (*Agelaius phoeniceus*), most of the progesterone is located in the periphery, estradiol is found in the centre and testosterone is evenly distributed between peripheral and central areas of the yolk (Bahr et al., 1983, Lipar et al., 1999). One

of the steroids, testosterone is important in the determination of embryonic development (Schwabl, 1993, 1996). However, when interpreting the results, it is important to know whether the studies consider primary sex ratio – sex ratio of all fertile eggs, or secondary sex ratio – when only the sex ratio of the chicks that hatched is considered.

The testosterone content of the yolk of domestic chicken and peafowl eggs that contain male embryos is higher. In the same species, eggs of dominant hens contain more testosterone, and more chicks hatch from these eggs (conclusions based on primary sex ratio) (Müller et al., 2002, Pike and Petrie, 2005). Higher plasma and yolk estradiol levels shift the sex ratio towards females in zebra finch (*Taenopygia guttata*) (von Engelhardt et al., 2004). Nevertheless, DHEA plays a role in the optimal development of the male embryo, probably through its conversion to testosterone through androstenedione (Szőke et al., 2004). Stress inflicting on the mother bird affects the amount of corticosterone deposited into the yolk, which in turn negatively affects the offspring. In increased amounts it causes early embryonic death, and therefore can change the sexual ratio in hatchlings (Szőke et al., 2004).

Yolk substances are synthesised in the liver, primarily as a result of the substantially increased estrogen levels during puberty. Most estrogen is produced by the white follicles one or two weeks before laying the first egg. In the cytoplasm and nucleus of the hepatocytes of only female individuals, high-affinity estrogen receptors are formed by estrogen upregulation, which specifically bind estradiol and estrone, but do not compete with neither progesterone nor testosterone. Their amount is substantially lower in immature females compared to ones that have reached puberty (Turner, 1984). The effect of estrogen does not only result in the synthesis of yolk proteins and lipids, but also the stabilisation of the bird-specific apolipoprotein II-mRNA production, which is a prerequisite of increased liver cell function (Ratnasabapathy, 1995). Yolk synthesis is an energy-demanding process, and during the formation of preovulatory follicules it uses up to 40-50% of the daily energy demand of the female bird. During intensive yolk formation, basic metabolic rate increases by 13-41% in songbirds, while in some waterbirds this increase can reach 200% (Meijer and Drent, 1999). Yolk proteins and lipoproteins get transported into the ovarian follicules bound to plasma prealbumin and albumin. During egg laying, plasma vitellogenin levels can reach 1.4 mg/ml in zebra finch, while in the case of an inactive ovary this value is 0 (Salvante and Williams, 2002).

The transport of yolk into the oocyte occurs mostly by receptor-mediated endocytosis, stimulated by FSH., In addition to vitellogenin (VTG) and VLDL-uptake receptors, LDL receptors also take part in this process, which is formed in the theca externa and granulosa cells of the yellow follicles (Hummel et al., 2003). These LDL receptors import cholesterol necessary for steroid synthesis into the layers of the follicular wall. The uptake of lipoproteins by the oocyte is

ensured by an oocyte membrane-specific 95 kDa receptor, which primarily is responsible for VLDL. Another lipoprotein transport receptor is a 130 kDa protein, which takes part in the homeostatic regulation of cholesterol. This latter receptor is synthesised by somatic cells (Steyrer et al., 1990).

The cholesterol synthesised in the liver of juveniles is in a dynamic equilibrium between the VLDL and HDL fractions, however, this changes in mature birds. Estrogens decrease the amount of cholesterylester transfer protein (CETP) mRNA in the liver and the amount of transfer protein in the plasma, which are responsible for the equilibrium. This hypothesis is supported by a similar outcome of estrogen treatment in juvenile females. The results support estrogen regulation of the transfer protein gene, and the decrease in its translocation in the VLDL-HDL direction (Sato et al., 2007).

Further degradation of tight junctions between granulosa cells and the increase in high-efficiency paracellular transport are characteristic of this period. The structure of granulosa cells becomes loose, making it possible for the incorporation of yellow yolk, the amount of which, for instance, can reach 2 g daily in domestic chicken (Johnson et al., 2007). The incorporation of yolk into the large yellow follicle slows down 2-3 days before ovulation, and then it completely ceases, which can be associated with the substantial decrease in FSH secretion and the amount of FSH receptors (Hertelendy and Asem, 1984).

The local incorporation of yolk into the oocyte is not unifom. In the germinal disc area much fewer VTG and VLDL receptors are synthesised, and consequently, less yolk gets incorporated. Therefore, in the germinal disc area pronuclear movements associated with fertilisation can occur adequately.

Hormone production of the preovulatory (yellow) follicles typically shifts towards more intensive progesterone secretion, along with the decrease of estrogen synthesis (Forgó et al., 1988a, 1988b). This is based on that inhibin-A production in the large yellow follicles decreases FSH secretion, the amount of FSH receptors decreases in the granulosa, while at the same time substantially increases the amount of LH receptors. The driving force of increased progesterone synthesis is LH-activated cAMP production.

Progesterone production of the yellow follicles becomes more pronounced in the granulosa cells of the vegetal pole, distal from the germinal disc, especially in the largest follicle. The amount of progesterone that reaches the circulation (preovulatory progesterone peak) will trigger a peak in preovulatory LH secretion. A smaller amount of progesterone secreted in the granulosa will enter the theca layer in a paracrine way, where it serves as a precursor for androstenedione and testosterone. Increased amount of mitochondria, increased cytochrome P450scc activity and LH stimulus-triggered StAR protein expression will cause more intensive progesterone production in the granulosa cells of the vegetative pole.

Granulosa cells above the germinal disc (on the animal pole) are still mitotically active, producing a substantial amount of EGF, which inhibits LH receptor synthesis and progesterone production in these cells (Tischkau and Bahr, 1996).

In domestic hen, at the end of puberty, about 2 hours before the first ovulation, the significantly increased plasma LH level will cause the chromatida pairs of the oocyte nucleus that are in the diplotene stage to separate from each other, and the first meiotic division progresses into the diakinesis phase. In the next step, the nuclear membrane degenerates, the germinal vesicle disintegrates and the chromosomes move into the equatorial plate (metaphase). After this, as a result of kinetochores attached to the diplosome, the chromosomes migrate towards the poles (anaphase), and finally two haploid daughter nuclei are formed (telophase). As a result of this fast process, one of the two recently formed nuclei becomes the nucleus of first polocyte, and it gets ejected from the now secondary oocyte, surrounded by a small amount of cytoplasm. Following the ejection of the first polocyte, the second spindle microtubules, responsible for the second division, start to form immediately in the secondary oocyte, but mitosis, which is the second step of maturational division, will only occur after ovulation, during fertilisation in the oviduct.

The primary to secondary oocyte transformation is triggered by very high levels of plasma LH concentration, the so-called preovulatory peak, which triggers ovulation. The substantially higher number of LH-receptors in the wall of the largest follicle (F1) binds a large amount of LH, and this intensive LH effect – only here, in the F1 follicle – will substantially increase progesterone synthesis locally. This will restart the thus far blocked meiosis, and by activating proteases also triggers the rupture of the follicular wall (Johnson et al., 2007).

Local effects of estrogens play an important role in follicular differentiation, as (α and β) estrogen receptors can be detected in the stroma, as well as in the theca and granulosa cells, fewer in juvenile birds, more in laying hens. The amount α-receptors is higher in all layers and all cell types compared to β-receptors (Hrabia et al., 2008). In the active ovary of laying geese the amount of α-, and β-receptors is also higher compared to prepubertal birds, and more FSH and prolactin receptors can be detected (Kang et al., 2009).

Local effects of tyroid hormones in the follicular wall is an important regulating factor of follicular growth and development, in both prehierarchical and preovulatory follicles. In vitro T3 treament decreases basal and LH-stimulated estrogen secretion in white follicles and in the theca layer of preovulatory follicles, while increases progesterone secretion in F2 and F1 follicles. In vivo, T3 decreases plasma LH and estradiol levels, and increases progesterone. In the wall of white follicles there was no difference in the amount of tyroide hormone receptors (TR-α and TR-β0), while in the granulosa layer of preovulatory

follicles, the amount of TR-α receptors was higher compared to TR-β0), as opposed to the theca layer, where no such difference was detected (Sechman et al., 2009).

2.1.2. The structure of the active ovary

The avian ovary is typically a single organ, located on the left side in the body cavity lined by a serosa fold and under the abdominal air sac (cavum serosum genitale). It is covered by tunica albuginea, with a relatively large number of lymphocytes. There is a single-layer epithelium under the capsule, tighly connected to the mesenchymal stroma of the outer cortex. The structure of the lower, medullar region of the stroma is looser, as a result of a large numbers of arteries, veins, and lymphatic lacunae. The stroma matter contains several adrenergic, postganglionic and cholinergic nerve bundles, which form plexuses mostly around follicles of various sizes in the cortex and inside their developing theca layer. (Figure 8, 9, 10, 11, 12)

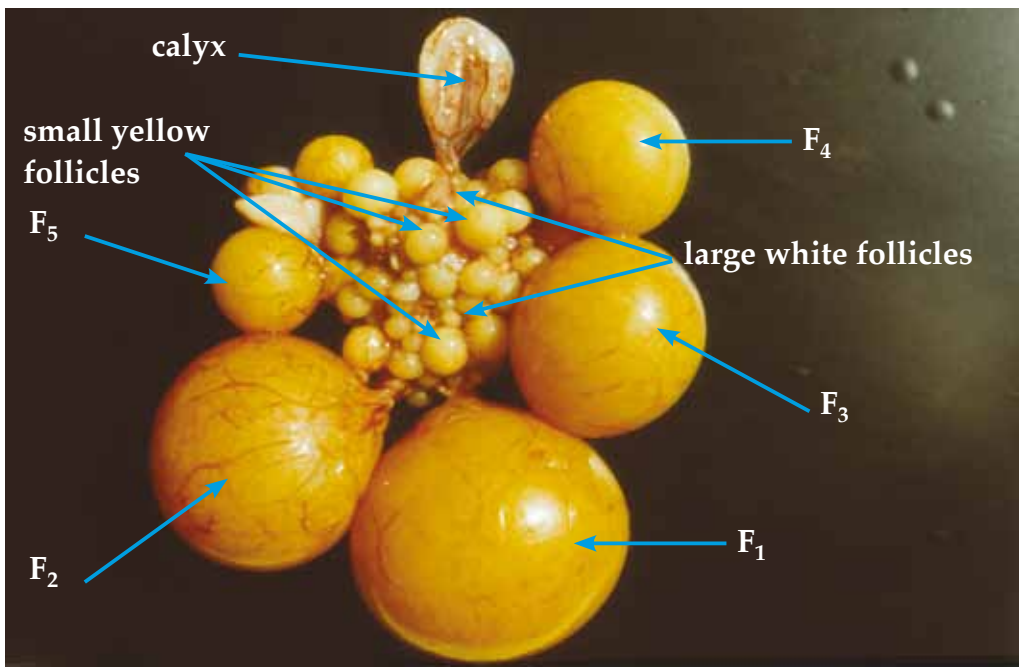

Figure 8. Ovary of egg-laying domestic goose

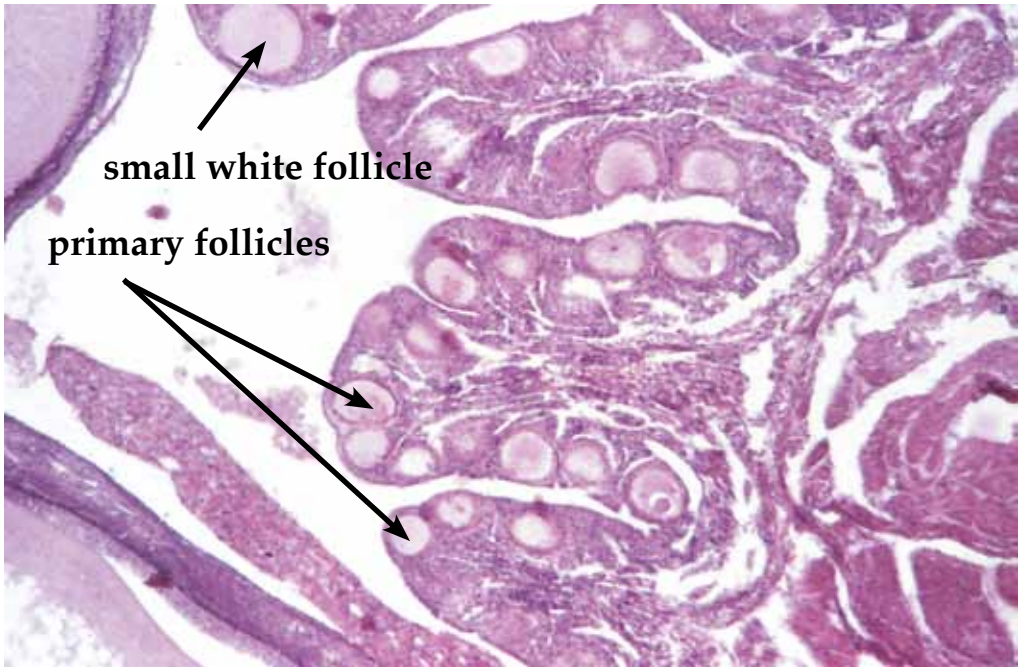

Figure 9. Primary and white follicles of domestic goose ovary

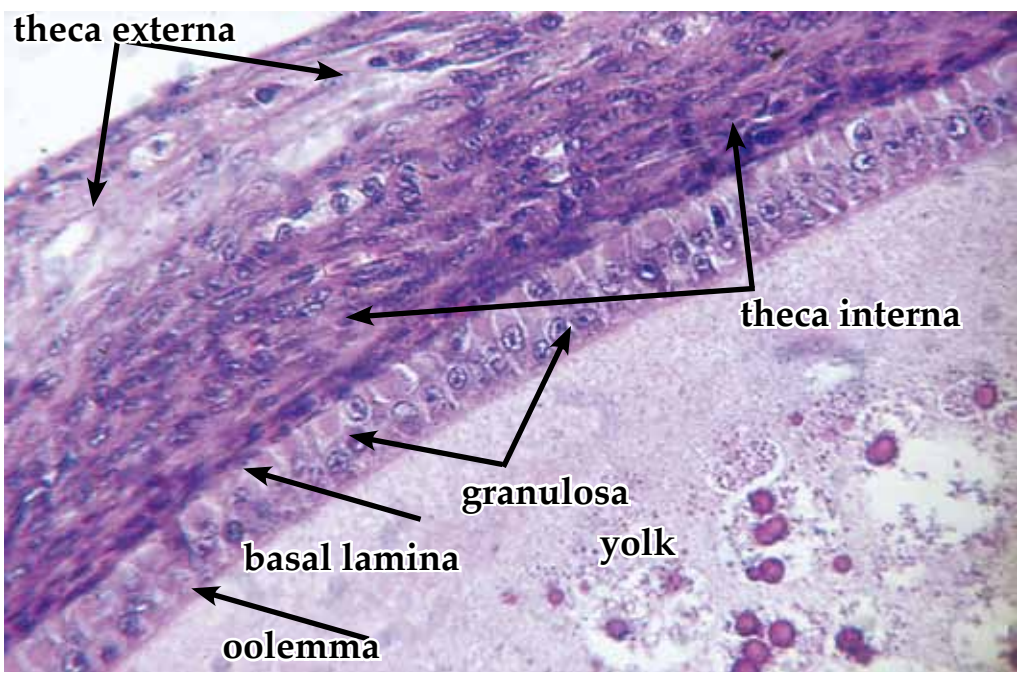

Figure 10. Cross section of the follicular wall of a small white follicle

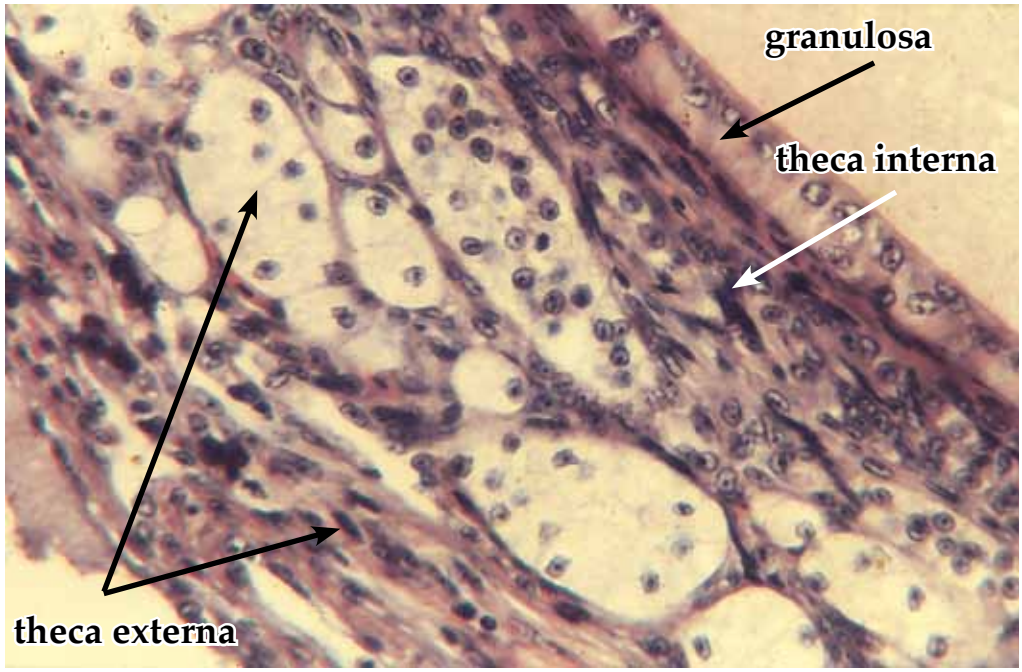

Figure 11. Cross section of a follicular wall of a larger white follicle a domestic goose ovary

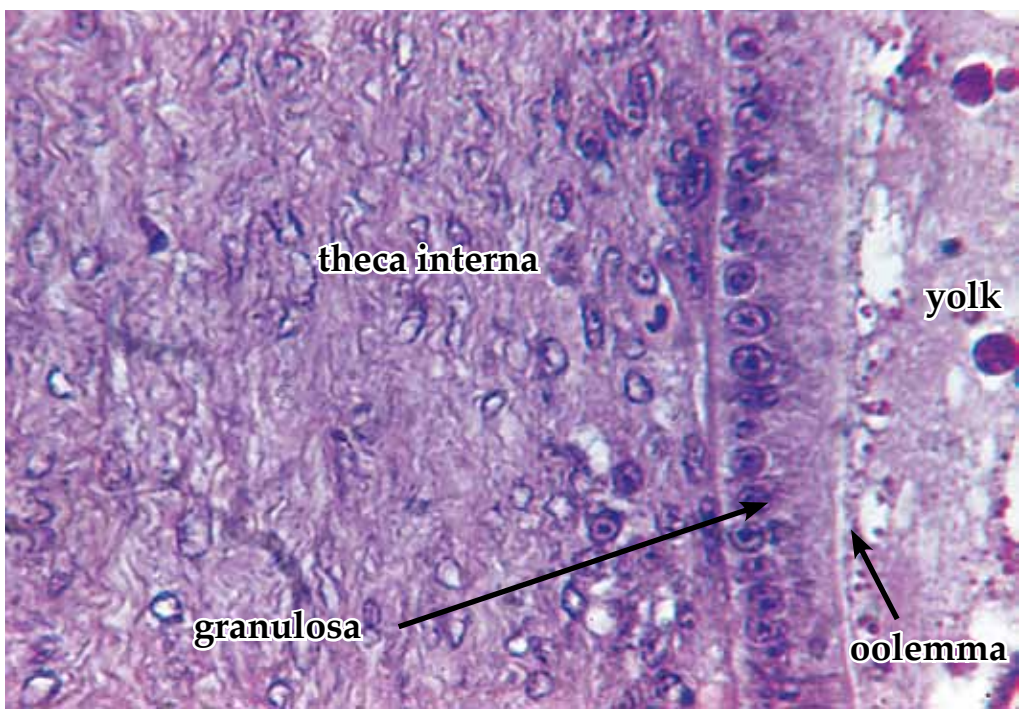

Figure 12. Cross section of the follicular wall of a large yellow follicle of a domestic goose ovary

The arterial blood supply of the ovary is delivered by the renal artery, which branches out in the stroma tissue. The stalked follicles receive 2-4 arterial branches from this system, which furher branch out in the theca tissue. The connective tissue of the stigma area is thin and it does not have blood vessels. The granulosa layer does not receive arterioles, its oxygen and nutrient needs are supplied indirectly, through the theca. The ovarian veins directly open into the vena cava posterior, with several thin, short branches.

The large amount of lymphocytes and macrophages in the stroma and theca layer of the follicles produce cytokins. The activity of the immune system of the developing and mature follicles is characterised by the intensive formation of major histocompatibility complex (MHC) molecules (Johnson et al., 2007).

In sexually mature birds the cortex is stongly lobulated, and contains white and yellow follicles of different developmental stages. Most of the mass of the active ovary consists of the 8-12 small, 4-6 medium-large, 2-4 large and 1 preovulatory large yellow follicle, which makes the structure of the ovary resemble a bunch of grapes. In the cortex there are also a large amount of white follicles of different sizes and regressing follicles in different stages of atresia.

The granulosa layer of the smallest white follicles (primary follicles) is single layered, and they lack theca. In domestic chicken, the diameter of these follicles is 0.05-0.1 mm. When the connective tissue theca appears, the granulosa becames multilayered in the small white follicules of 1-4 mm diameter size. This developmental stage is characterised by lipid vacuole-rich, steroidogenic cells that appear in a nestlike arrangement in the theca. In the largest (6-10 mm) white follicles, the thickened theca starts to differentiate into two layers: theca externa, the outer layer, which is rich in fibres and theca interna, with has less fibres, but it contains fibroblasts and steroidogenic cells, which form a more and more continuous layer.

In the small yellow follicles of 10-15 mm size, the theca separates into two distinct layers: theca externa (which is richer in connective tissue fibres, blood vessels and nerves) and theca interna (the layer of steroidogenic cells). The number of cell layers decreases in the granulosa, and among them, accessory cells appear, that divide often. The larger yellow follicles emerge more and more from the surface of the ovary, and their theca layer gets covered by the thin fibrous connective tissue of the stroma and a single-layered germinal epithelium. The relatively thick stalk that connects them to the medullary tissue is rich in blood vessels and nerves. The glandular cell-nests of the outer layer disappear, and the fibrous substance loosens. The granulosa gets converted into single-layered columnar and cuboidal epithelium. In the wall of the stalked large yellow follicles (F4-F1) the theca gets thinner, the cells of the inner layer lose their steroidogenic ability, and the capillarisation of the layer increases. The granulosa is formed from a single layer of columnar epithelium, and as tight junctions disappear and paracellular channels are formed, the cells

become more loosely arranged. The proliferation of mitochondria and smooth endoplasmic reticulum, associated with the substantially increased progesterone production, are characteristic of the F1 follicule a few hours before ovulation (Forgó et al., 1988a). (Figure 13.

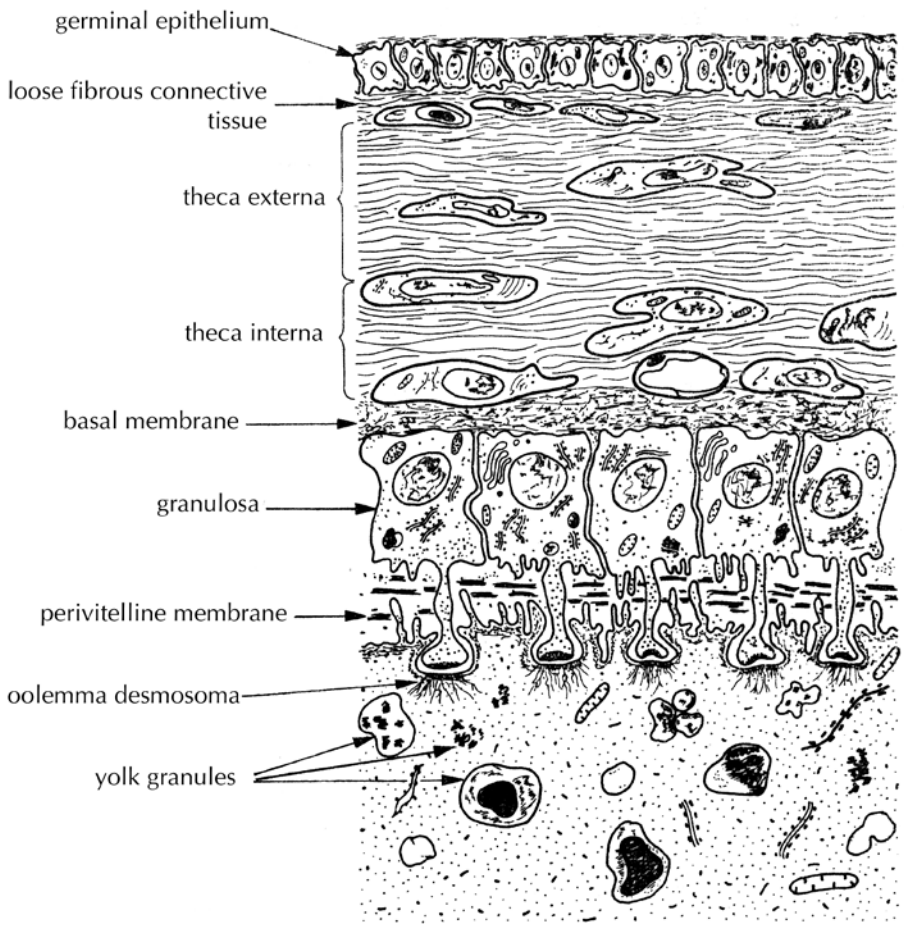

Figure 13. Electon microscopic structure of the wall of small yellow follicles. Modified after Péczely (1987)

The primary oocyte is located in the central area of the follicles, and contributes most to the mass of the follicle. The oocyte is surrounded by a special membrane, the vitelline membrane. The vitelline membrane has two layers: the inner layer is the oolemma, which is the actual cell membrane that contains microvilli, indentations and pinocytotic invaginations. The oolemma contains more and longer microvilli at the area covering the animal pole that contains the ooplasm and the nucleus of the oocyte, compared to the the oolemma over the vegetative pole and the areas of deuteroplasm that contain yolk.

The outer layer of the vitelline membrane is called perivitelline membrane (membrana perivitellina), and it is formed in the gap between the oocyte and the granulosa cells, as a product of the oocyte and mostly the granulosa cells. Therefore it can be considered a secondary oocyte sheath. The perivitelline layer consists of protein fibres embedded in a mucopolysaccharide matrix, arranged parallel to the oolemma. In diagonal position among the protein fibres are the microvilli of the oolemma and the basal processes of the granulosa. Therefore, in this way a layer resembling the zona radiata is formed, that has a cartwheel-like structure, which is most developed in the generations of large yellow follicles, and is already regressed in the follicule right before ovulation. The perivitelline sheath is analogous to the mammalian zona pellucida, and they share a similar chemical structure. It has two glycoprotein components, the 32 kDa compound is homologous to ZP3 in mammals, and the other 95 kDa compound is homologous to ZP1 of the mammalian zona pellucida. This structure has been detected not only in domestic fowl ovary, but also in Japanese quail, domestic duck, goose, pheasant and turkey. A glycoprotein equal or similar to the mammalian ZP2 has not been detected in the previtelline membrane of the avian oocyte. Avian ZP3 is synthesised by the granulosa cells of the follicle, and this process is regulated by testosterone. In contrast, ZP1 proteins are produced by the liver of the female bird, controlled by estrogen (Stepinska and Bakst, 2007).

The germinal disc is an elliptical structure located under the oolemma, excentrically in relation to the animal pole of the oocyte, and it contains the nucleus of the oocyte. In the laid egg it is found parallel to the longitudinal axis of the egg, floating on the denser, yolk-saturated deuteroplasm. The cytoplasm of the oocyte contains only a few mitochondria and a relatively large nucleus, with one or two nucleoli inside it. At this time, the oocyte still has a cytocentre, which will get expelled from the cell during ovulation, as part of the first polocyte. The deuteroplasm consists of layers of carotenoid-poor white and carotenoid-rich yellow yolk, and has few mitochondria and lysosomes. Outside the oocyte, under the oolemma there is a very thin coat of white yolk (formerly known as the nucleus of Pander), which surrounds the germinal disc as a funnel, and underneath it continues as a column of white yolk, the latebra, towards the centre of the oocyte. The majority of the deuteroplasm consists of alternating layers of thick yellow and thin white yolk arranged in a series of concentric rings around the latebra. Following ovulation, (a part of) the granulosa – unlike in mammals – does not stick to the ovulated oocyte to form a corona radiata-like structure. Therefore, the expulsed oocyte is only covered in the double-layered vitelline membrane, consisting of the inner oolemma and the outer perivitelline layer (Barua et al., 2001).

After ovulation, the wall of F1 follicle transforms into a large, goblet-like postovulatory follicle (calyx), with a lifespan of a few days (72-120 hours). It is

not homologous to the mammalian yellow body (corpus luteum). In the active ovary of continuously laying birds, it is often possible to observe one early, large and two to three continuously regressing postovulatory follicles. During ovulation, all granulosa cells remain the postovulatory follicle, and unlike in mammals, corona radiata is not formed around the oocyte. The outer part of the postovulatory follicle consists of the theca and cortical stroma material, covered by germinal epithelium. After ovulation, granulosa cells undergo intensive proliferation and more or less fill the cavity of the postovulatory follicle. These cells that originate from the granulosa have a greately increased amount of smooth endoplasmic reticulum and tubular mitochondria, suggesting increased steroid (progesterone) production.

In the postovulatory follicle apoptotic processes start to initate and they spread from the elements of granulosa origin to the whole structure. This is similar to the inflammatory regression of the mammalian corpus luteum, during which cytokins (IL-1β, IL-6, GM-CSF, IFN-γ and TGF-β2) and chemokins (chCXCLi2, chCCLi2, 4 and 7) express and activate. Lymphocyte subpopulations also appear, and the amount of apoptotic cells increases. In 36-48 hours more lipid vacuoles build up and progressive lipid degradation initiates, followed by a large increase in lysosomatic activity. As a result of these autolytic processes, the postovulatory follicle rapidly disappears, its location only indicated by connective scar tissue. In about 10 day (domestic hen), even its residues cannot be detected in the stroma (Sundaresan et al., 2008)

2.1.3. Follicular atresia

Different types and levels of atresia can be observed in the ovarian follicules from prepuberty, but especially from puberty. In domestic chicken, approximately 20% of developing follicles undergo atresia (Gilbert et al., 1983). It is after puberty, i.e., during breeding, when most follicular atresia occur (Figure 14).

In birds and reptiles follicular atresia occurs before the selection to enter the preovulatory hierarchy takes place, meanwhile in mammals it happens during the selection to develop one (or several) dominant follicle.

Most detailed studies of the morphogenesis of atresia were conducted on domestic goose, where seven different types of regressing follicles can be observed by light microscope (Forgó et al., 1988b). The first three types have small white follicles of 0.1-0.5 mm diameter.

1. The granulosa loses its single layer characteristics, starts to proliferate vigorously and creates invaginations into the yolk. With the progress of this process, granulosa islands develop inside the yolk, which contain a large amount of macrophages. Some of the proliferated granulosa cells have a pyknotic nucleus and they disintegrate, while others have lipid vesicles and well-developed

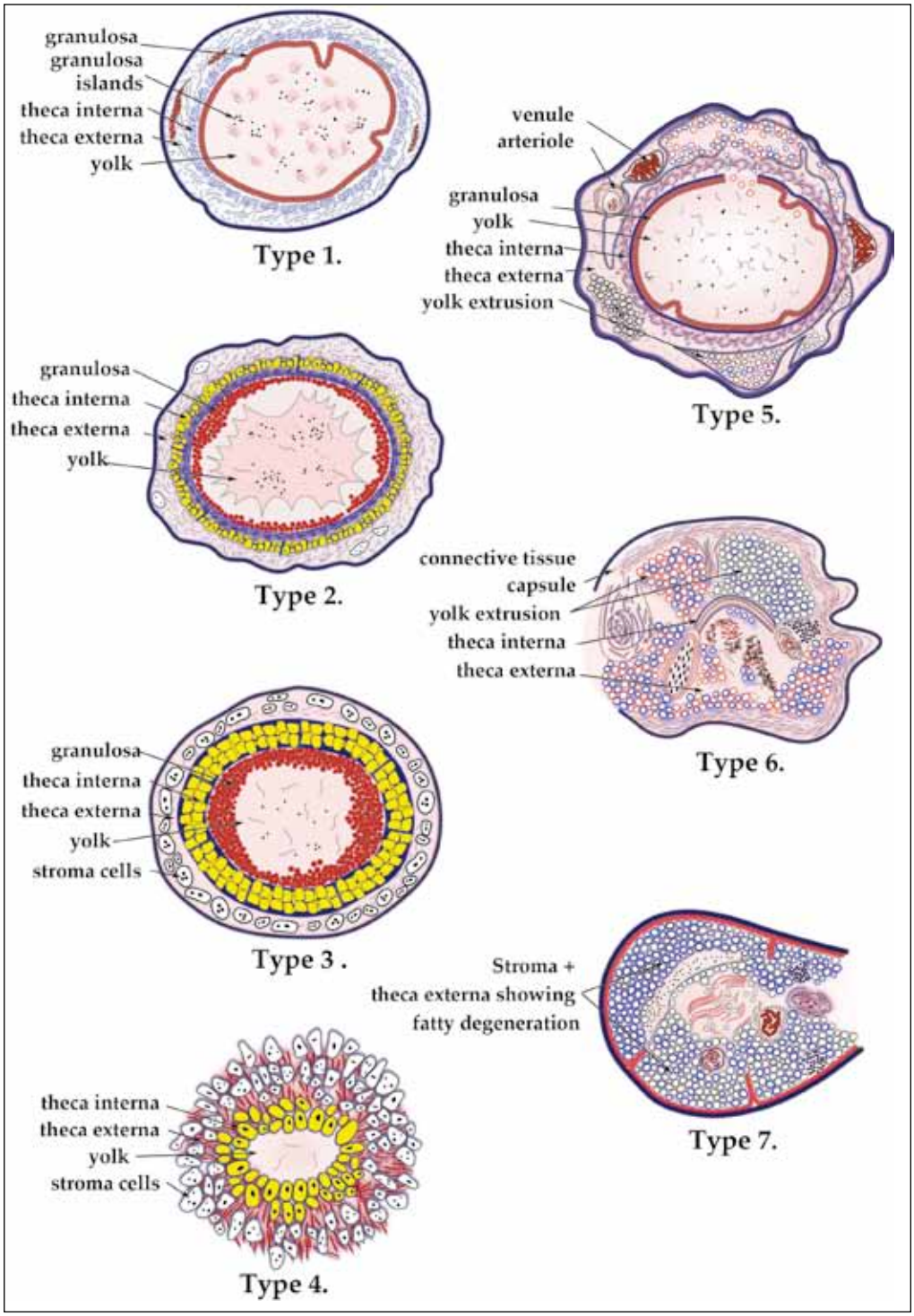

Figure 14. Types of regressing follicles in domestic goose ovary (drawn by Viktória Forgó)

smooth endoplasmic reticulum, suggesting active steroid production. Their theca layer is identical to those of "healthy" small white follicles.

2. The pseudostratified granulosa contains several vacuolated cells. Glandular cell-nests are formed within the theca interna, with connective tissue trabeculae among them.

3. A large amount of cell-nests appear within the theca externa, and several lipid vacuoles are visible in the cytoplasm of the cells. Strong fibrous proliferation is formed in the theca interna, which "pushes" the granulosa cells into the yolk.

4. This stage has structures that generally resemble white follicles of 0.5-2.0 mm diameter, but sometimes seem deformed, even to the naked eye. They are characterised by strong proliferation of the theca externa cells that have a cartwheel-like structure of connective tissue hedges towards the centre of the follicle. Because of the breaking up of the basal membrane, the cells of the granulosa and theca interna that underwent hyperplasia often mix.

5. The so-called bursting atresia type is formed by the breaking up of the oocyte membrane, with yolk leaking between the layers of the follicular wall. These follicular extrusions are often covered by a mass of stroma cells. Some of these stroma cells phagocyte yolk granules while others show fatty degeneration. The granulosa is single layered, the theca interna has a few cell lines and the theca externa is a loose fibrous connective tissue.

6. This stage has a compact structure, consisting of stroma cells that contain lipid vacuoles. Among the cells and the few connective tissue fibres, "leaks" are visible. These early-stage fatty degenerative structures are 6-7 mm in diameter.

7. Similar to the previous type, but the stroma cells show progressed fatty degeneration, and their nucleus is often completely pyknotic. Some cells transform into univacuolar adipose cells.

Ultrastructural studies of atresia have shown that independently from the type of regression, these follicles often consist of similar cell types, and they only differ in the ratio of these different cells. The first sign of atresia is the disappearance of transosomes. After this, lipid vacuoles begin to accumulate, which are covered by smooth membranes connected to rough endoplasmic reticulums and tubular mitochondria can also be observed. These signs support the hypothesis that atretic cells synthesise steroids at the beginning of regression. Later the cisternae of the endoplasmic reticulum expand, and the vacuoles appear, containing a dense substance, which are possibly lysosomes. With the progress of regression, the cytoplasm become dark and saturated with lysosomes (Kovács et al., 1992). Comparative studies of the enzyme content of normally developing and atretic follicles show that the amount of enzymes of lysosomal origin (mostly catepsin-D) is substantially higher in atretic follicles (Forgó et al., 1988b).

In some follicles, atresia serves to bypass lysosomal degradation and fatty degeneration that usually follow apoptotic processes. It is a non-inflammatory process that results in fast reabsorption of follicular matter, and it has physiological importance.

Bridgham et al., (2003) studied the molecular processes of atresia in domestic hen ovary. According to their results, proapoptotic processes in the granulosa cells lead to cell death by bypassing the effects of the antiapoptotic system, which protects normal structure and function. The effects of intrinsic (nutrients necessary for the functioning of the cell, production of EGF and IGF-I, decreasing amount of FSH and LH receptors and oxidative stress), as well as extrinsic factors together lead to apoptosis.

The extrinsic system is initated by the binding of cytokines to tumor necrosis factor (TNF)-type membrane receptors. The ligand-activated "death" receptors result in the homodimerisation of the death-domain of cytoplasmic adaptor proteins, which activates the caspase-8 enzyme. This enzyme cuts the cytoplasmic BH3 cell-protector protein, and consequently causes the outflow of mitochondrial proteins into the cytoplasm, including cytochrome-C, which contributes to the synthesis of the apoptosis protease activating factor (Apaf-1). Apaf-1 is one of the activators of caspase-3. Besides caspase-8, it directly activates caspase-3, which by destroying several important structural and functional proteins, directly triggers apoptosis.

Another physiologically important role of atretic follicles can be that for some time they produce a significant amount of progesterone, testrosterone and estradiol. Comparative measurements of steroid content suggest that with the progression of the reproductive cycle, it is mostly the amount of progesterone that increases, and it is specially pronounced in regression type 6. In the end of the cycle, the amount of progesterone that gets into the circulation from atretic follicles can therefore also increase the hormone concentration in the systemic circulation (Forgó et al., 1988b).

2.1.4. Steroid hormone biosynthesis in the ovary

Similar to other vertebrates, there are three types of endocrine cells in the avian ovary. These are the granulosa and theca cells that develop in the cortex and the follicular wall and the stromal cells of the cortex and the medulla. Enzymes of the steroid biosynthesis are located in the endocrine cells, such as δ-5-3β-hydroxysteroid-dehydrogenase, 17β-hydroxysteroid-dehydrogenase, δ-5-δ-4 izomerase, 3-reductase, 5α-, 5β- reductase, a 20- hydroxysteroid-dehydrogenase and 20- reductase (Gilbert, 1971, Lofts and Murton, 1973, Murton and Westwood, 1977, Bryndová et al., 2006).

Cholesterol can enter steroidogenic cells from three sources: 1. the LDL-VLDL fraction of the plasma, 2. cholesterol earlier imported into steroidogenic

cells, stored in lipid droplets, 3. in situ, de novo synthesised from isoleucine through squalene. In the case of the gonads and the adrenal cortex, steroid hormone biosynthesis begins with the uptake of cholesterol (C27), which was synthesised in the liver, mostly esterified instantly and transported into the steroidogenic organ by the blood stream.

The uptake of cholesterol into the cell occurs by receptor-activated phagocytosis of the complex macromolecules, LDL and VLDL, through the apical membrane of steroidogenic cells. Prerequisite for this activated phagocytosis is that FSH and LH receptors have developed earlier in the steroidogenic cells and the LH-activated adenyl cyclase-cAMP-protein kinase phosphorylation chain reaction is functional. As a result, a large amount of LDL-VLDL receptor components express, the formed receptors move into the apical membrane, and the uptake of lipoprotein macromolecules initiates. Inside the cell, with lysosomal assistance, lipoprotein molecules get split into apoprotein, lipid and cholesterol-ester elements, and these components enter different biosynthetic processes.

Cholesterol-esthers are stored intracellularly, possibly inside lipid droplets, while de-esterified cholesterol enters into the tubules of the smooth endoplasmic reticulum, and through this gets into the tubular mitochondria. Two proteins assist the transport of cholesterol into the mitochondria: the steroidogenic acute regulating protein (StAR) and the mitochondrium-specific 18 kDa cholesterol transport protein (TSPO), which bind cholesterol onto the inner membrane of mitochondria (London and Clayton, 2010).

The biosynthesis of steroid hormones begins on the inner membrane of mitochondria, with the first and rate limiting step being the cholesterol-pregnenolone transformation. A prerequisite of this step is the activation of cytochrome-P450 side chane cleavage (cyt-P450scc) enzyme coded by the CYP11A gene. The synthesis of a 21 carbon atom pregnenolone is dependent on a 4-member oxydo-reductase enzyme chain, the pregnenolone synthase. First, the formed superoxide-peroxide reaches the C20, C22 carbon atom of cholesterol, where it causes directed hydroxylation to form 20α, 22-dihydrocholesterol. From this molecule 20α, 22-27 desmolase cuts the 22-27 carbon part, an isocapronaldehyde is sliced off, and the 21 carbon atom **pregnenolone** is formed. Pregnenolone has no steroid hormone activity, it is an inactive metabolite (Furr and Pope, 1970, Ozon, 1972).

In the cytoplasmic "microsome" fraction the biosynthesis starting from pregnenolone can proceed following two different pathways: δ4 and δ5. One option (the δ5 pathway) is when 17α-steroid hydroxylase, which is coded by CYP17, through 17α-hydroxypergnenole leads to the formation of 17α-17,20-lyase **dehydroepiandrosterone (DHEA)**. The dehydroepiandrosterone intermediate can be both a metabolite and a final product. As a final product, this 19-carbon atom androgen has relatively small potential, and it is produced in the ovary,

as well as the testis and the adrenals of birds (Péczely, 1985). In the ovary, it is mostly synthesised by the theca interna cells, and – if not a final product destined for the blood stream – it gets converted into **androstenedione (A)**, which is a common precursor of androgens and estrogens, by the 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme, coded by several 3β-HSD gene isoforms. Alternatively, androstenedione can enter the blood stream as a low-potenital androgene final product, or as a precursor it can diffuse from the theca interna into the theca externa, to become the starting compound for estrogen synthesis.

The other option (δ4 pathway) occurs assisted by δ5-3β hydroxysteroid dehydrogenase (HSD) and produces 5pregnen-3-20-dion. This is converted into **progesterone (P4)** by δ5-δ4-isomerase. It is mostly the δ5 synthesis chain that operates in the theca layer, and as precursors, a substantial amount of dehydroepiandrosterone and androstenedione are produced. On the other hand, in the granulosa cells of the yellow follicles the δ4 synthesis chain is dominant, which produces a large amount of progesterone. The amount of progesterone produced increases as yellow follicles mature, as indicated by the increase in the activity of δ5-3β-hydroxysteroid dehydrogenase in the granulosa cells of these follicles (Hertelendy and Asem, 1984). Progesterone serves as a precursor in the smaller yellow follicles, which after entering the theca gets into the androgen and estrogen biosynthesis, while in the granulosa cells of the large (F5-F1) yellow follicles it is the final product of steroid synthesis. Under in vitro conditions, the treatment of granulosa cells from F5-F1 follicles with ovine LH causes increases P4 production, and the amount of this increase is proportional to their devepmental status (Porter et al., 1989). Additionally, the results of an in vivo study show that just before ovulation rapidly raising LH levels cause increased progesterone production, especially in the wall of the two largest yellow follicles (F2-F1), and a large increase in fluctuating plasma P4 levels will trigger an ovulatory peak in LH. At the same time, the level of DHEA that forms from pregnenolone greatly decreases (Lee et al., 1998).

LH-induced protein kinase-C (PKC) and mitogene-activated protein-kinase/Erk-signalling play an important role in the progesterone synthesis-stimulating effect of LH in the granulosa cells, stimulating steroidogenesis, as part of a cascade (Woods and Johnson, 2007).

Kinases that affect energy metabolism can modulate progesterone synthesis. AMP-activated protein kinase (AMPK) is such a modulator, which achieves its inhibitory effect on the granulosa cells of the yellow follicles through extracellular signal-regulated kinase (ERK), with its efficiency depending on the size of the follicle (Tosca et al., 2006). A part of this modulator system is IGF-1, which induces proliferation of the granulosa cells and also increases their progesterone production. The effect of IGF-1 is modulated by AMPK, stimulating it in the granulosa cells of the F1 follicle and inhibiting it in the F3-F4 follicles. These effects suggest different modulation of follicular energy metabolism (Tosca et

al., 2008). An important influencing factor of ovarian steroid synthesis is the ovarian 9-cis retinoic acid, which acts differently in certain follicle types and has a different effect on estrogen and progesterone synthesis. Regarding progesterone synthesis, it increases basic P4 production in granulosa cells of the preovulatory follicles, has no effect on LH-stimulated P4 secretion of the F2 and F3 follicles, but decreases LH-stimulated P4 production of the granulosa cells of the F1 follicle (Pawlowska et al., 2008).

Following the δ4 biosynthetic pathway, 17α-steroid-hydroxylase converts progesterone into **17α-hydroxy-progesterone**, which is an intermediate metabolite in birds and it is converted into **androstenedione** by 17α-hydroxy-progesterone-aldolase. The 19 carbon atom androstenedione therefore can be synthesised via both δ5 and δ4 pathways, i.e., it can also be synthesised form DHEA by 3β-HSD. Androstenedione – primarily in the small, medium and larger white follicles and in the smaller yellow follicles – gets converted into **testosterone (T)** by 17β-hydroxysteroid dehydrogenase. The synthesis of testosterone is substantial in the theca interna cells, but insignificant in the theca externa. Its biosynthesis can receive a progesterone precursor from the granulosa cells (Gilbert, 1971, Shababi et al., 1975, Huang and Nalbandov, 1979, Forgó et al., 1986, Kato et al., 1995). Generally, testosterone is the second androgen end product of the ovarian steroid biosynthesis. It is secreted in large amounts at the beginning of maturation and later its secretion gradually decreases and less is found in the plasm. During egg laying it participates in the regulation of ovulation, when its production becomes fluctuating.

A smaller amount of testosterone gets aromatised in the theca externa cells into 17β-estradiol.

The majority of C-18 estrogens are synthesised from androstenedione, but they can also originate from testosterone. In the first step, 19-steroid hydroxylase converts androstenedione into 19-hydroxyandrostenedione, and this compound gets oxidised by 19-steroid oxydase into 19-oxoadrostenedione. P450-aromatase, coded by CYP19, converts this compound by rearranging the δ4-3-1 type "A" ring double bonds typical to androgens into a phenol-type aromatic "A" ring, forming **estrone (E1)**. 17β-hydroxysteroid dehydrogenase can reversibly convert estrone into **17β-estradiol (E2)**, which is the most important peripheral estrogen hormone in birds, similarly to mammals. 17β-estradiol can also form directly from testosterone by the effect of aromatase. The third estrogen hormone, **estriol** (**E3**) is not synthesised in the avian ovary, or in the peripheries (Sturkie, 1976, Péczely, 1985).

Estrogen concentration is significant in the wall of the larger white and small yellow follicles, and its amount gradually decreases as the follicles grow. Aromatase, the key enzyme of estrogen biosynthesis is only found in the theca externa cells. In vitro, a high percentage of androstenedione and testosterone can be converted into estrogen in the theca cells of the F5 follicles, but this

process cannot be observed in the theca externa of F1 follicles, which suggests a strong decrease in aromatase activity during follicular maturation (Porter et al., 1989).

2.1.5. The transport, receptor binding, peripheral effects and catabolism of sexual steroids in female birds

Progesterone

Most of the progesterone reaches the target cells bound to proteins. The majority of these bound steroids is attached to the plasma albumin and prealbumin fraction, which has high (practically infinite) binding capacity, but low specific activity (Kd = 10^{-5} mol/ml), while a smaller amount binds to transcortin or corticosteroid-binding globulin (CBG), which is a transport globulin. CBG, which is also present in birds, forms a non-covalent bond with corticosteroids and progesterone, which has high affinity (Kd = 10^{-7}, 10^{-9} mol/ml) and low binding capacity. This causes competition between the two steroid groups when binding to CBG. CBG is synthesised by the liver, androgens inhibit, while progesterone, estrogens and thyroid hormones stimulate its production (Seal and Doe, 1966, Péczely, 1985). A lower proportion of the progesterone that gets into the blood stream is found in the plasma in a free form, not bound to proteins.

There is a dynamic equilibrium in the plasma between the amounts of free and protein-bound progesterone. Only the free fraction can be considered biologically active, i.e. able to diffuse into cells and bind to specific receptors, and also be exposed to plasma steroid enzyme systems causing degradation. They also get metabolised in the steroid catabolic system of the liver, and their metabolites get transported into the duodenum through the bile. The degradation percentage of active steroid molecules is reflected by the metabolic clearance rate (MCR), which is 5.72 ml/minute/kg body weight in mature female Japanese quail (Péczely, 1985). The hormone supply of the target cells depends on the secretion rate (SR) of the steroidogenic organ, as well as on MCR, which in turn is largely determined by the amount of free and protein-bound steroids (progesterone) in the plasma and the actual amount of transport proteins. As the fraction of plasma albumin remains constant, the amount of transport proteins is determined by the current amount of CBG (Péczely, 1985). Biological inactivation of progesterone can be characterised by a change of its speed, i.e., the biological half life (t1/2). This is 64 min in mature, egg-laying turkey and 92 min in immature females, 12 min in mature female Japanese quail and 17 min in immature females – i.e., the half life is longer in sexually inactive birds (Mashaly and Weinthworth, 1979, Péczely, 1985).

Several factors affect the steroid hormone-metabolising capacity of liver cells, with the aim of maintaining homeostasis. The most important factor

among these is the stability of the free fraction of the particular steroid hormone. Because of this, increased secretion is followed by increased metabolism, i.e., the increase of periferial degradation (e.g., increased thyroid function, increased plasma corticosterone levels) increases secretion to maintain stable hormone levels.

During progesterone metabolism in the liver, it is first converted into di-, and tetrahydo-derivates. The C-atom in position 20 gets hydroxylated to form 4-pregnen-20β-ol-3-on, which, by the addition of an H to the δ4 position gets converted into 5-β-pregnan-2-0β-ol-3-on. Sulphokinase converts these di-, and tetrahydo-derivates into sulphate conjugates, significantly increasing their water solubility, so they can be discharged from the body with faeces and urine (Péczely, 1985).

In the peripheries, progesterone acts through specific cytosolic-nuclear receptors. The structure and function of progesterone receptors has been studied in most details in chicken oviduct (Schrader et al., 1975, Schrader and O'Malley, 1978, Denner et al., 1990, Spelsberg et al., 1996, Camacho-Arroyo et al., 2007). The cytoplasmic progesterone receptor is a phosphoprotein, which consists of two polypeptide isoforms: a 79 kDa „A" and a 110 kDa „B". These receptor elements in a non-activated form (as aporeceptors) bind to a heat-shock protein complex of three components: hsp 90 (kDa), hsp 70 (kDa) and hsp 59 (kDa). When transfering across cell membranes by diffusion, progesterone molecules bind to the hormone-responsible section at the carboxy-terminal end of the A and B receptor isoforms, activating them by hyperphosphorylation, i.e., by causing a conformational change. The activated A and B elements detach from the heat-shock protein complex, and become capable of transporting a reversibly bound progesterone molecule in the cell, one each. Activated A and B isoforms either form a heterodimer complex, e.g., in the oviduct, or they are produced in different amounts, independently from each other, and they fullfill their own specific function as a homodimer, e.g., in different brain regions.

In the case of the oviduct, the dimer progesterone receptor and two progesterone molecules get transferred through the nuclear pores by using tubules of the endoplasmic reticulum, and when they reach the caryoplasm, they bind to the DNA. Before binding, the dimer separates into its two components, which attach to a region of the DNA not covered in histones, i.e. where it is not repressed. Two regions form on the non-repressed DNA: one element of the dimer binds to a non-histone protein acceptor, while the other also binds to a non-histone protein effector that covers the DNA. The central region of the receptor dimers is a DNA-responsible element, which consists of 60 amino acid residues. This section is bound by two zink-finger elements, which raise finger like, are zink based, and contain eight cysteine residues. The section between them binds to the acceptor and effector proteins, and through them to the DNA,

along with the two progesterone molecules. The formation of this structure is reversible, and it initiates transcription. The elment bound to the effector activates RNA polymerase, and therefore initiates doubling of the actual, despiralised DNA to form mRNA. Then the mRNA fragment moves to the cytoplasm through the pores of the nucleus, where by the insertion of transferRNA in the polysomes, a new specific polypeptide is formed. The cytoplasmic polypeptide chains formed by translation move into the system of activated Golgi cisterns, and get converted into new protein components (e.g., ovalbumin).

Later, in the target cells, the progesterone receptor isoforms undergo phosphorylational inactivation, catalysed by the EGF receptor (Ghosh-Dastidar et al., 1984).

Progesterone is an important intermediate of intraovarian steroid biosynthesis. When it gets into the blood stream, it regulates (triggers) ovulation, and along with estrogens, also plays a determining role in the functional differentiation of the oviduct and egg formation. Progesterone receptors are also located in different brain regions, where this hormone affects the appearance of certain behavioural form, and serves as a regulating factor of feather growth and moulting processes in the feather follicules.

The effect of progesterone on the ovary usually manifests after estrogen priming or simultaneously with estrogens, so we will detail its effects later, when introducing the effects of estrogen.

Additionally, progesterone can also act alone: in the mucosa of the uterus it increases prostaglandine F2α synthesis without the synergic effect of estrogen, and therefore it increases Ca^{2+} transport towards the lumen of the uterus (Lundholm, 1992).

Estrogens

Two estrogen hormones occur in the plasma of birds: 17β-estradiol (E2) and estron (E1). Most of our knowledge is about E2, given that in the past 10-20 years, data about plasma estrogen practically only referred to E2 levels, because of technical reasons (E1 and E2 could only be distinguished in fractions separated by chromatography). According to earlier data, E1 and E2 occur in nearly equal quantitites (10-300 pg/ml concentration) in domestic chicken, Japanese quail, domestic turkey, domestic geese, Eurasian collared dove (*Streptopelia decaocto*) and American tree sparrow (*Passerella arborea*). The amount of E1 was several times higher than E2 only in rook (*Corvus frugilegus*) (Péczely, 1981).

Because the sexual steroid-binding protein (SSBG) that transports androgens and estrogens in mammals is lacking in the plasma of birds, earlier all androgens and estrogens were thought to be transported to the target cells by the albumin fraction of plasma. However, it was found in several bird species that the actual amount of plasma CBG (transcortin) is significantly affected by

plasma testosterone level, which is, therefore, an important link that forms a reciprocal relationship between the functioning of the adrenal gland and the gonad (Péczely, 1979, 1985). In dark-eyed junco, plasma CBG binds corticosterone and progesterone with equal affinity (100%), while its testosterone binding affinity is approximately 20% (Deviche et al., 2001). On the other hand, in spite of the lower binding affinity, over 90% of plasma testosterone is bound to CBG according to studies in juncos and white-throated sparrow (*Zonotrichia albicollis*) (Deviche et al., 2001, Swett and Breuner, 2008). However, there are only indirect observations about estrogen binding of avian plasma CBG: corticosterone and progesterone completely block testosterone binding in the plasma of chicken embryo, but estrogen only shows slight competition (Savu et al., 1986). If the higher binding capacity of CBG is also present in the case of estrogens, it is possible that besides albumin and prealbumin – in spite of its low binding affinity – globulin also plays an important part in estrogen transport.

Estron plays a central role in the peripheral degradation of estrogens. This compound, acting as an active hormone, metabolises directly in tissues with estrogen receptors. Additionally, estron is formed during the first step of 17β-estradiol catabolism, by adding a keto group to the 17th carbon atom. Next, in Japanese quail, estron gets hydroxylated on the 16th carbon atom, forming 16α-hydroxyestron. In female turkey, however, 15α and β metabolites are formed (Brown et al., 1979, Péczely, 1985). 15 and 16hydroxy-estron sulphokinase convert estron into suphate, which gets eliminated through the kidney. Glucuronic acid conjugates are not known to form in birds.

Among estrogens, biological half life and metabolic clearance rate is only known for 17β-estradiol. Biological half life is similar in juvenile and sexually mature female Japanese quail: 17 and 19 minutes, respectively, while the metabolic clearance rate (MCR) is 6.86 ml/min/kg. Secretion rates (SR) calculated based on plasma levels of progesterone and 17β-estradiol, as well as MCR, show that in Japanese quail, within a given time frame, about three times as much progesterone is synthethised than 17β-estradiol: 9.63 and 3.12 ng/min/kg, respectively (Péczely, 1985).

In the target cells, estrogens bind to cytoplasmic α and/or β receptors or also α and/or β-type membrane receptors. In mammals, estrogen α-receptors were discovered in 1986, β-receptors in 1996 and membrane α and β receptors in 2009.

Estrogen α-receptor was first detected in the mucosa of chicken oviduct by Krust et al., (1986), which is relatively (80%) similar to the human estrogen α-receptor with a 66 kDa molecular weight and 589 component amino acids. Its structure is also basically equal to that of the known mammalian receptor. The first 185 amino acids of the estrogen α receptor peptide form the transctiption-activating domain (domain A/B), which performs ligand-independent transactivation function. Domain C, which provides DNA binding, consists of

66-68 amino acids, with high cysteine and basic amino acid content. The next, short section (D) carries the nuclear localisation signal. The hormone-binding domain E is responsible for specificity and hormone-dependent transcription activity, and is stongly hydrophobic in chicken. The carboxy terminal section is part of receptor F.

Estrogen β receptor consists of a slightly smaller, 485-member amino acid chain. Its DNA-binding domain is 96% similar to that of the α-receptor, while their hormone-binding domains are only 58% similar. The considerably shorter (103 amino acid) A/B domain, and the composition of D and F domains show larger differences (Mosselman et al., 1996).

The steroid-receptor co-activator (SRC-1) plays an important role in the formation of gene-expressing effects of estrogen, modulating ligand-dependent transactivation of the receptors. SRC-1 is mostly found in steroid-sensitive brain regions. The amino acid sequence of SRC-1 prepared from Japanese quail brain, shows approximately 70% similarity to the peptide prepared from rat brain, and it contains three boxes (LXXLL) that link the steroid receptor to the nuclear binding site. In male birds, experimental blocking of SRC-1 inhibits the effect of estrogens and androgens that are responsible for the development of sexual dimorphic brain regions and for reproductive behaviour (Charlier et al., 2002, 2005).

Estrogen membrane receptors mainly perform their steroid-binding and cell-function regulating function in nerve cells. Their effect is fast and non genomic. Peptides identical to the estrogen α and β receptors of the cytosol enter the membrane bound to cytokeratin intermediate filaments. Here, assisted by caveolin peptides, they bind to metabotropic glutamate receptors, which is a type of membrane glutamate receptors, and activate it. The presence of glutamate is not necessary to activate this glutamate receptor. The formation of a G-protein-coupled cAMP (stimulating effect) and the incorporation of β-arrestin2 into the membrane indicate the activation. β-arrestin2 is also responsible for the internalisation of the receptor that follows the activation (Mermelstein, 2009, Sanden et al., 2010).

Estrogen membrane receptors (without indicating their type) have also been isolated from the cell membrane of the pars nervosa in laying hen. The dissociation constant (Kd) of these receptors is equal in non-laying and laying hens. Their functional role is demonstrated by maximum binding capacity (Bmax) of the receptor fraction being lower in laying hens compared to non-laying chickens, and that one hour after estrogen intake the specific binding capacity markedly decreases, as well as three hours before oviposition, when the plasma estrogen level is increasing (Takahashi and Kawashima, 2009).

Reproduction-related, or peripheral activies of estrogens occur in many areas. These studies mostly focused on 17β-estradiol, which occurs in the highest concentrations. Larger amounts of estrogen receptors have been found in

the stroma, granulosa and theca layers of the ovary (higher concentrations of α, than β type), in different sections of the oviduct, in the liver, bone tissue (mostly in medullary bones) and in different brain regions.

According to studies conducted on female Japanese quail, the receptor kD is $2x10^{-10}$ M and the amount of binding sites in liver cells of juvenile birds is 0.15 pmol/mg DNA, while in mature, egg-laying females it is 0.47 pmol/mg DNA. Estrogen receptors could not be detected in males, but after a treatment with 17β-estradiol, a substantial amount of estrogen receptors appeared in these birds, their numbers proportional to the dose. In liver cells the synthesis of estrogen receptors is triggered by the increasing plasma estrogen level during puberty, and their numbers are also upregulated by it (Turner, 1984).

Estrogen also triggers the synthesis of yolk matter in the liver cells, causing mRNA expression and accumulation of vitellogenin, VLDL, LDL and apo-VLDL-II, while the expressed RNA binds to a stabilising cytosol protein, which protects it from the effects of endonucleases. This protein is mostly apolipoprotein, which to a smaller degree, also assures stability of serum albumin and vitellogenin synthesis during puberty and egg laying (Ratnasabapathy, 1995). Increased estrogen secretion by sexually mature birds also plays an important role to assure the synthesis of the riboflavin-binding protein in the liver. This protein enters the plasma and transfers riboflavin into the yolk of the oocyte and into the albumen that is formed in the magnum. Along with other yolk-precursors (lipids, phospholipoproteins, and vitellogenin carrying inorganic ions), riboflavin-binding protein enters the oocyte bound to a 95-kDa protein receptor, which is specific, but has a wide-affinity range (Mac Lachlan et al., 1994). Estrogen increases the synthesis of retinol-binding protein (RBP) in the liver and its transport into the blood stream. Retinoids and vitamin A transported by RBP get incorporated into the yolk matter of the oocyte in the growing follicles (Vieira et al., 1995).

Estrogens can affect factors influencing the basal metabolism at several levels. Estradiol treatment decreases plasma glucose levels, the glucose content of the body and glucose metabolism. It also decreases plasma insuline levels. Plasma T3 concentration and basic metabolic rates increase in laying hens after the administration of estrogen (Jones and Manning, 1987, Jaccoby et al., 1995).

Estrogens play a role in the regulation of leptin receptor expression and therefore indirectly regulate lipid metabolism. The amount of leptin receptors, which are 1148 amino acid-long in chicken and show a 60% similarity to the mammalian "long" leptin receptor, increases only in the intestine during sexual maturation. Estrogen treatment of juvenile chicken increases mRNA expression of the leptin receptor only in the intestine (Ohkubo et al., 2000).

Estrogens play a basic regulatory role in the actual lipid levels in liver, plasma and connective tissues. The subcutaneous injection of one dose of 17β-estradiol in chickens fed an adequate diet substantially raises plasma trigliceride

concentration, reaching 137–2263 mg/dl. Plasma phospholipid, cholesterol and VLDL levels also increase, and the apolipoprotein composition of VLDL changes. During egg laying, typical changes occur in the liver with regard to the expression of genes associated with yolk production: estrogen increases the synthesis of vitellogenin-I, apo-VLDL-II, ethanolamine kinase, G-protein γ-5 subunit and leucyl-t-RNA syntetase (Ding et al., 2007). In their liver, there is also significant increase in phospholipid (phosphatidylcholin, phosphatidylethanolamide and phosphatidylinositol) transfer activities, which suggest increased plasma lipoprotein biosynthesis (Rusinol and Bloj, 1989). In laying domestic hen changes in the lipid production of the liver are also reflected by the size of VLDL particles: in periferial tissue their size is 70 nm, while the ones deposited into the oocyte yolk are smaller, of 30 nm diameter (Salvante et al., 2007). Fatty liver can often form following estrogen treatment, as a result of substantial triglyceride accumulation. The amounts of malate, glucose-6-phosphate dehydrogenase, and ATP citrate-lyase also significantly increase in the liver. The increased fat content of the liver can ocassionally cause fatty liver haemorrhagic syndrome (FLHS), which is a symptom of estrogen hyperproduction (Pearce and Balnave, 1976, Lee et al., 2010).

The increase of plasma estrogen level stimulates both the uptake and the enzymatic degradation of lipids. Hormone-sensitive lipase content of peripheral adipose tissue did not change following estrogen treatment of optimally fed chickens, but in fasted birds the treatment singnifically increased the activity of this enzyme as well as lipid catabolism. In chicken, estrogen treatment does not only increase plasma lipid concentration, but also the levels of plasma malonaldehyde, which suggest oxidation of lipids (Leszczynski et al., 1987, Park and Cho, 1988, 1990, Cho and Park, 1990). The tissues of estrogen-treated chickens also show altered fatty acid and mucopolysaccharide composition: the ratio of unsaturated fatty acids substantially increases, and the amounts of hexosamine, hexuronic acid, hydroxyproline, and sialic acid change in connective tissue, depending on the organ (Bruce and Anastassiadis, 1977).

The amount of estrogen receptors, and the mediated steroid effect play a crucial role in cytodifferentiation, as well as the functioning of the active oviduct. In the oviduct of juvenile chicken the concentration of estrogen receptors is around 1000 receptors per cell, while in laying hens this increases to 2000 receptors/cell (Best-Belpomme et al., 1975). A more detailed analysis of estrogen receptors shows that in Japanese quail oviduct only estrogen α-receptors express, estrogen β-receptors were not detected (Das et al., 2006).

Analysis of oviduct proteins of 5, 35 and 65 week-old, as well as mature chickens shows that among the approximately 300 proteins the amount of eight at least doubled in 35 week-old egg laying hens. Five of these proteins were identified: calumenin, acidic ribosomal phosphoprotein(s) (ARP), prohibitin, heart fatty acid-binding protein and anterior-gradient2 (AGR-2). The

amount of ARP and AGR-2 increased particularly in the magnum of laying hens. Though to a lesser degree, the amount of these proteins also increased in the isthmus. The activity of the promoter region of AGR-2, which contains the estrogen-binding site, has greatly increased during puberty (Kim et al., 2007).

Estrogens can also regulate ovarian function indirectly, independent from other ovarian steroids, therefore they can alter the motility of the uterus and the sperm storage function of the uterovaginal section. Estrogen treatment increased uterus mass and the mRNA of arginine vasotocine in the mucosa and its oxytocin-like receptor in the myometrium in photorefractory Japanese quail. In treated quails, uterus motility increased after the administration of arginine vasotocine compared to birds not pre-treated with estrogen (Srivastava et al., 2007). The arginine vasotocine responsible for increased contractility probably expressed locally, and acted in a paracrine way (Sirastava et al., 2008). In the mucosa of the uterovaginal junction the amount of estrogen α-receptors decreases following repeated insemination. This effect can be related to the sperm storage function, as there was no change detected in receptor numbers in the area of the uterus (Das et al., 2006).

Estrogens can regulate the increased calcium turnover of laying birds by themselves. Estradiol implanted into juvenile chickens – which increases plasma E2 levels – increased calcium and magnesium content in the uterus, and the activity of Ca-Mg-ATP-ase, signifying increased calcium transport in the uterus. Increased estrogen levels during puberty stimulates alkaline phosphatase activity in the intestine, which causes more intensive absorption of calcium (Quin et al., 1993, Quin and Klandorf, 1993). Estrogens locally affect the expression of cellular calcium-transport regulating calbindin in the intestine, ensuring by this way the amount of calcium necessary for eggshell formation. This process also needs active vitamin D. In juvenile chicken the D3 vitamin treatment alone does not increase the expression of calbindin-D28K in the intestine, it needs a pre-treatment with estradiol. On the other hand, co-treatment with estradiol and active vitamin D3 does not affect the calbindin production of the uterus (Bar et al., 1990).

Plasma calcium level – through intestinal absorption, metabolisation of medullary bones and decreased renal excretion – is affected by the actual estrogen production. In laying hen, parathyroid hormone Kd and Bmax values in the kidney and the bones substantially decrease three hours before laying an egg. A similar decrease occurs in these parameters following estrogen and possibly progesterone treatment. These data prove the ovarian steroid hormone dependence of parathyroid hormone receptors of the bones and the kidney (Yasuoka et al., 1996). In moulting birds, plasma estrogen levels are significantly lower, and the calcium content of the plasma also decreases, while plasma estrogen and calcium levels increase simulatenously after moulting (Yosefi et al., 2003). Estrogen affects bone development, causes the rearrangement of bone tissue

and changes its calcium transport. Treatment with 17β-estradiol depolarises the cell membrane of osteoclasts within seconds, which suggests that osteoclasts posess membrane estrogen receptors. This depolarisation takes place by opening membrane channels, as it can be avoided by inhibiting Na^+Ka^{+-}ATPase and blocking K^+ channels (Pederson et al., 1997, Brubaker and Gay, 1999). Estrogen increases early gene expression (e.g., c-fos and c-jun), which occurs within 30 minutes of estrogen treatment, decreases cytoskeletal, membrane-linked tyrosine kinase activity and lysosomal catepsin-B and acid phosphatase activity, which leads to decreased resorption of bone tissue (Pascone and Oursier, 2001).

Primary estrogen effect initiates the development of progesterone receptors in the mucosa of the oviduct, which – after binding P4 – will lead to the initiation of secretory activity of the tubular gland cells. Hora et al., (1986) suggested that the effect of estrogen manifests in increasing the progesterone-binding capacity of nuclear acceptor sites, and therefore the amount of P4-induced mRNA (transcription). However, the production of ovalbumin, avidin and lysozyme is continuous and requires additional estrogen (Palmiter and Wrenn, 1971, Oka and Schiemke, 1969a, 1969b, Joensuu et al., 1990).

In interaction with androgens and – to a greater degree (and also with details better known) – with progesterone, estrogens affect the structural differentiation of the oviduct, the functioning of unicellular epithelial and tubular propia glands, and their differentiated secretion. The first step of this process is the activation of estrogen receptors of the epithel cells in the juvenile ovary, which, by a transcription-translation mechanism, results in the differentiation of epithelial cells. At the same time, the interaction between mesenchymal and epithelial cells begins, during which the mesenchymal cells that contain progesterone receptors submerge in the subepithelial layer of the mucosa, and stimulate the differentiation of epithelial cells, and the formation of tubular propia glands (Joensuu et al., 1990). In the next step, progesterone receptors appear in the unicellular and tubular gland-forming secretory cells of the mucosa, and they cause the production of certain ingredients of albumen (Tuohimaa et al., 1989). A basic hormonal condition of the tissue differentiation of the oviduct is that first an estrogen-triggered mitotic active growth and proliferation should start in the epithelial cells of the mucosa, which leads to the formation of tubular glands, to be followed by the gland-stimulating effect of progesterone. If it happens in the opposite order, progesterone will inhibit cell differentiation and gland formation.

Estrogen treatment at the beginning of puberty will first increase the amount of lysosime mRNA in the cells of the oviduct (in 24 hours) and after 3 days lysosime protein can also be detected in significant amounts. The synthesis of ovalbumin is somewhat slower to initiate, and needs the presence of both estrogen and progesterone from the beginning (Oka and Schimke, 1969a, 1969b). Under experimental conditions, chronic treatment with estrogen and progesterone

together affects tissue proliferation first in a synergistic then antagonistic way: after some time the differentiating tubular gland cells stop invaginating the propria, but the secretory activity of glands becomes more intensive (Palmiter and Wrenn, 1971). According to other studies, in chicken the two steroids together increase ovalbumin in a synergistic way and their effect is long lasting, however, in the case of conalbumine, its synthesis will first decrease, and later increase, reaching a higher level then in individuals only treated with estrogen. Comparing a single administration of estrogen and progesterone shows that in estrogen-treated birds the amount of estrogen receptors increased in the magnum 6 hours after the injection, while it decreasead after progesterone injection. However, after 18-24 hours the pattern turns around, the group treated with progesterone had a higher amount of estrogen receptors (Sutherland et al., 1980).

Co-administration of estrogen and progesterone modifies the local effect of vitamin D3 in the uterus on the synthesis of calcium-binding protein (CaBP). Estrogen increases CaBP synthesis in the absence of vitamin D3 and also following vitamin D3 stimulation, while progesterone alone does not have an effect, but inhibits (decreases) the stimulating effect of estrogen (Navickis et al., 1979).

The estrogen effect can also be detected in the pars nervosa, acting through the estrogen receptors of the cell membrane. We will detail the role of estrogen receptors in the nervous system when introducing the central regulation of reproductive processes.

Androgens

Androgen production in the ovary primarily refers to the formation of an intermediate metabolite of estrogen biosynthesis. The progesterone that gets from the granulosa to the theca interna serves as a precursor for androgen synthesis via the δ4 and δ5 pathways. In the δ4 pathway 17α-steroid hydroxylase converts progesterone into 17α-progesterone, and in a second step 17α-hydroxyprogesterone aldolase converts 17α-progesterone into androstenedione. Androstenedione has two functions: being one of the weaker androgens it can get into the blood circulation and can reach the peripheral androgen receptors, and also it can remain an intermediary metabolite, and it can move into the theca externa cells as an element of estrogen biosynthesis to get converted into estrone by aromatisation.

In the theca interna, through the δ5 pathway 17α, 17, 20 lyase converts 17α-hydroxypregnenolone into dehydroepiandrosterone (DHEA). DHEA also has two functions. On one hand, it can get into the blood stream as a weak-activity androgen end product (nevertheless, it can have some specific functions). Its mechanism of action in the periphery is not yet known in birds, i.e.,

its binding affinity towards androgen receptors is insignificant, however, indirect evidence suggests its physiological effects in the regulation of breeding, moult and aggressive behaviour, which assumes specific receptor binding.

In mammals, DHEA can be partly responsible for the opitimalisation of muscle mass and skeletal composition. Its amount in the plasma is also known to decrease with age, which suggests specific physiological relevance. There are no studies confirming the presence of a specific DHEA receptor in birds, but in mammals several experiments confirm that DHEA does not bind to "classic" androgen receptors, but peroxisome proliferator-activated receptor alpha (PPAR-α), pregnane-X receptor (PXR), and estrogen-receptor-β have a high affinity towards DHEA. Additionally, several memberane receptor types (caveole-associated receptor, Gq/11 protein-coupled membrane receptor, membrane estrogen receptor-α and β) can possibly function as specific DHEA receptors (Webb et al., 2006). As a metabolic intermediate, DHEA can be converted into androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD), which can be converted into a testosterone with more efficient androgen/anabolic steroid activity by 17β-hydroxysteroid dehydrogenase both in the brain tissue and in the periphery.

Testosterone, and possibly also androstenedione get transported to the target cells bound to the albumin and prealbumin fraction of the plasma as well as to transcortin (CBG), while DHEA is probably transported by albumin and prealbumin proteins.

Testosterone, dihydrotestosterone and androstenedione bind to specific androgene cytosol-nuclear receptors in the target cells, which show a generally similar structure to other steroid receptors (which have been introduced through the progesterone receptor). In birds, a 61.2 kDa peptide was first identified in chicken oviduct, which corresponds to an androgen receptor structure of 703 amino acids. The structure of the androgen receptor is characterised by a relatively long AB domain, which triggers transcription activation (559 amino acids). The amino acid sequency of the DNA-binding (C) domain and the ligand-binding (E) domain are homologous among species. It binds sexual steroids, primarily androgens, with a great affinity. Its binding affinity is highest in 5α-dihydrotestosterone, and decreases through testosterone to progesterone and estradiol. The Kd value was found to be 10^{-9}, while Bmax was 0.13 nM. The Kd value was equal in juvenile and laying birds, while Bmax was higher in laying hens (Ellis and Danzo, 1989, Kawashima et al., 1999). Later the complete cDNA of the androgen receptor was identified and cloned, and it was found to be 2109 bp (Katoh et al., 2006).

In female birds, the androgen receptor is present in several organs. During embryonic development, it was found to express more in the left ovary compared to the testis. The presence of androgen receptors is crucial in the morphogenesis of the ovary: the receptor-antagonist flutamide inhibits differentiation

in the ovarian cell-bundles in the developing embryo and it can play an important role in the stimulation of aromatase expression of ovary cells (Katoh et al., 2006). In the ovary of sexually mature hens, both the granulosa and theca layers contain a large number of androgen receptors, but they cannot be detected in the stroma cells. The amount of androgen receptors does not change during the ovulation cycle, but their amount significantly decreases in the postovulatory follicles (Yoshimura et al., 1993). A large amount of androgen receptors are found in the ovary of the chicken, and also in the uterus (Ellis and Danzo, 1989, Kawashima et al., 1999).

The peripheral mechanisms of androgens was only studied in detail in male birds, and only sparse data are available about females (Furr, 1969, Furr, Pope, 1970). Besides testosterone, androstenedione and androstanedione have been detected from domestic chicken ovary and peripheral blood, which suggests that androgen catabolism has similar steps in females as in males: di- and tetrahydro metabolite (4androstene 3, 17-dione, 5-androstanedione) is produced, which gets converted into androsterone by hydroxilation. Suphokinase converts androsterone into water-soluble androsterone-suphate in the kidney (Péczely, 1985).

Androgens, and in particular, testosterone, play an important role in the neuroendocrine system of female birds. The role of androgens in the regulation of ovulation is disputed (and it will be discussed in detail in the section about the regulation of ovulation). While it is accepted that progesterone plays a primary trigger role, considering recent data, among other ovarian steroids the effect of testosterone seems to be crucial too. In laying hens, the administration of flutamid (androgen receptor antagonist) strongly decreased egg production, in a dose dependent manner. Additionally, the rate of increase of preovulatory plasma progesterone, estradiol and LH levels – but not that of testosterone – decreased, which suggests that inhibiting the effect of testosterone (ligand-receptor relationship) blocks ovulation-triggering hormonal processes (Rangel et al., 2006). The results of active immunisation with testosterone were similar. Immunisation blocked the typical preovulatory progesterone peak, which indicates that testosterone directly (through androgen receptors) stimulates progesterone production by the follicles, in a paracrine way. The hypothesis that the administration of testosterone increases progesterone production in the granulosa cells has been confirmed by in vitro studies. This stimulation cannot be further increased by administering LH. However, the addition of flutamide decreased progesterone production in the granulosa cells, and therefore the specific, preovulatory stimulating effect of testosterone that manifests through androgen receptors (Rangel et al., 2007).

Androgens also play a specific role in the fuctional maturation of the ovary. An estrogen plus androgen effect can be detected in both the epithelial layer of the oviduct mucosa and in the differentiation of tubular glands (Joensuu et

al., 1992). Administration of dihydrotestosterone to chickens pretreated with estrogen increased ovomucoid mRNA production in the epithelial cells without affecting ovalbumin mRNA production. However, in an in vitro system, dihydrotestosterone can increase the expression of ovalbumin and ovomucoid genes on its own, even if the effect of estrogen is blocked by tamoxifen (Compere et al., 1981). In prepubertal chickens, treatment with testosterone and 5α-dihydrotestosterone causes the increase of vaginal mass. The effect of androgen causes the strengthening of the circular and longitudinal muscles and increases the differentiation of ciliated and calyx cells in the epithelial layer of the mucosa. The specific binding of 3H-testosterone in the vaginal tissue indicates that in this area several androgen receptors express (Grau et al., 1985).

During puberty, androgens have a crucial role in the regulation of material transport processes in medullary bones. Androgen and estrogen receptors (at least some of them) are found in the osteoblast membrane, and they open membrane Ca^{2+} channels and free Ca^{2+}up from intracellular calcium pools (Armen and Gay, 2000). The results of in vitro experiments suggest that 17β-estradiol, testosterone, or the two together promote proliferation (mitogenesis), DNA synthesis and cell cycle, as well as alkaline phosphatase activity of osteoblasts. The steroid effect is manifested through the activaton of growth factors (IGF-I, TGF-β and FGF). Besides this, the androgen plus estrogen effect inhibits apoptosis of the osteoblasts, but testosterone alone results in osteoblast apoptosis (Kasperk et al., 1990, Chen et al., 2010).

Androgen receptors are found in the brain of male and female birds, mainly in the hypothalamus and in the limbic system. Their occurance in the central nervous system and their functional role will be introduced in detail in the section about the central regulation of reproductive processes.

2.1.6. The mechanism and hormonal regulation of ovulation

The process of egg and follicle maturation is a determing element of the avian reproductive cycle, which also means the coordinated functioning of the ovary and the oviduct, as part of the ovulatory-ovipositional cycle, including its complicated neuroendocrine regulation. The temporal aspects and complex hormonal regulation of the maturation of the oocyte, the ovulation, the egg formation and laying were first elucidated by Ralph and Fraps (1959a,b, 1960). Most of their more important – often indirectly obtained – observations are still valid.

The regulation of the ovulatory cycle can be characterised by the hormonal events of the whole clutch and also by the neuroendocrine changes occurring during each ovulation.

Hormonal regulation of the clutch

Within a clutch, the time between each ovulation is determined by the time it takes to form an egg (i.e., the amount of time spent in the oviduct). In domestic hen, egg forming takes about 24.5 hours, and the next ovulation occurs 30-75 minutes after laying an egg. This means that egg laying is 1-1.5 hours late every day compared to the previous day. The time of oviposition shows a species-specific daily pattern, and falls within a certain 8-9 hour period of the day. Domestic ducks lay in the second half of the night and early morning, domestic hens in the first part of the day, and Japanese quail mostly in the afternoon. Domestic geese, on the other hand, lay nearly continuously during the day. Considering the temporal connection between egg laying and ovulation, this means that ovulation falls in a determined "open period" of the 24 hours of a day, when the trigger effect can release LH. The time of the first ovulation of the clutch (C1) signifies the beginning of the open period, subsequent ovulations occur daily, delayed by a species-specific time interval, and the last ovulation (i.e., oviposition) (Ct) signifies the end of the open period. During the so-called rest days, following the last lay of the clutch, the mechanism regenerates in a way that the first ovulation of the next clutch will coincide with the beginning of the open period, and the biological regulating system (clock) of the bird will be reset to the earlier time.

With regards to hormonal regulation, the open period signifies that this is when the "sensitive period" of the neuroendocrine system occurs, which is set daily by the clock genes. This makes it possible the accurately timed positive feedback of progesterone on LH, which eventually results in the rupture of the F1 follicular wall (Figure 15.)

An important element of the functioning of the ovulatory mechanism is the accurate regulation of the timing, which aligns particular elements of the clutch with the circadian rhythm of the bird. Evidently, the LH-progesterone axis, and the metronome characteristics of the trigger effects of progesterone have a determining role in the timing of ovulation. It is not clear however, what sets the preovulatory peaks according to the circadian rhythm, i.e., where is the "biological clock" and how does it work. This clock can be central, driven by GnRH secretion, which is regulated by the neuroendocrine system, or it can be located in the periphery, as the metronome function is directly responsible for the increase in progesterone secretion of the ovary and the timing of peak value (Fraps, 1965, Péczely, 1987).

Known elements of the central biological clock that sets ovulation time are the suprachismatic nucleus of the hypothalamus, the neurons of the retina and the epiphysis. In all areas there is melatonine secretion that fluctuates according to the circadian rhytm, and there are transcription-translation dependent oscillation mechanisms at work. The so-called "clock genes", such as the Period-2

(Per2), Period-3 (Per3), as well as the Clock and Brain and muscle Arnt-like protein (Bmail-1) genes, form the base of the metronome functions (Okano et al., 2001, Yoshimura et al., 2000, Hastings et al., 2003). GnRH-I neurons of the hypothalamus are in functional connection through intermedier neurons – especially with the suprachiasmatic nucleus. Metronome functions of the brain and the clock genes responsible for its timing are going to be introduced more in detail when discussing the hypothalamo-gonadotrop system.

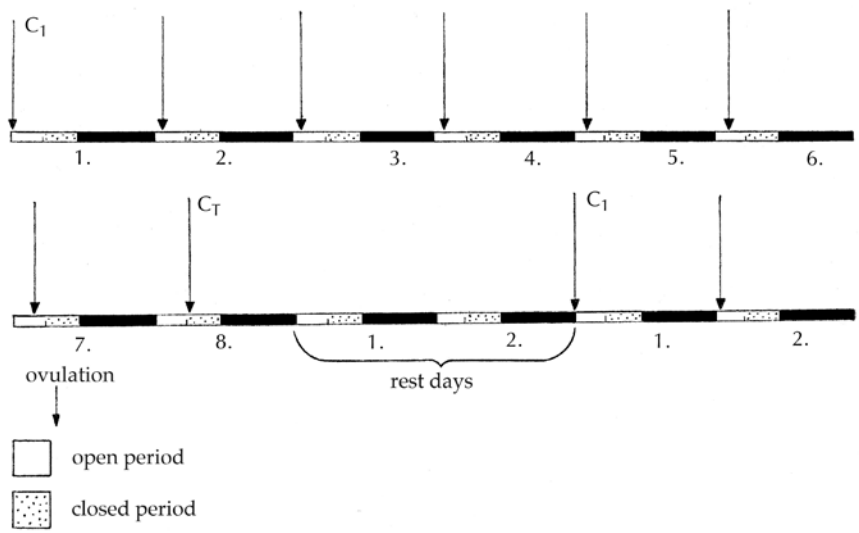

Figure 15. The relationship between the regulation of the egg laying cycle and the light period, modified after Péczely (1987)

According to recent studies, there is a peripheral biological clock in the avian ovary, which plays a role in the preovulatory regulation of progesterone synthesis (Nakao et al., 2007). Elements of the progesterone biosynthesis are well known: the VLDL receptor, responsible for the uptake of lipoprotein by the granulosa cells, the sterol-carrier protein-2 (SCP-2), responsible for intracellular cholesterol transport, steroidogenic acute regulatory protein (StAR), which transports cholesterol across the mitochondrial membrane, adrenodoxin reductase (AdRed) and adrenodoxin (Adx), which transport electrons to the mitochondrial P450, and finally p450, the enzyme that converts cholesterol into pregnenolone and 3β-hydroxysteroid dehydrogenase, which converts pregnenolone into progesterone.

Clock genes detected in the brain are also responsible for the timing of the cholesterol transport system and the regulation of progesterone synthesis in the avian ovary. All of them (like all clock genes) are E-box stimulating elements, which bind to the CLOCK/BMAIL-1 heterodimer protein and therefore enhance gene transcription. The expression of Per2 and Per3 genes in the

granulosa cells shows a circadian pattern, but only in the F1 follicle, the smaller yellow follicles lack rhythmic activity. StAR protein, a key factor in progesterone synthesis, fluctuates in way exacly like the circadian rhythm of Per2. Therefore, in the granulosa cells of F1, the Star gene-StAR protein system is the target of the impulses of the clock genes, which consequently produces a preovulatory peak in progesterone synthesis. This "peripheral type" rhythmic regulation is enforced by the central, also rhythmically regulated LH effect, which directly, as well as in an additive way, increases the expression of the StAR gene, and therefore progesterone synthesis.

Growth factors expressing in the theca layer of the follicular wall and in the oocyte itself play a role in the regulation of the amount of progesterone secreted. Bone morphogenetic protein-15 (BMP-15), a member of the TGF-β group, is synthesised in the oocyte, and it inhibits progesterone synthesis in the granulosa cells in a paracrine way. Here, after a gonadotrop effect, BMP-15 strongly inhibits the expression of StAR protein, which stimulates steroidogenesis (Elis et al., 2007). Bone morphogenetic protein-6 (BMP-6), another member of the TGF-β group, which originates in the theca, increases the amount of gonadotrop receptors in the granulosa in a paracrine way, and therefore increases LH-Star gene (and protein)-regulated progesterone secretion (Al-Musawi et al., 2007).

The hormonal regulation of the entire clutch and the end of the ovulation can be interpreted in several ways. One of the most obvious hormonal changes is the gradual decrease of the preovulatory LH peak, and also that the LH peak gets closer in time to the rupture of the follicular wall and the expulsion of the oocyte. At the same time, progesterone preovulatory maxima also gradually decrease.

The change in LH and progesterone levels suggest receptor desensitisation. This is confirmed by the decrease of the efficiency of injected LH. In the study group LH induced ovulation with a 100% efficiency in case of the first ovulation of the clutch, but only 40% of the time of the second. Another experiment investigated the effects of injected GnRH. For about six hours before C1 ovulation, the level of LH substantially increased, and the level of progesterone also increased five fold for the same time period. Before C2 ovulation the increased LH level only lasted one hour, along with a one-hour doubled level of progesterone. The in vivo results were in agreement with studies of the progesterone and LH-dependent adenyl cyclase production of isolated granulosa cells, confirming that with the progression of the ovulatory sequence, the amount of GnRH-triggered LH and the progesterone production of the LH-stimulated granulosa cells decrease. Therefore, according to the desensitisation theory, given the same intensity of GnRH secretion, the sensitivity of GnRH and LH receptors decreases, and though the subsequent ovulations within a clutch, the amount of LH and progesterone decreases, and this later, if it gets under a

threshold value, can not trigger follicular rupture. The process therefore leads to Ct ovulation and the conclusion of the clutch (Bahr and Johnson, 1984).

Other studies could not detect desensitisation in the adenohypophysis. Following Ct ovulation, it was possible to trigger a new ovulation by injecting GnRH (Reeves et al., 1973), and domestic chickens treated with a GnRH analogue for 12 days had nearly the same plasma LH level increase following each injection (Sterling and Sharp, 1984).

The regulation of the entire clutch and the timing of the Ct ovulation can also be approached from endogenous metronome functions. The theories – none of them proven – build on decreasing or increasing coincidence between hormonal peaks. We should consider three theories.

1. During individual ovulations of a clutch, the time of the follicular rupture gradually gets further away from the circadian crepuscular LH surge, which has an oscillator function, and this leads to a gradual weakening of the preovulatory LH peak (Cunningham et al., 1984).

2. The preovulatory LH peak that gets temporally shifted away also causes it to get further from the circadially regulated (depending on the current light conditions) temporally fixed morning corticosterone maximum, which upregulates the progesterone receptors of the hypothalamus and therefore makes them sensitive to the trigger mechanism. The increasing time between the high corticosterone concentration and the LH peak value can also directly decrease the progesterone production of ovarian granulosa cells locally. Therefore, the increase of the time interval between the corticosterone maximum and the preovulatory LH peak can gradually weaken central and peripheral hormonal mechanisms that lead to follicular rupture (Muray et al., 1980c., Péczely et al., 1980, Wilson and Cunningham, 1980).

3. The postovulatory increase in LH, which occurs in the next eight hours following ovulation, can also have a regulatory function over the whole clutch. As this smaller peak also occurs after Ct ovulation, it is not directly connected with the hormonal system that regulates follicular rupture, but it can be an intrinsic element of LH secretion. This high LH value during clutch laying approaches the crepuscular LH peak, and following resting days increases it by positive interference, so it can trigger a preovulatory LH peak that in turn triggers C1 ovulation to initiate a new clutch (Cunningham et al., 1984). (Figure 16.)

Hormonal regulation of individual follicular ruptures

The rupture of the follicular wall along the stigma is the complex result of several processes, in which the cascade-like activation of the gonadotrop-gonadal axis has a determining role. The growth and differentiation of the large yellow follicles (in the F5-F1 direction) is the result of the intensive FSH secretion, which along with cytoplasmic structures and mitochondrial enzyme systems,

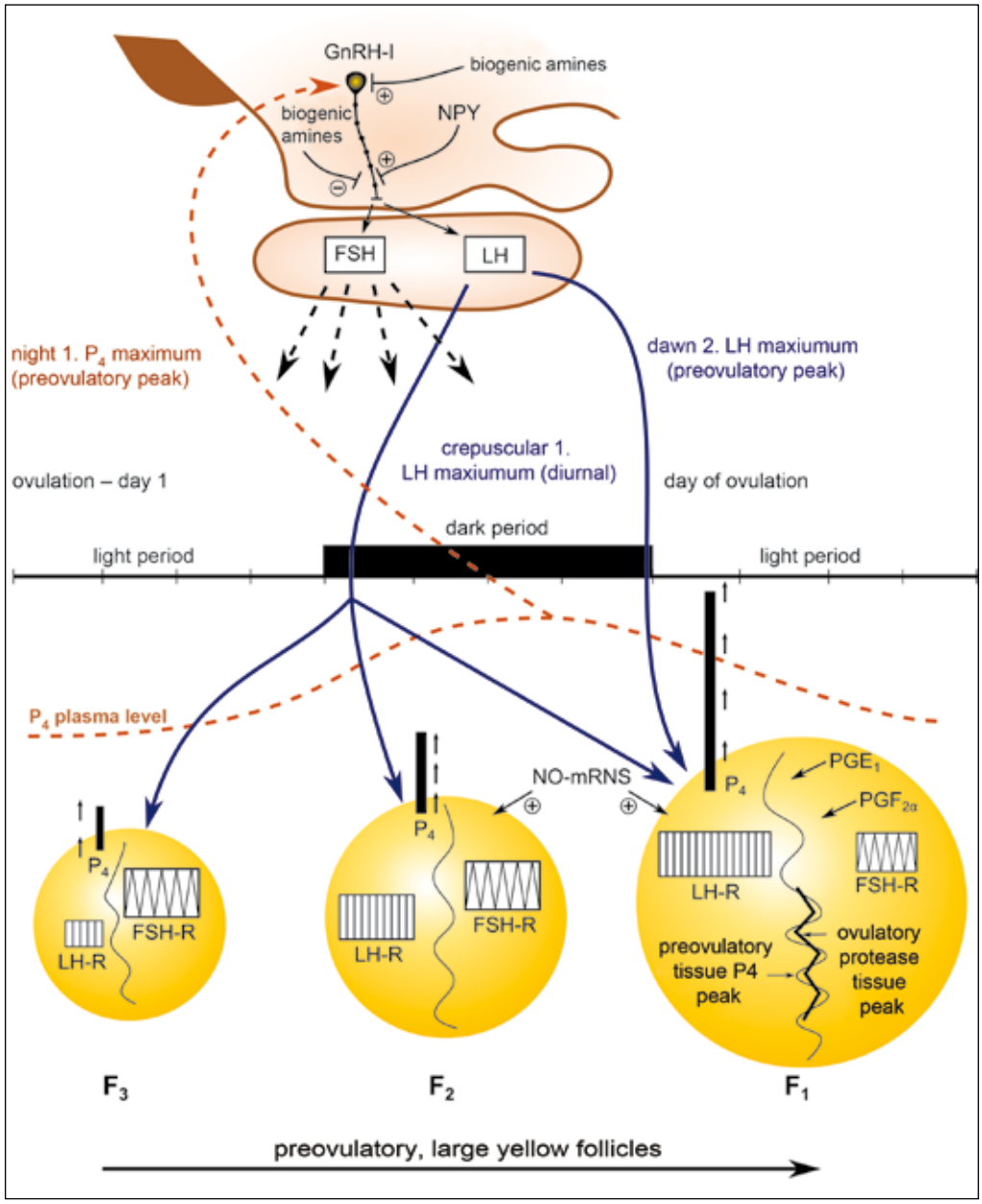

Figure 16. The hormonal regulation of follicular maturation and oocyte expulsion

increases the number of its own receptors (by upregulation) and the number of LH receptors in the preovulatory (F1) follicles. A large amount of LH receptors are characteristic of the preovulatory F1 follicle, while smaller (F3-F5) follicles have a lots of FSH and few LH receptors (Bahr and Johnson, 1984, Zhang et al., 1997, Yamamura et al., 2001). TGF-β type growth factor (bone morphogenetic

protein-6 (BMP-6)), which originates in the theca layer of the follicles, also increases the amount of LH and FSH receptors in the large yellow follicles. BMP-6 increases the amount of LH receptors in the granulosa cells of the F1 follicles and the amount of FSH receptors in the F1, F2 and F3 follicles.

The role of the inhibin-activin system in the regulation of the ovarian cycle is less known. The inhibin-A concentration of the plasma shows a maximum that coincides with the forming of the preovulatory LH peak and it reaches its lowest level about 10 hours later, when after ovulation the F2 follicle converts into the next F1. The high LH level ensures inhibin-A production in the preovulatory follicle, which maintains continuous, non-fluctuating FSH secretion (Lovell et al., 2000).

The intensive LH binding makes it possible for the F1 granulosa cells to secrete progesterone a few hours before the first ovulation of the clutch, reacting to the so-called crepuscular surge in LH level, which is light regulated, and shows circadian fluctuation with an excessively strong (30-40 times the normal value). A large proportion of the high amount of progesterone gets into the systematic circulation, causing a preovulatory progesterone peak, which is 3-5 times higher than the base level. By positive feedback (trigger) effect, the high progesterone level causes an intensive discharge of GnRH-I from the hypothalamus into the adenohypophysis, where it drastically increases LH production. The GnRH stimulus causes a preovulatory LH peak in the plasma 1-2 hours after the preovulatory progesterone peak (Knight et al., 1984). The preovulatory LH peak in the wall of the F1 follicle causes a further surge in the tissue progesterone concentration, which, as the driving force of the ovulatory mechanism, activates proteases (among others, collagenases), which act along the stigma of the follicular wall, which became thin as a result of apoptotic processes, to rupture the wall of F1. This second peak in progesterone production does not appear in the blood stream, and can only be measured in the wall of F1 (Lague et al., 1975; Tanabe et al., 1980).

The preoptic-paraventricular area of the hypothalamus and the median eminence play a determining role in the central regulation of preovulatory LH release. The axons of the GnRH neurons in the preoptic-paraventricular area terminate in the zona externa of the median eminence, in both the anterior and posterior areas. Axon terminals end on and among the glial bundles of the portal capillaries (Péczely and Kovács, 2000). 4-6 hours before ovulation (follicular rupture), during the high (preovulatory) plasma LH peak, the GnRH content of both the preoptic area and the median eminence decreases by about 50%, which confirms the intensive GnRH release and transport from the preoptic-paraventricular area. The following decrease in LH level coincides with the increase in the amount of GnRH (Knight et al., 1984).

The LH trigger effect of progesterone has been proven in laying hens: 90-180 minutes after a progesterone injection, GnRH-I content of the median

eminence (posterior hypothalamus) decreases substantially, while plasma LH level increases to a great degree (Wilson et al., 1990). Progesterone receptors are mainly located in the preoptic area. Their amount is higher in laying hens compared to non-laying individuals. During the ovulatory cycle, 21-18 hours and 6-3 hours before the next ovulation, the cytosol concentration of progesterone decreases in the preoptic area, but at the same time, the amount of receptors increases in the nuclear fraction. These changes prove the active participation of progesterone-binding sites in the preovulatory release of GnRH (Kawashima et al., 1994).

The modulation (the facilitation / inhibition ratio) of GnRH regulation occurs in the median eminence. The stimulating NPY and inhibiting β-endorphine neuron terminals that end in the zona externa influence GnRH release by axon-axon connections. In the preovulatory phase the NPY tone dominates. GnRH release-inhibiting dopamine and serotonine axon terminals also end in the median eminence. Injecting dopamine or serotonine before the expected preovulatory LH peak inhibits it from happening and blocks ovulation. The concentration of biogenic amines in the hypothalamus characteristically changes four hours before the preovulatory LH peak: in the frontal areas increases the amount of dopamine, serotonine and adrenaline, while their concentration decreases in the median eminence (Sharp et al., 1989). In case of stress (e.g., food deprivation) dopamine inhibits the ovulatory cycle at the level of median eminence (Advis and Contijoch, 1993).

The determining role progesterone plays in ovulation has been proven in several experiments. For instance, progesterone injection triggered an LH release similar to the preovulatory LH peak in intact, as well as ovariectomised domestic chickens. Progesterone antiserum treatment 14 hours before the expected ovulation prevented ovulation in intact chickens, if pregnenolone (and progesterone) synthesis was blocked with aminoglutethimide, the preovulatory LH peak did not occur. However, if treated animals were given progesterone injection, the missed gonadotropin peak was triggered, followed by normal ovulation and egg laying (Péczely, 1987).

In addition to the primary trigger effect of progesterone, other steroid hormons and tissue factors also participate in the regulation of ovulation.

The plasma level of estrogens during the ovulatory cycle fluctuates irregularly. In certain cases, these irregular estrogen peaks coincide with the preovulatory progesterone maxima, but their regular co-occurrence cannot be observed (Shababi et al., 1975; Péczely et al., 1980). Therefore estrogens can probably influence the formation of the preovulatory LH peak. This regulation also occurs by positive reinforcement, however it is not a direct trigger effect, but a priming on the hypothalamo-hypophyseal system (Wilson and Sharp, 1976). Their role is more pronounced in increasing the activity of the GnRH-LH axis and modulating the amount of progesterone and GnRH receptors during

the ovulatory cycle (Kawashima et al., 1992a,b; Kawashima et al., 1993). Preovulatory peak and ovulation still occur after treatment with E2 antiserum, as opposed to after the treatment with progesterone antiserum (Furr and Smith, 1975). Treatment with tamoxifen (synthetic estrogen receptor antagonist) did not inhibit the forming of a preovulatory LH peak (Wilson and Cunningham, 1981).

Testosterone probably also takes part in the regulation of ovulation, as suggested by earlier and more recent studies. According to earlier studies, testrosterone fluctuates more or less regularly during the ovulatory cycle (Etches and Cunningham, 1977; Péczely et al., 1980) and testosterone injection results in a preovulatory-type LH release and the rupture of follicle F1 in domestic hen (Fraps and Fevold, 1955; Croze and Etches, 1980). Treatment with an androgen receptor antagonist (cyproterone acetate) inhibits ovulation and causes ovarian regression (Luck 1982). Recent results confirm these findings, showing that blocking the effect of testosterone by passive or active immunisation inhibits ovulation (Rangel et al., 2005).

Acknowledging the important role of testosterone, the target point of ovulatory regulation is still a topic of debate. Detailed analysis of the inductive effect of ovulation showed that injecting testosterone is only effective after 9 hours, therefore its regulatory effect is more indirect, or priming, as opposed to causing direct LH release (Fraps, 1955). According to another study, it is only effective in doses so large that are physiologically relevant (Etches and Cheng, 1981), and testosterone would have a very wide spectrum of effects: treatment with flutamide, a non-steroidal androgen receptor antagonist blocks ovulation, but inhibits preovulatory increase in testosterone, estrogen, progesterone and LH in laying domestic hens.

The priming effect of testosterone on the preovulatory effect of progesterone in hypothalamo-hypophysis axis is less probable, but possibly occurs at the periphery, acting in the ovary. Testosterone synthesised in the theca would bind to the androgen receptors of the granulosa cells, stimulating their progesterone production in a paracrine way, and thereby playing a role in the regulation of ovulation. In domestic chicken, in vitro testosterone was shown to increase the production of StAR protein, P450scc and the LH receptor mRNA directly and along with them the synthesis of progesterone in the granulosa cells of the yellow follicles. On the other hand, the stimulating effect of testosterone on progesterone secretion also manifests along the LH stimulation of the granulosa cells (Rangel et al., 2006, 2007, 2009).

According to other authors, in constantly laying domestic hens, the fluctuating testosterone level shows a peak amount when the (previous) egg is in the uterus, undergoing the most intensive shell formation. The intensive phase of eggshell formation is known to be supported by androgen-estrogen synergism. According to this, the testosterone maximum that precedes the next

preovulatory progesterone peak by 8-9 hours, does not form part of the ovulation-stimulating preovulatory hormone mechanism, but belongs to the regulatory system of the shell-forming of the previous egg (Péczely et al., 1980). This hypothesis is supported by the findings of Etches et al., (1980), who found that during the preovulatory progesterone peak plasma testosterone levels reach minimum levels (Figure 17).

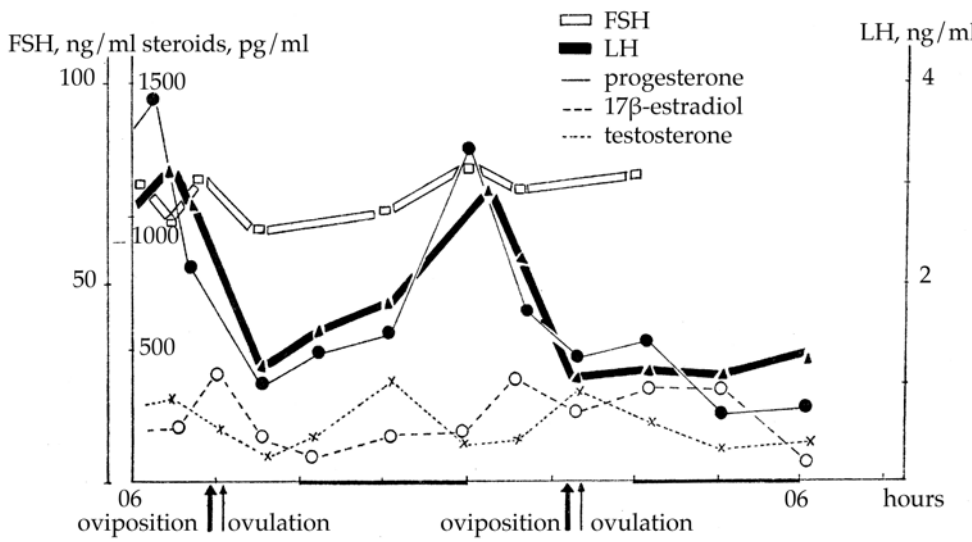

Figure 17. Hormonal changes in the ovulatory-ovipositional cycle in domestic chicken modified after Péczely (1987)

Retinoids and thyroid hormones can also play an important role in the modulation of preovulatory sexual steroid secretion. Under in vitro conditions, 9-cis retinoic acid decreases the E2 production in white and yellow follicles, while at the same time increases their progesterone secretion (Pawlowska et al., 2008). In vitro, T3 decreases basic and LH-stimulated E2 secretion in white and yellow follicles, but increases P4 levels (Sechman et al., 2009).

According to some studies, in addition to sexual steroids, actual secretion of corticosterone and its plasma levels can also play a part in the regulation of follicular maturation and in the ovulatory mechanism. The effects of in vivo and in vitro corticosterone treatment are not obvious. The injection of a large dose of corticosterone into domestic hens that had F1 follicles triggered ovulation. On the other hand, long-term intravenous application of corticosterone caused the ceasing of oocyte development and regression of the ovary (Etches et al., 1984a). When pregnenolone synthesis was inhibited by aminoglutethimide in domestic hens, leading to the ceasing of egg production, a corticosteroid injection lead to ovulation in 50% of the cases, even though neither the

progesterone, nor the LH preovulatory peak formed. Under in vitro conditions, however, corticosterone did not affect the increase in progesterone production that occurred as a result of administering LH to isolated granulosa cells (Etches et al., 1984b).

It seems more probable, that the increase in corticosterone secretion is an important regulating factor of the in whole process of rapid follicular maturation, as opposed to affecting directly the induction of ovulation. Plasma corticosterone level significantly increases along with the process of follicular maturation, and reaches its maximum level during periovulation. This can mean a synergistic effect, playing a part in the regulation of follicular morphogenesis and steroid synthesis (Wingfield and Farner, 1978; Péczely and Pethes, 1980, 1982).

Several factors are known to regulate ovulation locally, on a tissue level in the follicular wall. The role of growth factors in the development of the follicular wall and the oocytes has already been mentioned. A few hours before ovulation the amount of prostaglandine E1 and F2α concentrations significantly increase in the wall of the largest follicle. This occurs independently of the preovulatory LH peak, as LH does not affect the prostaglandine content of isolated granulosa cells under in vitro conditions, even in large doses. The trigger of their release can be an adrenerg impulse, suggested by the observation that the administration of adrenerg blockers into the F1 follicle inhibits ovulation. Prostaglandines affect the developed smooth muscle of the follicular wall, causing it to contract (Ferrando and Nalbandov, 1969; Hammond et al., 1980, Scanes et al., 1982).

In the preovulatory follicular wall nitric oxide (NO) can play a significant role in the tissue changes right before ovulation. The NO-mRNA concentration increases during the formation of F2-F1 follicles, and suddenly decreases sharply after ovulation. This observation can suggest its role in the mechanism of ovulation (Sundaresan et al., 2007).

Blood circulation in the follicular wall becomes more intensive during the days and hours before ovulation, resulting in increased intrafollicular pressure. The significantly increased intrafollicular turgor pressure together with the contraction of smooth muscle cells of the theca layer causes rupture in the follicular wall and the expulsion of the oocyte from the follicle (Péczely, 1987, Johnson et al., 2007).

The expulsion is further helped by the gradual weakening of the granulosa-oolemma connection, which results from the degradation of the transosomes in the end of the maturation-growth process of the yellow follicles. This results in a perivitelline gap and in this widening gap the amount of fluid increases (Yoshimura et al., 1993). Additionally, the degradation of the granulosa tight junction system weakens the connections among cells.

References 2/1.

1. Advis JP, Contijoch AM (1993): The median eminence as a site for neuroendocrine control of reproduction in hens Poult.Sci., 72, 932-939.
2. Al-Musawi SL, Gladwell RT, Knight PG (2007): Bone morphogenetic protein-6 enhances gonadotrophin-dependent progesterone and inhibin secretion and expression of mRNA transcripts encoding gonadotrophin receptors and inhibin/activin subunits in chicken granulosa cells Reproduction 134, 293-306.
3. Armen TA, Gay CV (2000): Simultaneous detection and functional response of testosterone and estradiol receptors in osteoblast plasma membranes J.Cell Biochem., 79, 620-627.
4. Asem EK, Feng S, Stingley-Salazar SR, Turek JJ, Peter AT, Robinson JP (2000): Basal lamina of avian ovarian follicle: influence on morphology of granulosa cells in vitro Comp.Biochem. Physiol., C. 125, 189-201.
5. Bahr JM, Johnson AL (1984): Regulation of the follicular hierarchy and ovulation J.Exp.Zool., 232, 495-500.
6. Bahr JM, Wang SC, Huang MY, Calvo FO (1983): Steroid concentrations in isolated theca and granulosa layers of preovulatory follicles during the ovulatory cycle of the hen Biol.Reprod., 29, 326-344.
7. Bar A, Striem S, Mavel-Afshar S, Lawson DE (1990): Differential regulation of calbindin-DK28K mRNA in the intestine and eggshell gland of the laying hen J.Mol.Endocr., 4, 93-99.
8. Barua A, Michiue H, Yoshimura Y (2001): Changes in the localization of MHC class II positive cells in hen ovarian follicles during the process of follicular growth, postovulatory regression and atresia Reproduction 953-957.
9. Best-Belpomme M, Mester J, Weintraub H, Baulieu EE (1975): Oestrogen receptors in chick oviduct. Characterization and subcellular distribution Eur.J.Biochem., 57, 537-547.
10. Board RG, Hornsey DJ (1978): Chemical Zoology, vol.10, 37. (eds.: M.Florkin, BT Scheer) Acad. Press New York – London
11. Bridgham JT, Wilder JA, Hollocher H, Johnson AL (2003): All in the family: evolutionary and functional relationships among death receptors Cell Death Differ., 10, 19-25.
12. Brown KI, Long DW, Bacon WL, Braselton WE (1979): Evidence for the presence of 15-hydroxylated estrogens in the peripheral plasma of the laying turkey Gen.Comp.Endocr., 39, 552-560.
13. Brubaker KD, Gay CV (1999): Estrogen stimulates protein tyrosine phosphorilation and Src kinase activity in avian osteoclasts J.Cell.Biochem., 76, 206-216.
14. Bruce KR, Anastassiadis PA (1977): Connective tissue constituens of the fowl. Effects of exogenous estrogen Poult.Sci., 56, 1073-1085.
15. Bryndová J, Klusonová P, Kucka M, Mazancová-Vagnerová K, Miksik I, Pacha J (2006): Cloning and expression of chicken 20-hydroxysteroid dehydrogenase J.Mol.Endocr., 37, 453-462.
16. Camacho-Arrovo I, Gonzalez-Arenas A, Gonzalez-Morán G (2007): Ontogenic variations in content and distribution of progesterone receptor isoforms in the reproductive tract and brain of chicks Comp.Biochem.Physiol.A Mol.Integr.Physiol. 146, 644-652.
17. Carlson JL, Bakst MR, Ottinger MA (1996): Developmental stages of primary oocytes in turkeys Poultry Sci., 75, 1569-1578.
18. Charlier TD, Balthazart J (2005): Modulation of hormonal signaling in the brain by steroid receptor coactivators Rev.Neurosci., 16, 339-357.
19. Charlier TD, Lakaye B, Ball GF, Balthazart J (2002): Steroid receptor coactivator SRC-1 exhibits high expression in steroid sensitive brain areas regulating reproductive behaviours in the quail brain Neuroendocr., 76, 297-315.
20. Chen X, Deng Y, Zhou Z, Tao Q, Zhu J, Li X, Chen J, Hou J (2010): 17beta-estradiol combined with testosterone promotes chicken osteoblast proliferation and differentiation by accelerating the cell cycle and inhibiting apoptosis in vitro Vet.Res.Commun., 34, 143-152.
21. Cho BK, Park JR (1990): Estrogen induces hyperlipidemia in fasted chicks Proc.Soc.Expl.Biol. Med., 193, 104-109.
22. Compere SL, McKnight GS, Palmiter RD (1981): Androgens regulate ovomucoid and ovalbumin gene expression independently of estrogen J.Biol.Chem., 256, 6341-6347.

23. Costagliola A, De Man JG, Majewski M, Lakomy M, Cecio A, Robberecht P, Pelckmans PA, Adriaensen D, Timmermans JP (2004): Coexistenceof non-adrenergic non-cholinergic inhibitory and excitatory neurotransmitters in a large neuronal subpopulation int he vaginal segment of the chicken oviduct Auton.Neurosci., 31, 112 37–48.
24. Croze F, Etches RJ (1980): The physiological significance of androgen-induced ovulation in the hen J.Endocr., 84, 163-171.
25. Cunningham FJ, Wilson SC, Knight PG, Gladwell RT (1984): Chicken ovulation cycle J.Exp.Zool., 232, 485-494.
26. Das SC, Nagasaka N, Yoshimura Y (2006): Changes in the expression of estrogen receptor mRNA in the utero-vaginal junction containing sperm storage tubules in laying hens after repeated artificial insemination Theriogenology 65, 893-900.
27. Denner LA, Weiger NL, Maxwell BL, Schrader WT, O'Malley BW (1990): Regulation of progesterone receptor-mediated transcription by phosphorylation Science 21, 1740-1743.
28. Deviche P, Breuner C, Orchinik M (2001): Testosterone, corticosterone, and photoperiod interact to regulate plasma levels of binding globulin and free steroid hormone in dark eyed juncos, Junco hyemalis Gen.Comp.Endocr., 122, 67-77.
29. Ding ST, Yen CF, Wang PH, Lin HW, Hsu JC, Shen TF (The differential expression of hepatic genes between prelaying and laying geese Poult.Sci., 86, 1206-1212.
30. Elis S, Dupont J, Couty I, Persani L, Govoroun M, Blesbois E, Batellier F, Monget P (2007): Expression and biological effects of bone morphogenetic protein-15 in the hen ovary J.Endocr., 194, 485-497.
31. Ellis DL, Danzo BJ (1989): Identification of an androgen receptor in the adult chicken oviduct J.Steroid Biochem., 33, 1081-1086.
32. Etches RJ, Cheng KW (1981): Changes in the plasa concentrations of luteinizing hormone, progesterone, oestradiol and testosterone and in the binding of follicle-stimulating hormone to the theca of follicles during the ovulation cycle of the hen (Gallus domesticus) J.Endocr., 91, 11-22.
33. Etches RJ, Croze F, Duke CE (1980): Plasma concentrations of luteinizing hormone, progesterone, testosterone and estradiol in follicular and peripheral venous plasma during the ovulation cycle of the hen In: Recent advances of avian Endocrinology, (eds.: G.Pethes, P.Péczely, P.Rudas) Pergamon Press – Akadémiai Kiadó Budapest 89-98.
34. Etches RJ, Cunningham FJ (1977): The plasma concentrations of testosterone and LH during the ovulation cycle of the hen (Gallus domesticus) Acta Endocr.,(Copenhagen) 84, 357-366.
35. Etches RJ, Petitte JN, Anderson-Langmuir CE (1984a): Interrelationships between the hypothalamus, pituitary gland, ovary, adrenal gland, and the open period for LH release in the hen (Gallus domesticus) J.Exp.Zool., 232, 501-511.
36. Etches RJ, Williams JB, Rzasa J (1984b): Effects of corticosterone and dietary changes in the hen on ovarian function, plasma LH and steroids and the response to exogenous LH-RH J. Reprod. Fertil., 70, 121-130.
37. Ferrando G, Nalbandov AV (1969): Direct effect on the ovary on the adrenergic blocking drug dibenzyline Endocrinol., 85, 38-42.
38. Forgó V, Afanasiev GD, Péczely P (1988a): Structural and hormonal changes during follicular maturation in the ovary of domestic goose Acta Biol.Hung., 39, 403-417.
39. Forgó V, Sass M, Péczely P (1988b): Light microscopic, enzyme biochemical and steroid analytical investigations of follicular atresia in the ovary of domestic goose Acta Biol.Hung., 39, 377-401.
40. Fraps RM (1955): Egg production and fertility in poultry. In: Progress in the physiology of farm animals (ed.: J.Hammond) Vol.2., 661-710.
41. Fraps RM (1965): Twenty-four-hour periodicity in the mechanism of pituitary gonadotropin release for follicular maturation and ovulation in the chicken Endocrinol., 77, 5-11.
42. Fraps RM, Fevold HL (1955): Delaying action of gonadotrophins on ovulation in the hen Proc. Soc.Exp.Biol.Med., 90, 440-446.
43. Furr BJ, Pope GS (1970): Identification of cholesterol, 7-oxocholesterol, pregnenolone, progesterone, 20-hydroxypregn-4-en-one epimers and 5-beta-androstane-3,17-dione in plasma and ovarian tissue of the domestic fowl Steroids 16, 471-485.
44. Furr BJ, Smith GK (1975): Effects of antisera against gonadal steroids on ovulation in the hen Gallus domesticus J.Endocr., 66, 303-304.

45. Furr BJA (1969): Identification of steroids in the ovaries and plasma of laying hens and the site of production of progesterone in the ovary Gen.Comp.Endocr., 13, 506.
46. Furr BJA, Pope GS (1970): Identification of cholesterol, 7-oxocholesterol, pregnenolone, progesterone, 20-hydroxypregn-4-en-3-one epimers and 5-beta-androstane-3, 17-dione in plasma and ovarian tissue of the domestic fowl Steroids 16, 471-485.
47. Ghosh-Dastidar P, Coty WA, Griest RE, Woo DD, Fox CF (1984): Progesterone receptor subunits are high-affinity substrates for phosphorilation by epidermal growth factor receptor Proc.Natl. Acad.Sci.USA 81, 1654-1658.
48. Gilbert AB (1971): Physiologie and biochemistry of the domestic fowl (Eds.: DJ Bell, BM Freeman) Vol., 3. Acad.Press New York – London
49. Gilbert AB, Perry MM, Waddington D, Hardie MA (1983): Role of atresia in establishing the follicular hierarchy in the ovary of the domestic hen (Gallus domesticus) J.Reprod.Fert., 69, 221-227.
50. Grau Y, Reichhart JM, Thiebold J (1985): Growth stimulation of the immature chick oviduct by androgens: the vagina as a new target tissue J.Embryol.Exp.Morphol., 85, 81-93.
51. Hammond RW, Olson DM, Frenkel RB, Biellier HV, Hertelendy F (1980): Prostaglandins and steroid hormones in plasma and ovarian follicles during the ovulation cycle of the domestic hen (Gallus domesticus) Gen.Comp.Endocr., 42, 195-202.
52. Hastings MH, Reddy AB, Garabette M, King VM, Chahad-Ehlers S, O'Brien J, Maywood ES (2003): Expression of clock gene products in the suprachiasmatic nucleus in relation to circadian behaviour Novartis Found.Symp., 253, 203-217.
53. Hertelendy F, Asem EK (1984): Steroidogenesis in granulosa cells during follicular maturation: evidence for desenzitization-resenzitization during the ovulatory cycle J.Exp.Zool., 232, 513-520.
54. Hora J, Gosse B, Rasmussen K, Spelsberg TC (1986): Estrogen regulation of the biological activity of the avian oviduct progesterone receptor and its ability to induce avidin Endocrinol.,119, 1118-1125.
55. Hrabia A, Wilk M, Rzasa J (2008): Expression of alpha and beta estrogen receptors in the chicken ovary Folia Biol (Krakow) 56, 187-191.
56. Huang ES, Nalbandov AV (1979): Testosterone synthesis by chicken follicular cells Adv.Exp.Med. Biol., 112, 197-202.
57. Hummel S, Lynn EG, Osanger A, Hirayama S, Nimpf J, Schneider WJ (2003): Molecular characterization of the first avian LDL receptor: role in sterol metabolism of ovarian follicular cells J.Lipid Res.,44, 1633- 1642.
58. Jaccoby S, Amon E, Snapir N, Robinzon B (1995): Effects of estradiol and tamoxifen on feeding, fattiness and some endocrine criteria in hypothalamic obese hens Pharmacol.Biochem.Behav., 50, 55-63.
59. Joensuu TK, Blauer MK, Sannisto T, Tuochima PJ (1992): The effect of dihydrotestosterone on the estrogen-induced cytodifferentiation of the chick oviduct J.Steroid Biochem.Mol.Biol., 41, 701-709.
60. Joensuu TK, Ylikomi TJ, Toft DO, Keinanen RA, Kulomaa MS, Tuohimaa PJ (1990): Progesterone-induced avidin as a marker of cytodifferentiation in the oviduct: comparison to ovalbumin Endocrinol., 126, 1143-1155.
61. Johnson AL, Solovieva EV, Bridgham JT (2002): Relationship between steroidogenic acute regulatory protein expression and progesterone production in hen granulosa cells during follicle development Biol.Reprod., 67, 1313-1320.
62. Johnson AL, Woods DC (2007): Ovarian dynamics and follicle development, In: Reproductive biology and phylogeny of birds (Ed.: BGM Jamieson Science Publishers, Enfield, Jersey, Plymouth 6A, 243-278.
63. Johnson AL, Woods DC (2009): Dynamics of avian ovarian follicle development: cellular mechanisms of granulosa cell differentiation Gen.Comp.Endocr., 163, 12-17.
64. Jones CS, Manning R (1987): Glucose kinetics in the oestrogen-treated male chicken (Gallus domesticus), measured after administration of (6-3H) glucose in vivo Int.J.Biochem., 19, 475-478.
65. Kang B, Guo JR, Yang HM, Zhou RJ, Liu ZX, Li SZ, Dong CY (2009): Differential expression profiling of ovarian genes in prelaying and laying geese Poult.Sci., 88, 1975-1983.
66. Kasperk C, Fitzsimmons R, Strong D, Mohan S, Jennings J, Wergedal J, Baylink D (1990): Studies of the mechanism by which androgens enhance mitogenesis and differentation in bone cells J.Clin. Endocr.Metab., 71, 1322-1329.

67. Kato M, Shimada K, Saito N, Noda K, Ohta M (1995): Expression of P450, 17 alpha-hydroxylase and P450-aromatase genes in isolated granulosa, theca interna, and theca externa layers of chicken ovarian follicles during follicular growth Biol.Reprod., 52, 405-410.
68. Katoh H, Ogino Y, Yamada G (2006): Cloning and expression analysis of androgen receptor gene in chicken embryogenesis FEBS Lett., 580, 1607-1615.
69. Kawashima M, Kamiyoshi M, Tanaka K (1993): Estrogen receptor binding in the hen hypothalamus and pituitary during the ovulatory cycle Poult.Sci., 72, 839-847.
70. Kawashima M, Kamiyoshi M, Tanaka K (1994): Changes in progesterone receptor binding of preoptic hypothalamus during an ovulatory cycle of the hen Poult.Sci., 73, 855-863.
71. Kawashima M, Takahashi T, Kondo S, Yasuoka T, Ogawa H, Tanaka K (1999): Identification of an androgen receptor within the uterus of the domestic fowl Poult. Sci., 78, 107-113.
72. Kawashima M, Takeo H, Kamiyoshi M, Tanaka K (1992): Luteinizing hormone-releasing hormone receptor bindings of the hen pituitary: difference between laying and nonlaying hens, effects of ovarian steroid hormones in vivo, and changes during an ovulatory cycle Poult.Sci., 71, 1079-1086.
73. Kawashima M, Ukai A, Kamiyoshi M, Tanaka K (1992): Effect of progesterone in vitro on luteinizing hormone production in hen pituitary cells pretreated with estrogen Poult.Sci., 71, 1215-1220.
74. Kim NS, Shen YN, Kim TY, Byun SJ, Jeon IS, Kim SH (2007): Expression of AGR-2 in chicken oviduct during laying period J.Biochem.Mol.Biol., 40, 212-217.
75. Knight PG, Glister C (2006): TGF-beta superfamily members and ovarian follicle development Reproduction, 132,191-206.
76. Knight PG, Wilson SC, Gladwell RT, Cunningham FJ (1984): Hypothalamic contents of LHRH and cathecholamines during the ovulatory cycle of the hen (Gallus domesticus) J.Reprod.Fertil., 71, 289-296.
77. Kovács J, Forgó V, Péczely P (1992): The fine structure of the follicular cells in growing and atretic ovarian follicles of the domestic goose Cell Tissue Res.,267, 561-569.
78. Kowalski KI, Tilly JL, Johnson AL (1991): Cytochrome P450 side-chain cleavage (P450scc) in the hen ovary I. Regulation of P450scc messenger RNA levels and steroidogenesis in the theca cells of developing follicles Biol.Reprod., 45, 955-966.
79. Krust A, Green S, Argos P, Kumar V, Walter P, Bomert JM, Chambon P (1986): The chicken oestrogen receptor sequence: homology with v-erbA and the human oestrogen and glucocorticoid receptors EMBO J. 5, 891-897.
80. Krzysik-Walker SM, Ocon-Grove OM, Maddineni SB, Hendricks GL, Ramachandran R (2007): Identification of calcitonin expression in the chicken ovary: influence of follicular maturation and ovarian steroids Biol.Reprod., 77, 626-635.
81. Lague PC, van Tienhoven A, Cunningham FJ (1975): Concentrations of estrogens, progesterone and LH during the ovulatory cycle of the laying chicken (Gallus domesticus) Biol.Reprod., 12, 590-598.
82. Lee BK, Kim JS, Ahn HJ, Hwang JH, Kim JM, Lee HT, An BK, Kang CW (2010): Changes in hepatic lipid parameters and hepatic messenger ribonucleic acid expression following estradiol administration in laying hens (Gallus domesticus) Poult. Sci., 89, 2660-2667.
83. Lee KA, Volentine KK, Bahr JM (1998): Two steroidogenetic pathways present in the chicken ovary: theca layer prefers delta 5 pathway and granulosa layer prefers delta 4 pathway Domest. Anim.Endocr., 15, 1-8.
84. Leszcinszki DE, Pikul J, Kummerow FA (1987): Lipid oxidation in plasma and tissues from estrogenized chicken hens Biochem.Med.Metab.Biol., 38, 182-189.
85. Li Z, Johnson AL (1993): Regulation of cholesterol side chain clevage messenger ribonucleic acid expression and progesterone production in hen granulosa cells Biol.Reprod., 49, 463-469.
86. Lin CT, Chan L (1981): Estrogen regulation of yolk and non-yolk protein synthesis in the avian liver. An immunocytochemical study Differentiation, 18, 105-114.
87. Lipar JL, Ketterson ED, Nolan V, Casto JM (1999): Egg yolk layers vary in the concentration of steroid hormones in two avian species Gen.Comp.Endocr., 115, 220-227.
88. Lofts B, Murton RK (1973): Avian Biology (eds.: DS Farner, JR King, KC Parker) vol.3., Acad.Press New York – London
89. London SE, Clayton DF (2010): Genomic and neural analysis of the estradiol-synthetic pathway in the zebra finch BMC Neurosci., 11, 46.

90. Lopez-Berjes MA, Recio JM, Planas J (1981): Plasma variation of transferrin- iron and phosvitin-iron during the laying period in chicken hens Poult.Sci., 60, 1951-1956.
91. Lovell TM, Vanmontfort D, Bruggeman V, Decuypere E, Groome NP, Knight PG, Gladwell RT (2000): Circulating concentrations of inhibin-related proteins during the ovulatory cycle of the domestic fowl (Gallus domesticus) and after induced cessation of egg laying J.Reprod.Fert., 119, 323-328.
92. Luck MR (1982): Effects of an antiandrogen in the laying hen (Gallus domesticus) J.Reprod.Fertil., 64, 381-385.
93. Lundholm CE (1992): Progesterone stimulates prostaglandin synthesis in eggshell gland mucosa of estrogen primed chickens Comp.Biochem.Physiol. B 103, 217-220.
94. Mac Lahlan I, Nimpf J, Schneider WJ (1994): Avian riboflavin binding protein binds to lipoprotein receptors in association with vitellogenin J.Biol.Chem., 269, 24127-24132.
95. Maddineni SR, Ocon-Grove OM, Krzysik-Walker SM, Hendricks GR, Ramachandran R (2008): Gonadotropin-inhibitory hormone (GnIH) receptor gene is expressed in the chicken ovary: potential role of GnIH in follicular maturation Reprod., 135, 267-274.
96. Malewska A, Olszcanska B (1999): Accumulation and localisation of maternal RNA in oocytes of japanese quail Zygote, 7, 51-59.
97. Mashaly MM, Wentworth BC (1979): Half-life of plasma progesterone in turkey layers and non-layers 58, 971-975.
98. Meijer T, Drent R (1999): Re-examination of the capital and incomedichotomy in breeding birds Ibis, 141, 399-414.
99. Mermelstein PG (2009): Membrane-localised oestrogen receptor alpha and beta influence neuronal activity through activation of metabotropic glutamate receptors J.Neuroendocr., 21, 257-262.
100. Mosselman S, Polman J, Dijkema R (1996): ER beta: identification and characterization of a novel human estrogen receptor FEBS Lett., 392, 49-53.
101. Muray T, Pethes Gy, Péczely P (1980): Changes of plasma progesterone and testosterone level in chicken (Gallus domesticus) from the first day of life to the beginning of oviposition Acta Vet. Acad.Sci.Hung., 28, 97-102.
102. Murton RK, Westwood NJ (1977): Avian Breeding Cycles Clarendon Press, Oxford
103. Muscarella DE, Rachlinski MK, Bloom SE (1998): Expression of cell death regulatory genes and limited apoptosis induction in avian blastodermal cells Mol.Reprod.Dev., 51, 130-142.
104. Müller W, Eising CM, Dijkstra C, Groothuis TG (2002): Sex differences in yolk hormones depend on maternal social status in leghorn chickens (Gallus gallus domesticus) Proc.biol.Sci., 269, 2249-2256.
105. Nakao N, Yasuo S, Nishimura A, Yamamura T, Watanabe T, Anraku T, Okano T, Fukada Y, Sharp PJ, Ebihara S, Yoshimura T (2007): Circadian clock gene regulation of steroidogenic acute regulatory protein gene expression in preovulatory ovarian follicles Endocrinol., 148, 3031-3038.
106. Navickis RJ, Katzenellenbogen BS, Nalbandov AV (1979): Effects of the sex steroid hormones and vitaminD3 on calcium-binding protein sin the chick shell gland Biol.Reprod., 21, 1153-1162.
107. Nitta H, Yoshio Y, Bahr JM (1991): Multiple steroidogenic cell populations in the thecal layer of preovulatory follicles of the chicken ovary Endocr., 129, 2033-2040.
108. Ohkubo T, Tanaka M, Nakashima K (2000): Structure and tissue distribution of chicken leptin receptor (cOb-R) mRNA Biochim.Biophys. Acta 25, 303-308.
109. Oka T, Schimke RT (1969): Interaction of estrogen and progesterone in chick oviduct development. I. Antagonistic effect of progesterone on estrogen-induced proliferation and differentiation of tubular gland cells J.Cell Biol., 41, 816-831.
110. Oka T, Schimke RT (1969): Interaction of estrogen and progesterone in chick oviduct development. II. Effects of estrogen and progesterone on tubular gland cell function J.Cell Biol., 43, 123-137.
111. Okano T, Yamamoto K, Okano K, Hirota T, Kasahara T, Sasaki M, Tanaka Y, Fukada Y (2001): Chicken pineal clocks genes: implication of BMAL2 as a bidirectional regulator in circadian clock oscillation Genes cells, 6, 825-836.
112. Ozon E (1972): Estrogens in fishes, amphibians, reptiles and birds. In: D.R.Idler (ed.) Steroids in non –mammalian Vertebrates 328-389. Acad.Press New York
113. Palmiter RD, Wrenn JT (1971): Interaction of estrogen and progesterone in chick oviduct development. 3. Tubular gland cell cytodifferentiation J.Cell Biol., 50, 598-615.

114. Park JR, Cho BH (1988): Changes in plasma lipids, lipoproteins, triglyceride secretion and removal in chicks with estrogen implants Lipids 23, 327-333.
115. Park JR, Cho BH (1990): Effects of estrogen on very-low-density lipoprotein triacylglicerol metabolism in chicks Biochim.Biophys.Acta 1045, 180-186.
116. Pascoe D, Oursier MJ (2001): The Src signaling pathway regulates osteoclast lysosomal enzyme secretion and is rapidly modulated by estrogen J.Bone Miner.Res., 16, 1028-1036.
117. Pawlowska K, Sechman A, Suchanek I, Grzegorzewska A, Rzasa J (2008): Effect of 9-cis retinoic acid (RA) on progesterone and estradiol secretion and RA receptor expression in the chicken ovarian follicles Folia Biol. (Krakow) 56, 65-72.
118. Pearce J, Balnave D (1976): The effects of estradiol administration of the hepatic activities of some enzymes of carbohydrate, aminoacid and lipid metabolism in hte immature pullet Horm.Metab. Res., 8, 181-183.
119. Péczely P (1985): The role of thyroid and adrenal cortical hormones in the modulation of the gonadal function in birds Acta Biol.Hung., 36, 45-70.
120. Péczely P (1987): A madarak szaporodásbiológiája, Mezôgazdasági Kiadó, Budapest, 11 – 46.
121. Péczely P, Kovács KJ (2000): Photostimulation affects gonadotropin-releasing hormone immunoreactivity and activates a distinct neuron population in the hypothalamus of the mallard Neurosci.Lett., 290, 205-208.
122. Péczely P, Ladjánszky V, Biczó A, Szőke Zs, Pintér O, Kelemen K, Végi B (2004): Dehydroepiandrosterone (DHEA): it's possible role inPorter TE, Hargis BM, the avian annual cycles Abstr.8th. Intern. Symp.Avian Endocr., Scottsdale 95.
123. Péczely P, Pethes Gy (1979): Alterations in pkasma sexual steroid concentrations in the collared dove (Sterptopelia decaocto) during sexual maturation and reproductive cycle Acta Physiol. Acad. Sci.Hung., 54, 161-170.
124. Péczely P, Pethes Gy (1980): Interrelation of gonad-thyroid and adrenal function of Japanese quail Abst., XXVIII.Intern.Congr.Physiol.Sci., Budapest 2548.
125. Péczely P, Pethes Gy (1982): Pajzsmirigy hormonok és kortikoszteron hatása a szexuál szteroidok perifériás metabolizmusára japánfürjön MÉT XLVII. Vándorgyûlésének Elôadáskivonatai, Pécs 84.
126. Péczely P, Pethes Gy (1982): Seasonal cycle of gonadal, thyroid and adrenocortical function in the rook (Corvus frugilegus) Acta Physiol.Acad Sci. Hung., 59,59-73.
127. Péczely P, Pethes Gy, Szelényi Z, Muray T (1980): Variations in plasma level of sexual steroids during the ovipostion cycle in laying hens (Gallus domesticus) Acta Vet.Acad.Sci.Hung., 28, 103-108.
128. Pederson L, Kremer M, Foged NT, Winding B, Ritchie C, Fitzpatrick LA, Oursier MJ (1997): Evidence of a correlation of estrogen receptor level and avian osteoclast estrogen responsiveness J.Bone Miner.Re., 12, 742-752.
129. Pike TW, Petrie M (2005): Offspring sex ratio is related to paternal train elaboration and yolk corticosterone in peafowl Biol.Lett., 1, 204-207.
130. Porter TE, Hargis BM, Silsby JL, el Halawani ME (1989): Differential steroid production between theca interna and theca externa cells: a three cell model for follicular steroidogenesis in avian species Endocr., 125, 109-116.
131. Porter TE, Hargis BM, Silsby JL, el Halawani ME (1991): Characterization of dissimilar steroid productions by granulosa, theca interna and theca externa cells during follicular maturation in the turkey (Meleagris gallopavo) Gen.Comp.Endocr., 84, 1-8.
132. Qin X, Klandorf H, Porter DW, Holt SB, Martin WG (1993): Estrogen enhancement of CA-,Mg-, and CA-Mg-stimulated adenosine triphosphate activity in the chick shell galnd Gen.Comp.Endocr., 89, 4-10.
133. Quin X, Klandorf H (1993): Effect of estrogen in relation to dietary vitamin D3 and calcium on activity of intestinal alkaline phosphatase and Ca-ATP-ase in immature chicks Gen.Comp.Endocr., 90, 318-327.
134. Ralph CL, Fraps RM (1959a): Effect of hypothalamic lesions on progesterone–induced ovulation in the hen Endocrinol., 65, 819-824.
135. Ralph CL, Fraps RM (1959b): Long-term effects of diencephalic lesions on the ovary of the hen Am. J. Physiol., 197, 1279-1283.

136. Ralph CL, Fraps RM (1960): Induction of ovulation in the hen by injection of progesterone into the brain Endocrinol., 66, 269-272.
137. Rangel PL, Lassala A, Gutierrez CG (2005): Testosterone immunization blocks the ovulatory process in laying hens without affecting ovarian follicular development Anim.Reprod.Sci., 86, 143-151.
138. Rangel PL, Rodriguez A, Gutierrez CG (2007): Testosterone directly induces progesterone production and interacts with physiological concentrations of LH to increase granulosa cell progesterone production in laying hens (Gallus domesticus) Anim.Reprod.Sci., 102, 56-65.
139. Rangel PL, Rodriguez A, Rojas S, Sharp OJ, Gutierrez CG (2009): Testosterone stimulates progesterone production and STAR, P450-cholesterol side-chain céevage and LH receptor mRNAs expression in hen granulosa cells Reproduction 138, 961-969.
140. Rangel PL, Sharp PJ, Gutierrez CG (2006): Testosterone antagonist (flutamide) blocks ovulation and preovulatory surges of progesterone, luteinizing hormone and oestradiol in laying hens Reproduction, 131, 1109-1114.
141. Ratnasabapathy R (1995): In vitro characterization of an estrogen-regulated mRNA stabilizing activity in the avian liver Cell.Mol.Biol.Res., 41, 583-594.
142. Reeves JJ, Harrison PC, Casey JM (1973): Ovarian development and ovulation in hens treated with synthetic (porcine) luteinizing hormone releasing hormone-follicle stimulating hormone releasing hormone (LH-RH-FSH-RH) Poult.Sci., 52, 1883-1886.
143. Robinson FE, Etches RJ, Anderson-Langmuir CE, Burke WH, Cheng KW, Cunningham FJ, Ishii S, Sharp PJ, Talbot RT (1988): Steroidogenic relationships on gonadotrophin hormones in the ovary of the hen (Gallus gallus) Gen.Comp.Endocr., 69, 455-466.
144. Rusinol AE, Bloj B (1989): Estrogen treatment increases phospholipid transfer activities in chicken liver J.Biol.Chem., 264, 6612–6614.
145. Salvante KG, Lin G, Walzem RL, Williams TD (2007): Characterization of very-low density lipoprotein particle diameter dynamics in relation to egg production in a passerine bird J.Exp. Biol., 210, 1064-1074.
146. Salvante KG, Williams TD (2002): Vitellogenin dynamics during egg-laying: daily variation, repeatability and relationship with egg-size J.Avian Biol., 33, 391-398.
147. Sanden C, Broselid S, Commark L, Andersson K, Daszkiewicz-Nilsson J, Martensson UE, Olde B, Leeb-Lundberg LM (2011): G protein coupled estrogen receptor 1 (GPER1)/GPER30 localizes in the plasma mebrane and trafficks intracellularly on cytokeratin intermediate filaments Mol. Pharmacol., 79, 400-410.
148. Sato K, Ohuchi A, Sato T, Schneider WJ, Akiba Y (2007): Molecular characterization and expression of the cholesteryl ester transfer protein gene in chickens Comp.Biochem.Physiol. B Biochem.Mol. Biol.148,117-123.
149. Savu L, Zouaghi H, Gérard A, Nunez EA (1986): High-affinity binding of testosterone in serum from normal developing chick embryos and during the grafz-versus-host reaction Biochim.Biophys. Acta 881, 15-21.
150. Scanes CG, Mozelic H, Kavanagh E, Merrill G, Rabii J (1982): Distribution of blood flow in the ovary of domestic fowl (Gallus domesticus) and changes after prostaglandin F-2 alpha treatment J.Reprod.Fertil., 64, 227-231.
151. Schneider WJ (1996): Vitellogenin receptors: oocyte-specific members of the low-density lipoprotein receptor supergene family Int.Rev.Cytol., 166, 103-137.
152. Schneider WJ, Carroll R, Severson DL, Nimpf J (1990): Apolipoprotein VLDL-II inhibits lipolysis of trigliceride-rich lipoproteins in the laying hen J.Lipid Res., 31, 507-513.
153. Schrader WT, O'Malley BW (1978): Structure of chick progesterone receptors Cancer Res., 38, 4199-4203.
154. Schrader WT, Sharon S, Heuer S, O'Malley BW (1975): Progesterone receptors of chick oviduct: identification of 6S receptor dimers Biol.Reprod., 12, 134-142.
155. Schuster MK, Schmierer B, Shkumatava A, Kucher K (2004): Activin A and follicle stimulating hormone control tight junctions in avian granulosa cells by regulating occluding expression Biol. Reprod., 70, 1493-1499.
156. Schwabl H (1996): Environment modifies the testosterone levels of a female bird and its eggs J.Expl.Zool., 276, 157-163.

157. Schwabl H. (1993): Yolk is a source of maternal testosterone for developing birds Proc.Natl.Acad. Sci,USA 90, 11446-11450.
158. Seal US, Doe RP (1966): Steroid dynamics (eds.: G.Pincus, T.Nakao, JF Tait) 63, acad.Press New York – London
159. Sechman A, Pawlowska K, Rzasa J (2009): Influence of triiodothyronine (T(3)) on secretion of steroids and thyroid hormone receptor expression in chicken ovarian follicles Domest.Anim. Endocr., 37, 61-73.
160. Shababi NA, Norton HW, Nalbandov AV (1975): Steroid levels in follicles and the plasma of hens during the ovulatory cycle Endocrinol., 96, 962-968.
161. Sharp PJ, Talbot RT, Macnamee MC (1989): Evidence for the involvement of dopamine and 5-hydroxitriptamine in the regulation of the preovulatory release of luteinizing hormone in the domestic hen Gen.Comp.Endocr., 76, 205-213.
162. Srivastava R, Cornett LE, Chaturvedi CM (2007): Effect of photoperiod and estrogen on expression of arginine vasotocin and its oxytocic-like receptor int he shell gland of the Japanese quail Comp. Biochem. Physiol A Mol. Integr. Physiol., 148, 451-457.
163. Srivastava R, Cornett LE, Chaturvedi CM (2008): Effect of estrogen and its antagonist on the expression of arginine vasotocin (AVT) and its oxytocin-like receptor VT3 in the shell gland of japanese quail, Coturnix coturnix japonica Comp.Biochem.Physio. A Mol.Integr.Physiol., 151, 551-559.
164. Sirotkin AV, Grossmann R (2007): Leptin directly controls proliferation, apoptosis and secretory activity of cultured chicken ovarian cells Comp.Biochem.Physiol. A Mol.Integr.Physiol., 148, 422-429.
165. Sirotkin AV, Grossmann R (2008): Effects of ghrelin and its analogues on chicken ovarian granulosa Domest.Anim.Endocr., 34, 125-134.
166. Sirotkin AV, Grossmann R, Maria-Peon MT, Roa J, Tena-Sempere M, Klein S (2006): Novel expression and functional role of ghrelin in chicken ovary Mol.Cell.Endocrinol., 26, 257-258.
167. Spanos E, Pike JW, Haussler MR, Colston KW, Ewans IM, Goldner AM, McCain TA, MacIntre I (1976): Circulating 1alpha,25-dihydroxyvitamin D in the chicken: enhancement by injection of prolactin and during egg laying Life Sci., 19, 1751–1756.
168. Spelsberg TC, Lauber AH, Sandhu NP, Subramaniam M (1996): A nuclear matrix acceptor site for the progesterone receptor in the avian c-myc gene promoter Rec.Progr.Horm.Res., 51, 63-96.
169. Srivastava R, Cornett LE, Chaturvedi CM (2007): Effect of photoperiod and estrogen on expression of arginine vasotocin and its oxytocin-like receptor in the shell gland of the japanese quail Comp. Biochem.Physiol. A Mol.Integr.Physiol., 148, 451-457.
170. Steinmetz A, Hermann M, Nimpf J, Aebersold R, Ducret A, Weinberg RB, Schneider WJ (1998): Expression and conservation of apolipoprotein AIV in an avian species J.Biol.Che., 273, 10543-10549.
171. Stepinska U, Bakst MR (2007): Fertilization In: Reproductive Biology and Phylogenie of Birds (ed.: BGM Jamieson) vol.6A, 553-587. Science Publishers Enfield, Jersey, Plymouth
172. Sterling RJ, Sharp PJ (1984): A comparison of the luteinizing hormone-releasing activities of synthetic chicken luteinizing hormone-releasing hormone (LH-RH), synthetic porcine LH-RH, and buserelin , an LH-RH analogue, in the domestic fowl Gen.Comp.Endocr., 55, 463-471.
173. Steyrer E, Barber DL, Schneider WJ (1990): Evolution of lipoprotein receptors. the chicken oocyte receptor for very low density lipoprotein and vitellogenin binds the mammalian ligand apolipoprotein E J.Biol.Chem., 265, 19575-19581.
174. Sturkie PD (1976): Avian Physiology, 3-rd ed. Springer, New York, Heidelberg, Berlin
175. Sundaresan NR, Saxena VK, Sastry KV, Anish D, Saxena M, Nagarajan K, Ahmed KA (2007): Nitric oxide: a possible mediator of ovulation and postovulatory follicle regression in chicken Anim. Reprod.Sci., 101, 351-357.
176. Sundaresan NR, Saxena VK, Sastry KV, Nagarajan K, Jain P, Singh R, Anish D, Ravindra PV, Saxena M, Ahmed KA (2008): Cytokines and chemokines in postovulatory follicle regression of domestic chicken (Gallus gallus domesticus) Dev.Comp.Immunol., 32, 253-264.
177. Sutherland RL, Geynet C, Binart N, Catelli MG, Schmelck PH, Mester J, Lebeau MC, Baulieu EE (1980): Steroid receptors and effects of oestradiol and progesteroneon chick oviduct proteins Eur.J.biochem., 107, 155-164.

178. Swett MB, Breuner CW (2008): Interaction of testosterone, corticosterone and corticosterone binding globulin in the white-throated sparrow (Zonotrichia albicollis) Comp.Biochem.Physiol.A Mol. Integr.Physiol., 151, 226-231.
179. Szôke Zs, Biczó A, Barna J, Péczely P (2005): Szteroid hormonok tojásszikbôl történô meghatározása, mint a stresszhatások és az anyai befektetés diagnosztikai lehetôsége madarakban Proc. Vadállatok szaporodásbiológiája, állatkerti tenyészprogramok, Budapest, 73-75.
180. Szôke Zs, Ferenczi Sz, Biczó A, Péczely P (2004): Effect of maternal handling stress on the steroid deposition into the yolk and on the offspring Abstr. 8th.Intern.Symp.Avian Endocr., Scottsdale, 104.
181. Takahashi T, Kawasima M (2009): Properties of estrogen binding components in the plasma membrane of neurohypophysis in hens and changes in its binding before and after oviposition Poult.Sci., 88, 2206-2211.
182. Tanabe Y, Nakamura T (1980): Biological rhytms in birds: Neural and endocrine aspects (eds.: Y.Tanabe, K.Tanaka,T.Ookawa) 179. Jap.Sci.Soc.Press, Tokyo
183. Tilly JL, Kowalski KI, Li Z, Levorse JM, Johnson AL (1992): Plasminogen activator activity and thymidine incorporation in avian granulosa cells during follicular development and the periovulatory period Biol.Reprod., 46, 195-200.
184. Tischkau SA, Bahr JM (1996): Avian germinal disc region secretes factors that stimulate proliferation and inhibit progesterone production by granulosa cells Biol.Repr., 54, 865-870.
185. Tosca L, Chabrolle C, Crochet S, Tesseraud S, Dupont J (2008): IGF-1 receptor signaling pathways and effects of AMPK activation on IGF-1 induced progesterone secretion in hen granulosa cells Domest.Anim.Endocr., 34, 204-216.
186. Tosca L, Crochet S, Ferré P, Foufelle F, Tesseraud S, Dupont J (2006): AMP-activated protein kinase activation modulates progesterone secretion in granulosa cells from hen preovulatory follicles J.Endocr., 190, 85-97.
187. Tso P, Liu M, Kalogeris TJ, Thomson AB (2001): The role of apolipoprotein A-IV in the regulation of food intake Ann.Rev.Nutr., 21, 231-254.
188. Tuohimaa P, Joensuu T, Isola J, Keinanen R, Kuunas T, Niemela A, Pekki A, Wallén M, Ylikomi T, Kulomaa M (1989): Development of progestin-specific response in the chicken oviduct Int.J.dev. Biol., 33, 125-134.
189. Turner RT (1984): The binding of estrogen to liver nuclei from japanese quail Gen.Comp.Endocr., 54, 457-461.
190. Vieira AV, Kuchler K, Schneider WJ (1995): Retinol in avian oogenesis: molecular properties of the carrier protein DNA Cell Biol., 14, 403-410.
191. Vieira AV, Lindstedt K, Schneider WJ, Vieira PM (1995): Identification of a circulatory and oocytic avian apolipoprotein D Mol.Reprod.Dev., 42, 443-446.
192. Vieira AV, White HB, Vieira PM (1996): An oocytic membrane receptor for biotin-binding protein FEBS Lett., 382, 183-185.
193. von Engelhardt N, dijkstra C, Daan S, Groothuis TG (2004): Effects of 17-beta-estradiol treatment of female zebra finches on offspring sex ratio and survival Horm.Behav., 45, 306-313.
194. Webb SJ, Geoghegan TE, Prough RA, Michael Miller KK (2006): The biological actions of dehydroepiandrosterone involves multiple receptors Drug Metab.Rev., 38, 89 – 116.
195. Wilson CM, McNabb FM (1997): Maternal thyroid hormones in japanese quail eggs and their influence on embryonic development Gen.Comp.Endocr., 107, 153-165.
196. Wilson SC, Chairil RA, Cunningham FJ, Gladwell RT (1990): Changes in the hypothalamic contents of LHRH-I and –II and in pituitary responsiveness to syn-thetic chicken LHRH-I and –II during the progesterone- induced surge of LH in the laying hen J.endocr., 127, 487-496.
197. Wilson SC, Cunningham FJ (1980): Concentrations of corticosterone and luteinizing hormone in plasma during the ovulatory cycle of the domestic hen and after the administration of gonadal steroids J.Endocr., 85, 209-218.
198. Wilson SC, Cunningham FJ (1981): Effects of anti-oestrogen, tamoxifen (ICI 45,474), on luteinizing hormone release and ovulation in the hen J.Endocr., 88, 309-316.
199. Wilson SC, Sharp PJ (1976): Effects of androgens, oestrogens and deoxycorticosterone acetate on plasma concentrations of luteinizing hormone in laying hens J.Endocr., 69, 93-102.

200. Wingfield JC, Farner DS (1978): The annual cycle of plasma irLH and steroid hormones in feral populations of the white-crowned sparrow, Zonotrichia leucophrys gambellii Biol.Reprod., 19, 1046-1056.
201. Woods DC, Johnson AL (2005): Regulation of follicle stimulating hormone- receptor messenger RNA in hen granulosa cells relative to follicle selection Biol.Reprod., 72, 643-650.
202. Woods DC, Johnson AL (2007): Protein kinase C activity mediates LH-induced ErbB / Erk signaling in differentiated hen granulosa cells Reprod., 133, 733-741.
203. Yamamura N, Takeishi M, Goto H, Tagami M, Mizutani T, Miyamoto K, Doi O, Kamiyoshi M (2001): Expression of messengerRNA for gonadotropin receptor in the granulosa layer during the ovulatory cycle of hens Comp.Biochem.Physiol.A Mol. Integr. Physiol., 129, 327-337.
204. Yasuoka T, Kawashima M, Takahashi T, Iwata A, Oka N, Tanaka K (1996): Changes in parathyroid hormone receptor binding affinity during egg laying: implications for calcium homeostasis in chicken J.Bone Miner.Res., 11, 1913-1920.
205. Yosefi S, Braw-Tal R, Bar A (2003): Intestinal and eggshell calbindin, and bone ash of laying hens as influenced by age and molting Comp.Biochem.Physiol. A Mol.Integr.Physiol., 136, 673-682.
206. Yoshimura T, Suzuki Y, Makino E, Suzuki T, Kuroiwa A, Matsuda Y, Namikawa T, Ebihara S (2000): Molecular analysis of avian circadian clock genes Brain Res.Mol.Brain Res., 78, 207-215.
207. Yoshimura Y, Chang C, Okamoto T, Tamura T (1993): Immunolocalization of androgen receptor in the small, preovulatory, and postovulatory follicles of laying hens Gen.Comp.Endosr., 91, 81-89.
208. Yoshimura Y, Okamoto T, Tamura T (1993): Electron microscope observations on LH-induced oocyte maturation in Japanese quail (Coturnix coturnix japonica) J.Reprod.Fertil., 98, 401-407
209. Zhang C, Shimada K, Saito N, Kansaku N (1997): Expression of messenger ribonucleic acids of luteinizing hormone and follicle-stimulating hormone receptors in granulosa and theca layers of chicken preovulatory follicles Gen.Comp.Endocr., 105, 402-409.
210. Zhu Y, Evans MI (2001): Estrogen modulates the expression of L-arginine:glycine amidotransferase in chick liver Mol.Cell.Biochem., 221, 139-145.

2.2. The oviduct

2.2.1. Postembryonic development of the oviduct

Two stages can be distinguished in the development of the oviduct: first is the one from hatching to the beginning of sexual maturity, which is the prepuberty (or juvenile stage) and the period that includes the fast changes happening during sexual maturation, called puberty (or maturation), when the oviduct becomes suitable for egg formation. This second stage usually lasts 10-14 days, and it coincides with the rapid maturation of the ovarian follicles, which suggests that the increase in sexual steroid production is responsible for the great amount of tissue differentiation.

2.2.1.1. Prepuberty

Before sexual maturation the oviduct is a thin, smooth, greyish-white tube located on the left side of the abdominal cavity, tightly attached to the kidney lobes. The development of the vaginal-cloacal connection is completed only in the days following hatching, when the Mullerian duct breaks through to the urodeum of the cloaca. Next, the juvenile oviduct continues to develop along with the abdominal cavity of the quickly developing hatchling (Gilbert, 1971, Shahin, 1973).

Macroscopically, four segments can be distinguished in the prepubertal oviduct: the slightly widening, funnel-forming infundibulum (oviduct funnel), the slightly thicker magnum (main segment), the uterus, which is also thick, and the thinner, slightly curved vagina. At this stage, certain sections of the oviduct cannot yet be distinguished histologically. There are only certain differences in the thickness of the outer connective tissue, or the amount of smooth muscle cells that appear in this tissue. The inner surface layer of the lumen-lining mucosa is single-layered, undifferentiated cuboidal epithelium with small microvilli. At this stage, glands in the lamina propria are not detectable, not even in a primordial stage. The base of the epithelium is bordered by a thin basal membrane, which is covered by connective tissue on the outer side. This tissue contains collagen and elastic fibres, fibroblasts and smooth muscle cells, especially in the uterus. The surface of the oviduct facing the abdominal cavity is covered by serosa, which consists of fibrous connective tissue and single-layered squamous mesothel epithelium (Péczely, 1987).

2.2.1.2. Puberty

During the short sexual maturation the length and width of the oviduct increases substantially. In domestic hen, the length of the oviduct increases

from 10-15 cm to approximately 70-80 cm and its weight increases a hundredfold. Behind this intensive growth is the development of a special large gland tissue that often forms a thick layer. This makes the oviduct prominently wrinkled. In this stage, the mesovarium, a longitudinally running dorsal and ventral peritoneal fold that suspends the oviduct, thickens and its arteries and veins characteristically dilate.

The most obvious sign of tissue differentiation in the oviduct is the development of large glandular fields. This process is regulated by the differentiated increase in steroid secretion of the ovarian follicles. First, thecal androgen and estrogen production initiates, and this stimulates the proliferation of the epithelial layer of the mucosa. As a result, the simple squamous epithelial cells are converted into differentiated pseudostratified epithelial cells, and by continuous cell division the undifferentiated simple cuboidal epithelial cells turn into the primordia of tubular gland cells that sink into the propria layer of the mucosa (invagination). Gland types, characteristic of the main segment and the uterus can already be distinguished in the early stage of differentiation. In the main segment, the microvilli on the surface of the cells are shorter and more densely distributed, while on the gland cells of the uterus the microvilli are longer and are somewhat more sparsely distributed.

Later, increasing progesterone secretion gradually pushes the ratio of plasma estrogen and progesterone towards progesterone. This new steroid balance inhibits further differentiation and division of the epithelial cells, while on the other hand, it initiates the development of non-ciliated exocrine gland (calyx) cells in the epithelium. At this time, there is a characteristic cytodifferentiation in the tubular gland cells in the propria layer – a substantial increase in the amount of rough endoplasmic reticulum and the enlargement of the Golgi apparatus, with first small, then larger areas of secretory vesicles appearing on it. Later, these vesicles unite in large droplets in the supranuclear region of the cell (Laugier et al., 1978, Sandoz et al., 1975).

The differentiation of the epithelium and the glands is accompanied by the thickening of the connective tissue of the mucosa, increased capillarisation, thickening of the outer muscle layer, especially characteristic in the uterus (Péczely, 1987).

The effects of estrogens and progesterone on the differentiation of the oviduct and the secretion of the glands have been introduced in detail the in the section “Ovary”.

2.2.1.3. The structure of the active oviduct and egg formation

The oviduct is a wrinkled, yellowish-white tube, generally thicker than the intestine, found on the left side of the body cavity, covered with serous membrane (cavum serosum genitale). It is attached to the vertebral column by a

ligament that originates from the dorsal peritoneal fold. This ligament contains blood vessels that support the oviduct, as well as sympathetic and parasympathetic nerve fibres. The ventral ligament connects the oviduct to the abdominal muscles and the ventral peritoneum. Inside, several longitudinally running bundles of smooth muscle appear, which are able to increase peristaltic movements of the oviduct in a great degree (Figure 18, 19, 20, 21, 22, 23, 24, 25).

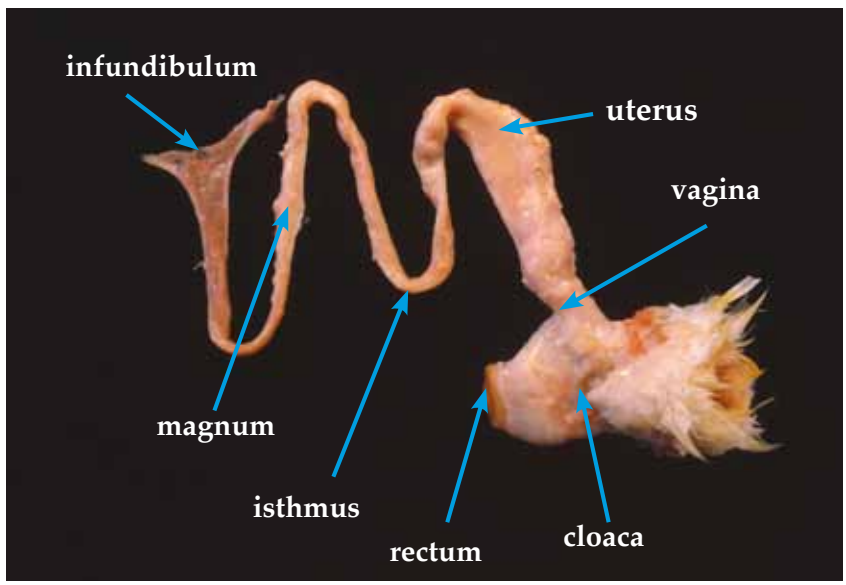

Figure 18. Oviduct of prepubertal domestic goose

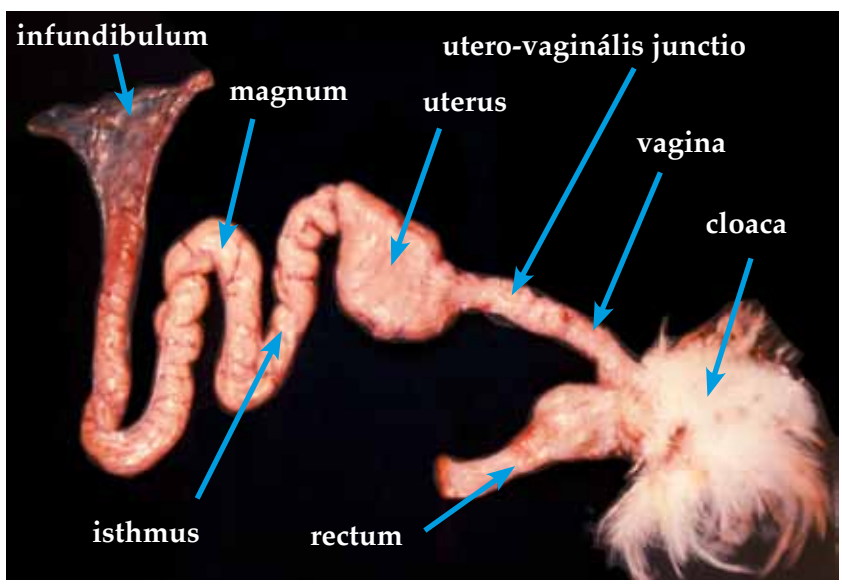

Figure 19. Oviduct of actively laying domestic goose

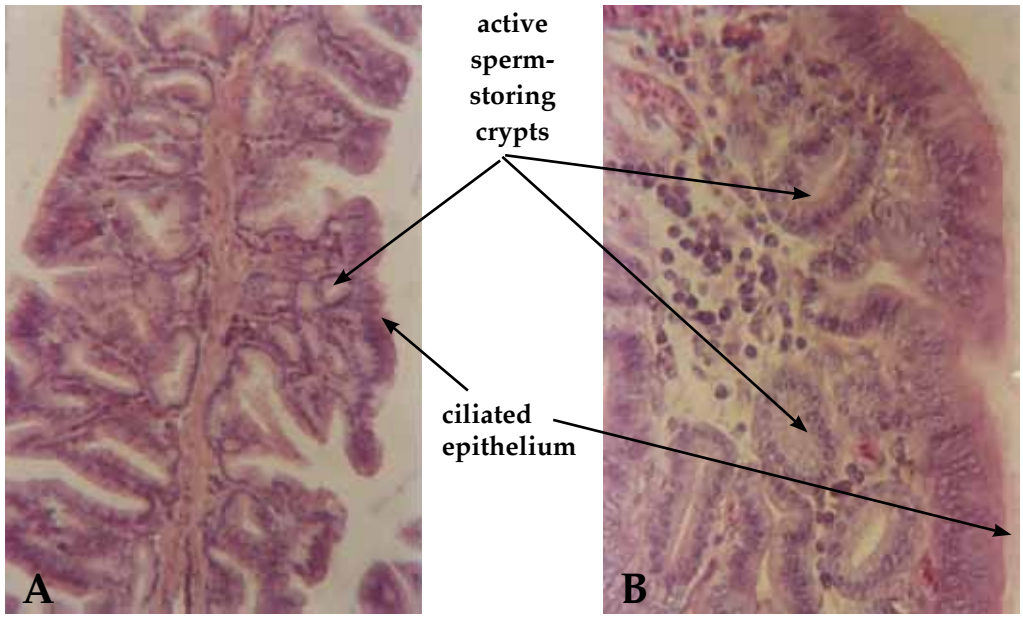

Figure 20. Infundibulum of actively laying domestic goose; A: transverse section of the mucosal ridge, B: a detail of the mucosal ridge enlarged

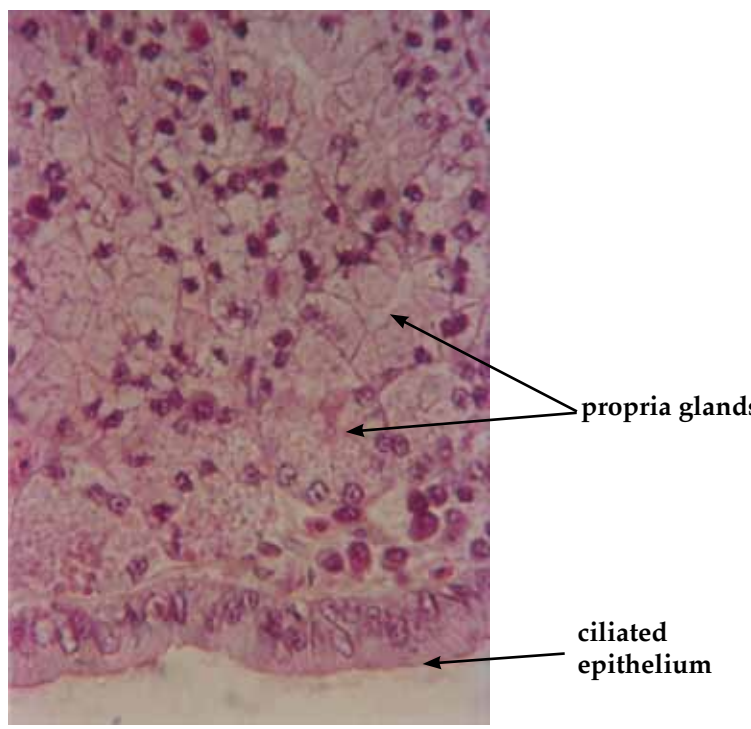

Figure 21. Magnum of actively laying domestic goose

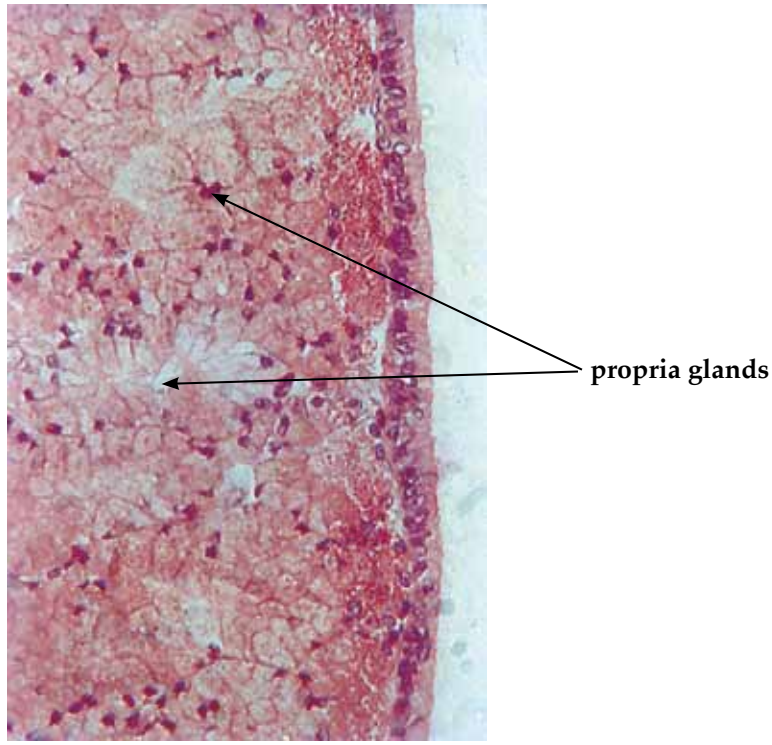

Figure 22. Isthmus of actively laying domestic goose

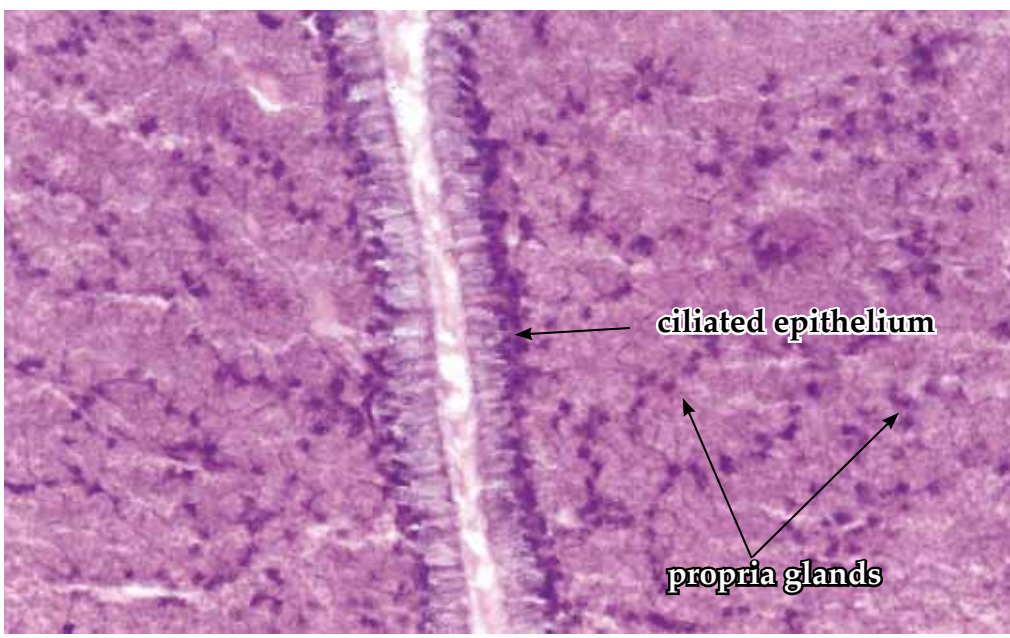

Figure 23. Uterus of actively laying domestic goose

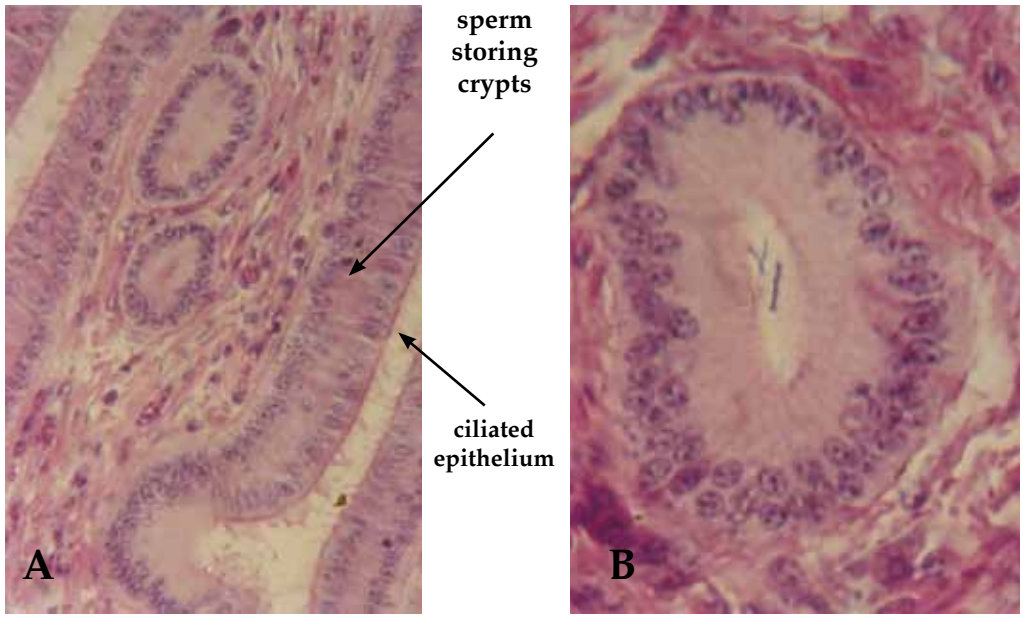

Figure 24. Utero-vaginal junction of actively laying domestic goose; A: transverse section of the mucosal ridge, B: active sperm nests (sperm storage tubules) with stored sperm

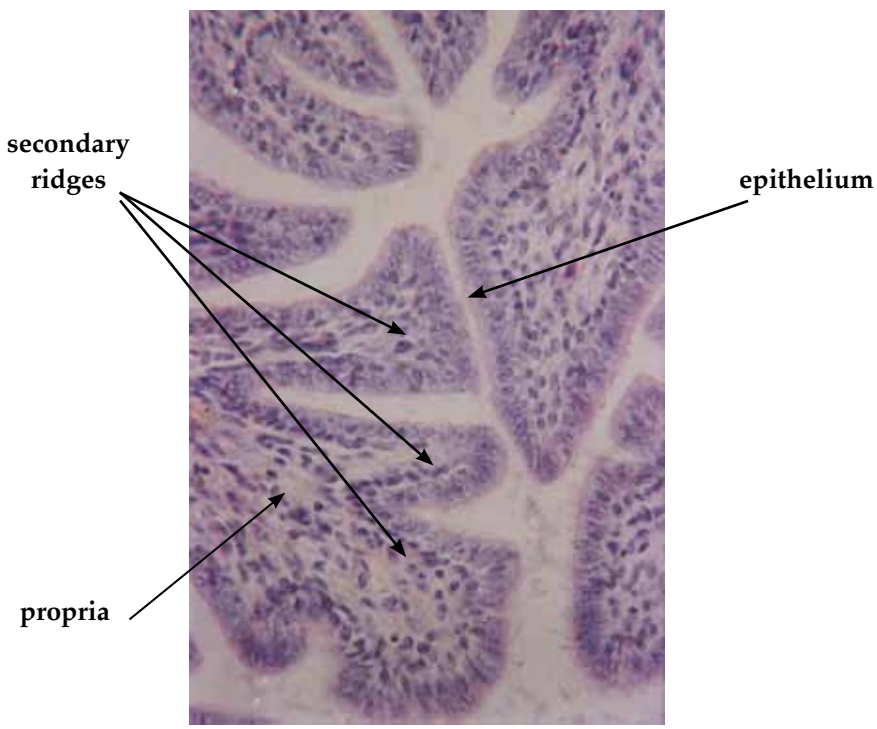

Figure 25. Vagina of actively laying domestic goose

Histologically, the oviduct can be characterised by three main layers. The development of the epithelium and the glands of the mucosa are specific to each section. Muscle layer thickness of the oviduct differs depending on the function of the segment: it is especially well developed and differentiated in the uterus and the vagina, i.e. it contains longitudinal connective tissue hedges. These septa make dilution possible for the developing and passing egg (Guzsal, 1981). The muscle layer contains a stronger, circular inner layer and a longitudinal outer layer that is further divided into bundles. Between these two layers there is an intrinsic neuronal network, equivalent to the myenteric plexus. This neuronal network (cells and fibres) connects with the sacral and hypogastric plexuses, as well as the symphathetic trunk, and their branches are partly spinal sensors, partly vegetative elements. The myenteric neurons of the plexus are so-called NANC motor elements: non-adrenerg, non-cholinerg neurons, which are capable of releasing VIP, NO and PACAP, and thereby afflict motor stimulation. Their role is to esstablish peristaltic movements during egg formation and laying (Costagliola et al., 2004). The outer surface of the oviduct is covered by a connective tissue layer (serosa).

The oviduct consists of six segments that are structurally and fuctionally specialised: infundibulum, main segment or magnum, isthmus, shell gland or uterus, cervix or utero-vaginal junction and vagina.

When discussing the regulation of oviduct functions, we should deal separately with the mophogenesis of glandular structures and the cyclic changes that accompany the development of each egg. During maturation, androgens, estrogens and progesterone form the glandular structures of the mucosa and after the first ovulation the same hormones are responsible for the regulation of the precise, cascade function of each segment. Systemic steroid and local tissue hormone (e.g., histamine) regulation both play an important role during egg formation. Especially important among steroids is progesterone, which is the main regulatory factor of protein synthesis in the magnum, but also affects the special function of each section. Its role is suggested by the large amount of progesterone receptors in the cytosol and the nucleus in all sections of the mucosa of the oviduct, and the change in their amounts during the cycle of egg development (Yu et al., 1987). Histamine concentration gradually decreases from the infundibulum towards the uterus, and its amount changes during the cycle, being exceptionally high in the infundibulum right before ovulation (Paczoska-Eliasiewitz et al., 1998). (Figure 26.)

The funnel, or infundibulum

The infundibulum is a funnel-shaped organ that ends in thin and wide finger-shaped structures called fimbria. Its lower section gradually narrows and its wall thickens. Its wide opening is always oriented towards the most protruding

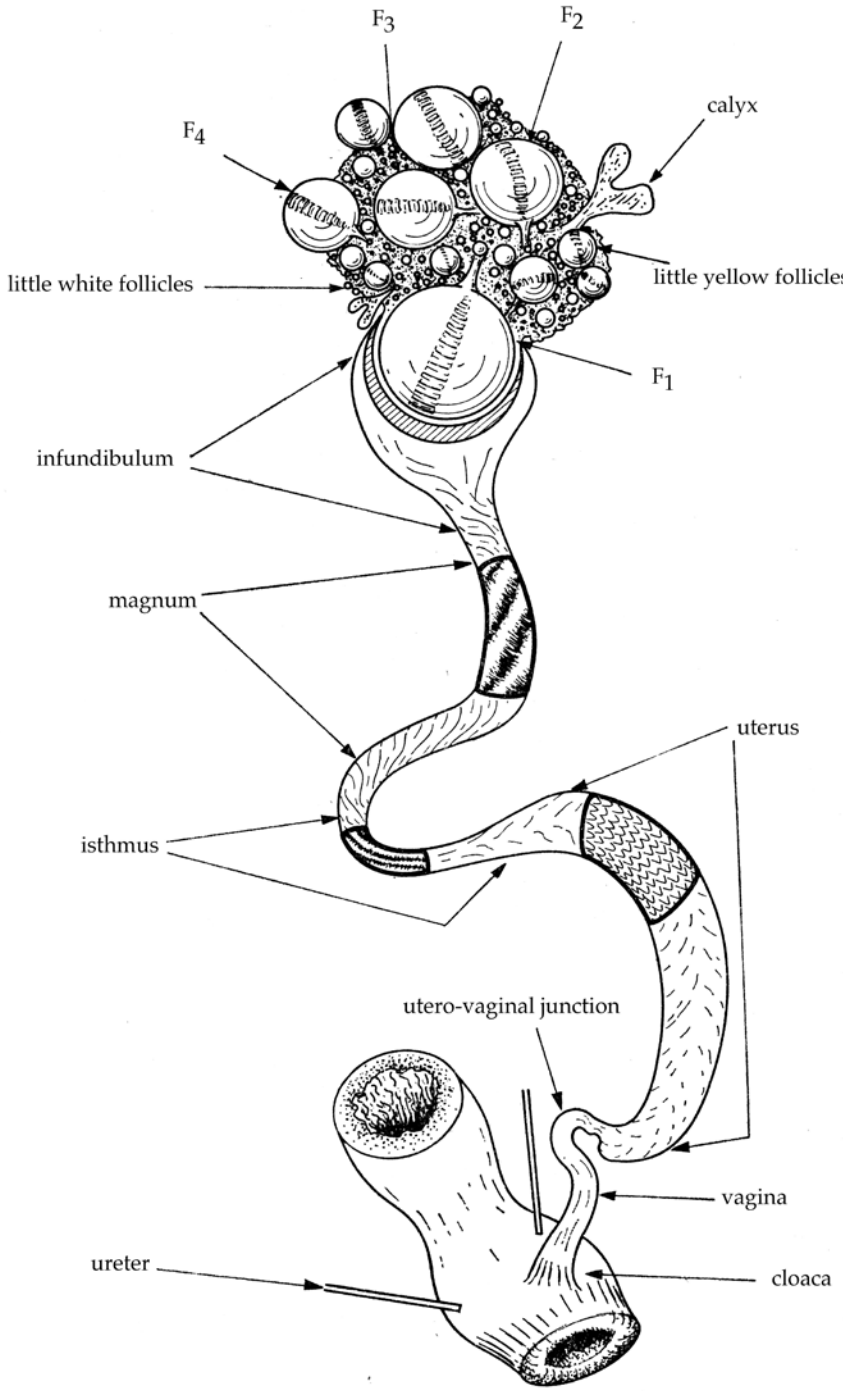

Figure 26. The structure of the ovary and the oviduct in a mature bird, modified after Péczely (1987)

preovulatory follicle (F1) by muscular ligaments, which originate at the opening and attach to the ribs. The movement of the infundibulum is also assisted by the smooth muscle bundles of the fimbria. Thus the ovulated oocyte nearly always gets into the oviduct. The infundibulum is about 8 cm long in domestic hen, and the egg passes through it in 25-30 minutes.

The funnel-like opening is covered in a serosa layer with squamous mesothelial epithelium, which gradually turns into the pseudostratified ciliated epithelium of the inner surface. In the transitional zone there is low and high cuboidal epithelium and its continuation is ciliated columnar epithelium. At the border of the ciliated epithelium and the pseudostratified epithelium some non-ciliated secretional (granular) cells appear. This cell type is characterised by granular endoplasmic reticulum in the basal area, and in the Golgi zone of the supranuclear area there are mucopolysaccharide drops (PAS positive, and alcian-blue staining) of different sizes. Sometimes these vesicles fuse and push the apical part of the cell into the lumen of the funnel (Guzsal, 1981).

The lower, neck segment of the infundibulum, also called a chalaza region, is substantially narrower compared to the opening. On its inner surface, the mucosa forms longitudinal, slightly spiral ridges that rise more and more from the surface. Calyx cells appear in the pseudostratified epithelium of the surface, which replace the granular cells of the opening. Calyx cells produce a mucin-containing secretion, and smaller and larger droplets of this liquid often fill most of the cytoplasm of the cell. In this, so-called storage phase, the nucleus is a thin, sickle-shaped structure, pushed into the basal cell membrane. The secretion of the granular and calyx cells of the infundibulum gets deposited on the oocyte as it passes by in the form of fibrous, reticulated tough membrane, and forms the outer, chalaza layer of the vitelline membrane (membrana extravitellina).

In the neck of infundibulum crypts are formed, which sink into the propria. They are similar to the tubular glands of the magnum and the isthmus, but they are more shallow depressions of the epithelial layer of the mucosa. At the edge of these gland-like crypts, the pseudostratified ciliated columnar epithelium becomes single-layer columnar epithelium, with PAS-positive secretion drops in its cells. The apical surface of the cells carries microvilli, but ciliae are not formed. These cells have a well-developed endoplasmic reticulum, Golgi apparatus, and several crista-type mitochondria. Previously, these crypts were thought to be sperm-host glands (Aitken, 1971., Koyagani and Nishiyama, 1981), which in accordance with the sperm-host glands of the utero-vaginal junction make it possible for the sperm to stay alive for several weeks after copulation and subsequently fertilise the oocyte. Recently this role has been questioned, as after copulation, as well as artificial insemination, only one or two sperm cells are found in their lumen (Bakst, 2011).

"Real" tubular glands appear at the border of the infundibulum and the magnum, however, these are even shallower and smaller compared to those in the main region. The secretion drops of their cells are smaller and more sparsely distributed than those in the magnum. Their different cell types represent functional phases as opposed to cells with different structure and function (Aitken, 1971).

The oocyte gets fertilised while passing through the infundibulum.

The main section or magnum

The magnum is the longest section of the oviduct, about 30-35 cm long in domestic hen. It has a thick wall and a narrow lumen, and the forming egg passes through it in 3 hours. This is the section where the majority of the protein (albumen) cover is added.

The well-developed, high ridges of its mucosa are spirally arranged, which assist the egg's passing through and deposit protein layers. The ridges of the mucosa are simple, without secondary structure. The epithelium consists of equal amounts of high, pseudostratified ciliated columnar epithelial cells and mucin-producing calyx cells. The loose connective tissue propria is nearly completely filled with long, narrow-lumened simple tubular glands. The end chambers and the constituting glandular cells are in different phases of the secretory cycle, which makes their appearance different. Some end chambers are in the secretion storage phase, with secretion drops fusing together, nearly completely filling out the cells, pushing the flattened nucleus onto the base of the cell. When the developing egg moves into the magnum, it gets in close contact with some mucosal ridges, from which it squeezes out a protein-containing secretion. When this occurs, the apical cell membrane opens up, the fused secretion drops get into the lumen of the tubular glands, and get deposited as layers on the downward-moving, spirally spinning egg. Other end chambers are in the stage of regeneration after having emptied their contents, while some reinitiating secretion production, with acidophil drops of different sizes located in cells rich in granular endoplasmic reticulum and with well-developed Golgi apparatus (Aitken, 1971).

According to ultastuctural studies conducted by Chousalkar and Roberts (2008), there are three types of tubular glands in the magnum. Types "A" and "C" consist of a single cell type, which is in different functional stages. Tubular gland type "B" is made of a different cell type. Type "A" glandular cells can be observed after the egg has passed through the magnum, but also on resting days. Typically, they have a large amount of dense secretory granules of different sizes in their cytoplasm, with less dense vesicles visible among them. Type "C" glands are most visible when the egg has passed through the magnum and when it is located in the isthmus. These cells are characterised by short bunches

of endoplasmic reticulum, and granules with different electron-density are visible in the Golgi area. Type "B" glands do not show cyclic changes, with the amount of amorph secretory granules is constant in their cells.

The glands of the magnum (both the calyx cells and the simple tubular structures) empty their secretion as a result of pressure that originates from the developing egg, as it passes through. The spirally arranged mucosal ridge and the peristaltic contractions from the muscle mass make the secretion empty out only from those mucosal ridges that are most exposed to the pressure. This means that when a particular egg passes through, only about a third of the mucosal ridges get in contact with the developing egg, and considering two thirds of the mucosal ridges are intact, there is sufficient time left to form a protein layer for a new egg in about every 25 hours (in domestic hen), while the gland cells of the emptied mucosal ridges continuously regenerate. Before the egg passes through, the colour of the mucosal ridges is yellowish-white (ivory), which turns light red when the protein secretion is emptied (Péczely, 1987).

Most of the albumen is secreted in the gland cells of the magnum, to which its amino acid components are continuously transported by the blood stream during egg laying. The processes of egg white synthesis and secretion are closely connected, and the glands of the magnum only store the synthesised proteins for a day or two. Synthesis and secretion of the near 50 proteins that make up the albumen occur in all gland cells of the tubular glands. There is some degree of division of function with the calyx cells of the epithelium of the mucosal ridge, which produce ovimucin, ovomucoids and avidin. Egg white protein synthesis is regulated by estrogens and progesterone.

In the lower section of magnum, which is 2-3 cm wide in domestic chicken, the mucosal ridges are less thick, and there are substantially more calyx cells on their surface. This area is separated for functional purposes, considered as the mucous-producing region of the magnum (Jacob and Bakst, 2007). This area – often together with the zone below it – is called magnum-isthmus junction (MIJ) and it possibly participates in the development of the peri-albuminal layer located between the egg white and the shell membrane (Mao et al., 2006). The alcian-blue positive glycoprotein is mostly formed in the calyx cells of the mucosa and it contains five proteins with molecular weights of 135–46 kDa.

The lower rim of the magnum is separated by the isthmus by a ring-like pinch (pars translucens). Here the mucosal ridges abruptly become lower, as they lack tubular propria glands. The epithelial layer of the mucosa consists of pseudostratified ciliated columnar epithelium with numerous calyx cells, however on the part closer to the isthmus calyx cells are absent and are replaced by so-called granular cells (Gilbert, 1971). The epithelium of the pars translucens stains well with alcian-blue, indicating glycoprotein production. In this area, between the eggwhite and the shell membrane, a particular kind of thin shell membrane is formed, indicated by peri-albumens (Sultana et al., 2003).

There is a relatively thick smooth muscle layer under the mucosa of the magnum, with circular inner and longitudinal external elements. The serosa that forms the external sheath is very rich in blood vessels.

The isthmus

In domestic hen, the isthmus is about 9-10 cm long, and the developing egg passes through it in about 1 or 2 hours. The upper part is yellowish-white with a thinner wall, and the lower part is thicker and more reddish because of the greater amount of blood vessels in the serosa. This section was formerly called "red isthmus", while recently it has been renamed "tubular shell gland" or the cranial part of the uterus. Mucosal ridges are longitudinally organised and are shallower then in the magnum. The muscle layer of the isthmus consists of a very strong, circular internal layer and longitudinal smooth muscle bundles. The rich intermuscular tissue between the external longitudinal muscle bundles make it possible for the wall to dilute extensively, which becomes necessary when the egg passes through.

The epithelial layer of the mucosa consists of pseudostratified ciliated epithelium, with three distinct cell types. There are microvilli among the ciliae on the surface of the ciliated cells. There are acidophil drops in their apical part, the amount of which is mostly consistent during egg development. The nucleus is centrally located. The surface of the secretory granular cells is covered in microvilli. Their apical region contains a large amount of PAS-positive glycoprotein granules, the amount of which changes significantly through the egg development cycle. When the cells are storing secretion, their apical part protrudes into the lumen. The nucleus of the granular cells is located basally in the cell. Cells of the third type are tall and thin, and they contain a large amount of elongated, oval mitochondria. There are microvilli on the cell surface, the cytoplasm lacks secretory granules and the location of the nucleus is variable. These are supposedly regeneratory replacement cells (Guzsal, 1981, Chousalkar and Roberts, 2008).

Compared to the magnum, the propria layer of the isthmus has fewer and smaller tubular glands. The pseudostratified epithelium of the mucosa surface – similar to the magnum – consists of two cell types: ciliated cells and non-ciliated, mucinous (granular) secretory cells. The apical part of the granular cells carries microvilli and the cytoplasm contains well-developed granular endoplasmic reticule and Golgi apparatus, as well as secretory granules in different densities. The nucleus is basally located, and is less flattened than those in the magnum. The cells of the tubular glands carry microvilli, with variable amounts of acidophil-staining and PAS-positive secretory granules. These contain neutral muco-polysaccharides and sulphur-containing proteins, similar to the granular cells. The secretion of the tubular glands empties into the lumen

in an amorphous form, where it condenses into fibrilla, and while the secretion gets pushed into the lumen of the isthmus, a polysaccharide sheath gets deposited on these fibres.

These fibres form the shell membrane that layers on the egg white, the material for which is mostly secreted in the upper part of the isthmus. According to some studies, the surface layer of the shell membrane is secreted by the tubular propria glands, while its deeper layers are produced by the granular cells of the surface epithelium. Other studies suggest that tubular glands produce the central protein part of the fibres, while the granular cells produce the outer, polysaccharide sheath of the fibres (Guzsal, 1981, Leach et al., 1981, Leach and Gross, 1983). The components of the shell membrane have a substantial β-galactosidase activity, which gets incorporated in the membrane here, in the area of the isthmus (Droba et al., 2006).

The shell membrane starts to develop as a uniform sheet, however following laying it separates into two layers, which are best visible at the blunt end of the egg, when the air space has developed. It consists of an irregular meshwork of thinner and thicker fibres with calcium-carbonate containing granules attached to them. Its composition is similar near the outer and the inner surface, however the number of thinner fibres is somewhat higher on the inner surface. The innermost part of the shell membrane, which is in contact with the eggwhite, permeates the outer, more liquid fraction of albumen, so its fibrous structure is less visible. Its outer layer, which is facing the calcareous shell, mostly consists of more loosely arranged, thicker fibres. There are pores within the meshwork of fibres, across which further liquid albumen components and water-soluble ions get into the protein layer. Where the fibres cross each other, neutral polysaccharides, rich is hexoses and hexoseamines get deposited, forming the so-called organic mammillary knobs. On these nuclei, calcium carbonate granules get deposited, which form inognic mammillary knobs, the starting points of calcification (Leach et al., 1981).

The secretion of the tubular glands of the isthmus also contains components, which are more liquid. These components have a basically equal composition to albumen, and in the lower section of the isthmus, they contain an increasing amount of dissolved calcium carbonate that are bound to the proteins. The liquid protein diffuses through the pores of the developing shell membrane, most of it spreads out directly underneath, and forms the outer, more liquidous layer of the egg white (Gilbert, 1971).

The calcification of the eggshell starts in the lower section of the isthmus. The apical microvilli of the tubular glands – similar to those of the uterus – contain calcium pump mechanisms and calbindin-D28k. These elements providing intensive calcium transport are absent in the area of the infundibulum and the magnum, as well as in the upper section of the isthmus (Wasserman et al., 1991).

During the first phase of the shell calcification, tiny (1-10 μm in diameter) calcium carbonate particles crystallise at the organic mammillary knobs that form at the crossing points of the fibres of the outer shell membrane. These structures are deposited very densely, at a rate of about 20,000 per mm^2. Because of the continuous calcite deposition, by the time the egg arrives to the isthmo-uterus transition zone, the size of the microparticles increases to 28-90 μm, the calcification points fuse, and thereby their number decreases to about 4,500 per mm^2. These spheric and later conic mammilla-hills consist of calcite crystals. The stuctures initiated in the isthmus as initiators keep growing further in the uterus, through a process called slow calcification (Sturkie, 1976, Tullett et al., 1975, Hincke et al., 2012).

The shell gland, or uterus

In domestic hen, the shell gland is about 10 cm long, its central part ampoule-like dilated, thick-walled section. Its anterior, thinner part is the continuation of the "red isthmus", and it is difficult to distinguish the border between the two. During its formation, the egg spends the longest time in the uterus, which is about 19-20 hours in domestic hen. The calcium carbonate-containing egg shell is formed in the lumen of the uterus (Figure 27).

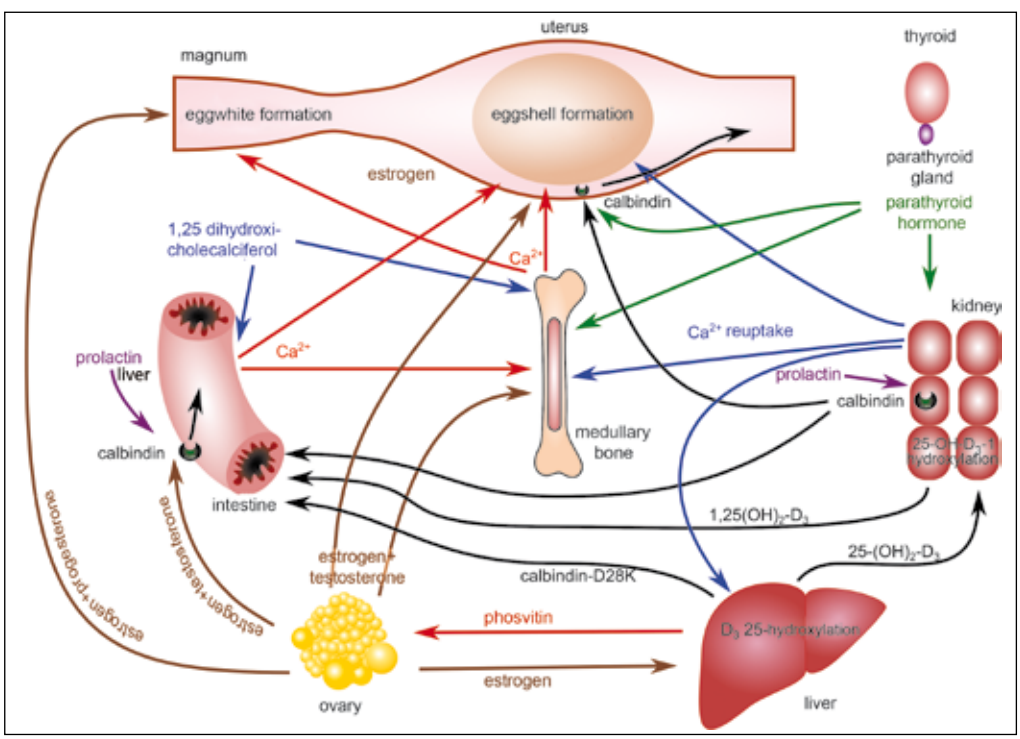

Figure 27. The complex regulation of egg shell formation

Mucosal ridges are arranged longitudinally in the anterior and posterior section of the shell gland, and in its central, ampulle-like dilated section they are arranged diagonally in relation to the longitudinal axis, and form characteristicly leaf-shaped, segmented sheets. The surface epithelium of the mucosa is an evenly arranged, pseudostratified ciliated columnar epithelium, with three separate cell types. The upper part of all three reaches the apical level, but their nuclei are organised in two definite lines. The nuclei of the ciliated cells are located in the upper level, while the nuclei of the microvilli-carrying, non-ciliated granular (basal) cells and the more sparsely distributed, mitochondria-rich cells are located in the basal layer. The basophil-staining secretion drops enclosed by membranes are located in the supranuclear zone of the ciliated cells. In the apical area of granular cells there is a large amount of secretion granules, which are PAS-positive and also react with acidic mucine. Following the excretion of the secretion, large empty vacuoles appear in the cytoplast, followed by the enlargement of the Golgi apparatus and the formation of new secretory granules. When the secretion is excreted, the intercellular gaps between epithelial cells substantially increase, which suggests that paracellular transport plays an important role in the secretory processes, as well as in the reabsorption of water and water soluble ions (Wyburn et al., 1973, Guzsal, 1981).

Secreting the organic matrix of the shell is an important function of the granular cells of the surface epithelium of the uteral mucosa. According to recent studies, tubular glands also contribute to this process. Matrix materials are important regulators of shell formation during biomineralisation. Some of them are located among the calcite crystals that form the calcareous shell, while others are within the crystals themselves, regulating the formation of the crystal nuclei, their growth and the aggregation of the centres of crystallisation. Generally, the matrix materials are acidic proteins, which are connected to other, hydrophobic macromolecules, forming a 3D network. This network consists of irregularly arranged 0.01 μm wide and about 10 μm long fibres, which contain 70% protein and about 11% polysaccharide. The polysaccharide component contains a significant amount of chondroitin sulphate, dermatan sulphate, galactose, mannose, glucosamine and galactosamine. Certain protein components get deposited in different layers of the shell: ovocleidin-17 into the mammillary and palisade layers, ovocleidin-116 mostly into the palisade layer, ovocalyxin-36 into the outer part of the palisade layer and the vertical crystal layer, ovalbumin into the mammillary layer, ovocalyxin-36 into the mamilla layer and the shell membrane, lysosim and ovotransferrin, osteropontin and dermatan-sulphate proteoglycan into the palisade layer. According to electron microscopic studies, ovocleidin-116 is mostly found in the vesicles of the matrix of the palisade layer, while osteopontin is located in the fibrous coating of the crystals (Lavelin et al., 2000, Hincke et al., 2012).

Ovocleidin-17 (in domestic hen) and the very similar ansocalcin (in domestic goose) have a very important role in the aggregation of single, rhombohedral calcite crystals into polycrystals, which is a determining step of shell formation. At the beginning of this process, there are strong interactions between the aspartic acid and glutamic acid elements of the protein and the increasing crystallising centres. As the concentration of these proteins gradually increases on the surface of the shell membrane, the aggregation of crystallising nuclei begins. The shape of the crystal aggregates changes from spheric to ellipsoid, as the concentration of matrix proteins continues to increase (Lakshminarayanan et al., 2002).

Both matrix peptides are C-type lectins. Ovocleidin-17 consists of 142 amino acids and its two phosphorylated serine molecules strongly bind calcium carbonate. It consists of three alpha helices and eight beta bundles. The flexibility of the protein is provided by two glycine molecules, while its stability by two cysteine disulphide bridges. Ansocalcin is another matrix protein with a similar structure, which forms a hydrophilic and a hydrophobic domain, and its calcium carbonate binding ability is provided by six asparagine acid-glutamine acid pairs (Reyes-Grajeda et al., 2004, Lakshminarayanan et al., 2002).

The matrix protein osteopontin also plays an important role in the regulation of eggshell biomineralisation. Its gene expresses in the epithelial layer of the oviduct mucosa. According to immunocytochemical localisation, the largest amount of this phosphorylated glycoprotein is produced by the unciliated epithelial cells of the uterus, and only when the developing egg arrives into the cavity of the uterus. In smaller amounts it can also be detected in the isthmus and the epithelial cells of the "red isthmus". In prepubertal chickens it does not yet occur, it appears after puberty, and only at certain times of the day. Its gene expression does not depend on calcium secretion, but gets initiated by the mechanical pressure of the egg. Osteopontin expression has two waves: when the egg gets in the uterus, the basal cells of the epithelium layer get activated first, and the cells located in a more apical position later. Gene expression substantially decreases one hour before egg laying. The osteopontin of the membrane-like stuctures that cover the calcite crystals in the filaments that form the egg shell matrix regulates the thickness of the shell by some kind of inhibitory action (Pines et al., 1995, Lavelin et al., 1998, 2000, Fernandez et al., 2003, Chien et al., 2008, 2009).

In the ciliated epithelial cells of the uterine mucosa, a 32 kDa protein has been detected that contributes to the organic matrix of the **cuticle** that covers the shell. This protein is produced in two phases, the first occuring a relatively long time after the laying of the previous egg (in Japanese quail 21 hours), while the second occuring four hours (in quail) before the laying of the next egg. During the first phase, 10 uM sheets get secreted onto the surface of the ciliated cells, and then these stuctures disappear in the second phase. The protein

creates a lining in the shell pore-tubule system, and also gets deposited as a cuticle on the shell surface (Rahman et al., 2009).

Porphyrin-derivate granules are also present in the apical plasm of the ciliated cells. These granules are responsible for the patterns and the colour of the eggshell, and are most characteristic of bird species with colourful eggs. According to earlier studies, these porphyrin derivates form in the liver from decomposed hemoglobin, get carried by the blood stream into the uterus, where they get depositied in the ciliated cells, which later secrete them and from there they enter the calcareous shell. According to recent studies, porphyrines also get synthesised in the uterine tissue from glycine, with the help of succinyl-coenzyme A through α-amino-β-keto fatty acids. This synthesis produces protoporphyrin, coproporphyrine, pentacarboxyl porphyrin and uroporphyrin, which form the characteristic pigment in the cuticle layer of the eggshell (Brush, 1978).

The tubular glands in the propria of the mucosa are shorter than the ones in the magnum, and their end chambers are located close to each other. The apical surface of the short columnar cells that form the tubular glands carry tall microvilli. The nucleus of the cells is most often located centrally, and is round or slightly elongated. In the supranuclear area – depending on the phase of the secretion – there are vacuoles that contain electron-dense granules of different sizes and empty, irregularly shaped smaller vesicles. Crista-type mitochondria with dense matrix are located mostly basally and along the side membranes. In these areas the cell membrane is strongly segmented and forms interdigitations, similar to the resorption cells of the proximal kidney tubules. The tubular propria cells produce a milky, liquid secretion that contains water soluble mineral salts, primarily calcium and hydrocarbonate ions, as well as proteins and glucose. The amino acid composition of the protein content is similar to that of egg white. The secretory activity of the propria glands is characterised by very fast and dynamic material transport: after the egg has passed, the secretion ceases immediately, and no secretion accumulation can be observed. They are capable of secretion into the lumen, as well as a high degree of reabsorption (Aitken, 1971, Guzsal, 1981).

About 96% of the egg shell is calcium carbonate, and the calcium (Ca^{2+}) necessary for this is transported by transcellular and paracellular mechanisms. Among the known transcellular processes calbindins (calbindin-D28k and calbindin-D9k) play an important role. These proteins, which bind calcium ions reversibly and with a high affinity, are located specifically in the tubular propria glands of the uterus and the "red isthmus" above it. This suggests that the highest amount of calcium is transported from the tubular glands towards the lumen of the uterus. The role of calbindin in calcium transport will be detailed in the chapter "Calcium metabolism of female birds".

Another active transcellular calcium transport mechanism is the Ca^{2+}-Mg^{2+}-ATPase, which is present in the tubular gland cells and surface epithelial cells of the uterine mucosa. The activity of this energy producing enzyme system is substantially higher in the mitochondrial and microsomal franction of the uterus in actively laying domestic hen and Japanese quail compared to juvenile and sexually inactive birds. Typically, in actively laying birds mitochondria are located apically in the gland cells, while in non-laying birds, they are evenly distributed in the cytoplasm of the tubular gland cells (Nys and de Laage, 1984, Yamamoto et al., 1985). The active Na^+ pump located at the base of the gland cells has an important role in this process. By mitochondrial Ca^{2+}-Mg^{2+}-ATP-ase active transport, this pump facilitates Ca^{2+} uptake from the blood, transports it towards the propria and the serosa and eventually to the apical pole of the cell and into the lumen of the uterus (Eastin and Spaziani, 1978a,b, Bar, 2009).

Passive, paracellular transport mechanisms also play an important role in this process, as a large amount of material gets transported in a short time (Sturkie, 1976). Even though the calcium content if the liquid in the lumen of the uterus is about four times higher than that of the blood plasma, most of it forms a stable complex with the secreted proteins. Therefore, the amount of free Ca^{2+} that contribute to the ion balance is in equal concentration between the blood plasma and the lumen of the uterus, with values of 6-7 mmol in laying hens. Consequently, there is no concentration gradient that would impede the diffusion of Ca^{2+} through the mucosa into the lumen of the uterus. Facilitated transport of calcium absorbed by the intestine towards the lumen of the uterus occurs because of the carbonic anhydrase activity of the uterine wall, which creates high HCO_3^- concentration in the in lumen. The electrochemical gradient created by the hydrocarbonate anions produce a certain "sucking" effect on the calcium ions in the circulation, which helps their transport into the lumen of the uterus (Bar, 2009).

The possibility of a passive calcium transport based on a difference in electrochemical gradient was suggested earlier by Hurwitz et al., (1973), who measured a 10-15 mV difference between the serosa and mucosa layers of the uterine wall in domestic hens and Japanese quails, with the mucosa being the negative side. This difference is sufficient to create and maintain Ca^{2+} transport into the mucosa. This electrochemical potential difference is created by an active Na^+ pump towards the serosa, which (also) compensates for the hydrocarbonate (HCO_3^-) flux towards the lumen of the uterus.

About 60% of the inorganic components of the egg shell is carbonate (hydrocarbonate), which binds calcium ions in the lumen of the uterus. There are two ways for carbonate to get into the lumen of the uterus: they are either transported from the blood plasma in a way similar to that of calcium, or are locally synthesised in the wall of the shell gland, as a metabolite of tissue respiration. Effererent venous blood from the uterus was studied after administering

calcium chloride and sodium hydrocarbonate isotopes intravenously. During egg shell development, the calcium content of the blood decreased by 57%, but the hydrocarbonate content remained stable, which suggests that hydrocarbonates necessary for egg shell development do not originate from the systemic circulation, but are synthesised form carbon dioxide produced in the uterine wall (Lörcher and Hodges, 1969). Carbonic anhydrase, which occurs in large concentrations in the cytoplasm of the mucosa epithelial cells and tubular propria cells, converts carbon dioxide of metabolic origin into hydrogen carbonate, which empties into the uteral lumen (Sturkie, 1976).

The binding of HCO_3^- and Ca^{2+} ions releases hydrogen ions in the lumen of the uterus. This hydrogen can get eliminated in several ways: H^+ ions can get exchanged with hydroxyl ions and ammonia produced during protein secretion can also bind hydrogen by the reaction $NH_2+2H^+ = NH_4^+$. Hydrogen ions can also be eliminated by passive diffusion, through the mucosa into the blood stream (Simkiss, 1975). As a result of these processes, the pH of the lumen of the uterus becomes basic, while the effluent blood and even the systemic blood becomes acidic. Therefore, in domestic hen during eggshell formation, the pH of systemic blood can decrease from 7.53 to as low as 7.41. This metabolic (egg shell development) acidosis is counteracted by the bird by increasing its respiratory rate and by forming acidic urine. When acidic urine is formed, phosphate ions from the decomposition of medullary bone material bind hydrogen ions, which leads to the formation of HPO_4^{2-} and H_2PO^{4-} (Sturkie, 1976).

In domestic hen, egg shell formation lasts about 18-20 hours, and during this process about 5 g of calcium carbonate gets deposited into the lumen of the uterus, through which the developing egg passes through. The partially liquid (cream-like) substance that contains proteins and calcium carbonate gets deposited on the shell membrane-covered developing egg. This process has three phases: the first phase, which lasts about five hours in domestic hen, is the initiating mineralisation.

During the first phase of secretion by the tubular propria glands, the concentration of water soluble calcium and hydrocarbonate ions increases gradually in the lumen of the shell gland. Consequently, when the egg is in the lumen, calcium carbonate deposition on the shell membrane reaches the intensity of 300 mg/h. The amount of water infux remains constant during this period (0.46 ml/h, per 1 g dry protein weight). During this "slow calcification" process the deposited calcite crystals form a radially growing mammillary layer that starts from the mammillary knobs and the formation of the palisade layer also initiates. An increasing amount of calcite crystals gets deposited on the mammillary knobs, which start growing laterally spoke-like, and then gradually also increase perpendicularly, creating columns towards the surface of the shell.

During this first phase of eggshell development, besides the gradually increasing amount of calcium carbonate deposition, a substantial amount of

water, water soluble protein and glucose diffuse from the liquid content of the lumen through the shell membrane into the egg white. As a result of this process, the shell membrane becomes more and more stretched and the pointed and blunt ends of the egg appear.

The second phase of eggshell development is the period of intensive calcium carbonate deposition. In this "fast calcification" phase, the influx of calcium carbonate into the lumen continues at the rate of 300 mg/h, same as the peak value of the first phase. In domestic hen, this lasts for about 10-13 hours. At the same time, the amount of water influx into the lumen substantially decreases, to a value of 0.04 ml/h per 1 g of protein dry weight. In this phase, the development of the palisade layer of the egg shell concludes, which is characterised by the layering of osteopontin on the surface of the crystal columns.

The third, and last phase of calcification is about 1.5 hours long. It is characterised by the supersaturation of uterine liquid by calcium and bicarbonate ions, which is accompanied (or maybe created) by inhibitory proteins (presumably mostly ovocalyxin-32) and the presence of phosphate anions that also have an inhibitory effect. Finally, with the decrease and complete ceasing of organic matrix material production, the intensive development of the exterior, dense vertical crystalline layer also concludes (Sturkie, 1976, Parsons, 1982, Fraser et al., 1999, Bar, 2009, Hincke et al, 2012).

The strong muscular layer of the uterus consists of interior circular and exterior longitudinal bundles. Thick bundles of blood vessels run in the intermuscular connective tissue hedges. The main arteries divide into secondary branches in the lower part of the mucosa, and the thinner branches form a periglandular and subepithelial system. The function of this rich capillary network is related to the intensive calcium transport and the two-way material transport. The serosa in the exterior layer of the uterine wall contains well developed veins.

The utero-vaginal junction (UVJ) or cervix

The utero-vaginal junction is located at the border of the uterus and the vagina. It is generally bent, in Anatidae in the shape "S". It can also be a tube with accordion-like ridges, which joins into the longer vagina. In hen it is only about 1-2 cm long. Its outer surface is covered by a thick connective tissue sheath, which grows from the lower part of the uterus into the urodeum, and because it is not closely conntected to the distal part of the oviduct, it makes it possible for the utero-vaginal junction and the vagina to move back and forth. The loose connection between the exterior connective tissue cover and the oviduct becomes important during the process of mating and even more during egg laying, when the vagina can slide forward in the cloaca, reaching over the opening of the cloaca and directly deposit the egg into the nest without faecal contamination.

The mucosa of the utero-vaginal junction shows well-developed, longitudinal ridges. It has a pseudostratified ciliated epithelium, which consists of a large number of ciliated and fewer non-ciliated granular cells. In the apical area of both cell types there are secretion granules that contain acidic mucopolysaccharides. In the subepithelial connective tissue, there are relatively short, almost straight tubules (crypts), which were thought to be tubular glands, but recently their function has been discovered to be sperm storage, and while they open to the surface of the mucosa, they have no secretory activity (Friess et al., 1978). These tubules are covered in single layer columnar epithelium, carrying microvilli on their apical part. In their supranuclear region there are some larger lipid drops, a well-developed Golgi apparatus and lysosomes. Around the nucleus, there is a weakly developed granular endoplasmic reticule. The nucleus is elongated, and it is located in the basal area of the cell. There are no myoepithel cells visible around the tubular structures in the propria.

Following mating, the tubular, gland-like structures of the utero-vaginal junction fill up with sperm cells, which orientate towards the base of the tubules. These sperm will spend some time in the lumen, and when they get out, they move upwards in the oviduct to fertilise the oocyte. The function of the sperm storage crypts of the utero-vaginal junction will be discussed in the chapter "Fertilisation".

The circular muscles in the muscle layer of the utero-vaginal junction are particularly well developed, and function as a utero-vaginal sphincter for the whole section, to protect the developing egg in the higher sections of the oviduct from bacterial infections that can move up from the cloaca (Figure 28).

The vagina

In hen, it is a 6-7 cm long, bent tube with a muscular wall. Its lumen is narrow, but thanks to the intermuscular connective tissue hedges in its wall, it can dilate to a great degree, which is necessary during egg laying. Its mucosa consists of high, thin ridges that are longitudinally arranged and divide into secondary ridges. In the lower part of the vagina the mucosal ridges become circular, and together with the circular muscles that lay above them form a strong sphincter.

The epithelial layer of the mucosa consists of ciliated cells and calyx cells, the amount of latter gradually increasing towards the opening of the cloaca. The secretion of the calyx cells does not only make the surface of the egg slippery, but also contributes to the formation of glycoprotein materials of the cuticle, which form the outer layer of the eggshell. During egg laying, the strong circular and longitudinal muscles play a role in the forward movement of the vagina into the cloacal cavity. The vagina opens to the ridges located at the junction of the urodeum and the proctodeum, not to the area of the typical urodeum (Dahm et al., 1980). At the cranial part of this region, the epithelial layer

of the vagina consists of the single-layered columnar epithelium of the urodeum, which has longer microvilli, and on the caudal part it is bordered by the single-layered columnar epithelium of the proctodeum with shorter microvilli. At the junction, in the propria, there is a large amount of migratory leukocytes.

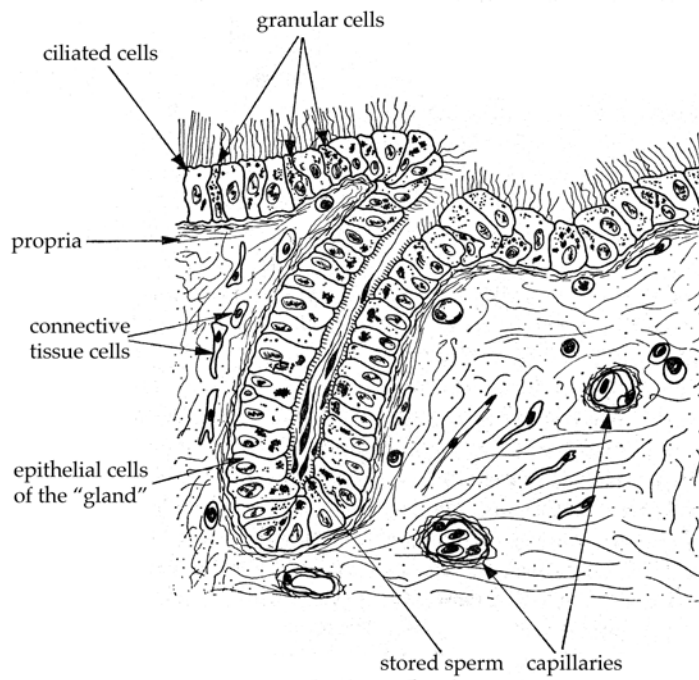

Figure 28. Tissue structure of the sperm-storing crypts of the utero-vaginal junction, modified after Péczely (1987)

2.3. The structure and composition of the egg

The avian egg consists of three structural parts. The inner, yellow, round central part, the yolk, is the mature oocyte (ovum), which forms in the ovarian follicle. The development and structure of the ovum was introduced earlier, when describing the functioning ovary.

The ovum is covered by the **vitelline membrane**, which has three layers. The innermost, the oolemma, which is practically the cell membrane (primary egg-sheath), which is covered by the perivitelline membrane (secondary egg-sheath), a joint product of the ovum and the granulosa layer, and finally, the outermost layer, the extravitelline membrane (first element of the tertiary egg-sheath), which is produced by the infundibulum. The development and structure of the oolemma and the perivitelline membrane are discussed in the

chapter about the functioning ovary. Fibres separate out in the periphery of the extravitelline membrane at the two poles of the ovum and form bundles, which make a connection between the vitelline membrane and the inner layer of the membrane testacea through the eggwhite. This tough, elastic bundle is the chalaza, which sticks to the yolk of the spirally rotating egg as it moves down the oviduct, and as it also rotates spirally, it forms a spring-like structure. Chalazae suspend the ovum by attaching along the longitudinal axes of the egg to the pointed and blunt ends and also to the shell membrane, ensuring that when the egg rotates the ovum would rotate too and the germinal disc under the vitelline membrane would always maintain its position in the upper part, which has an important role in normal embryonic development (Figure 29).

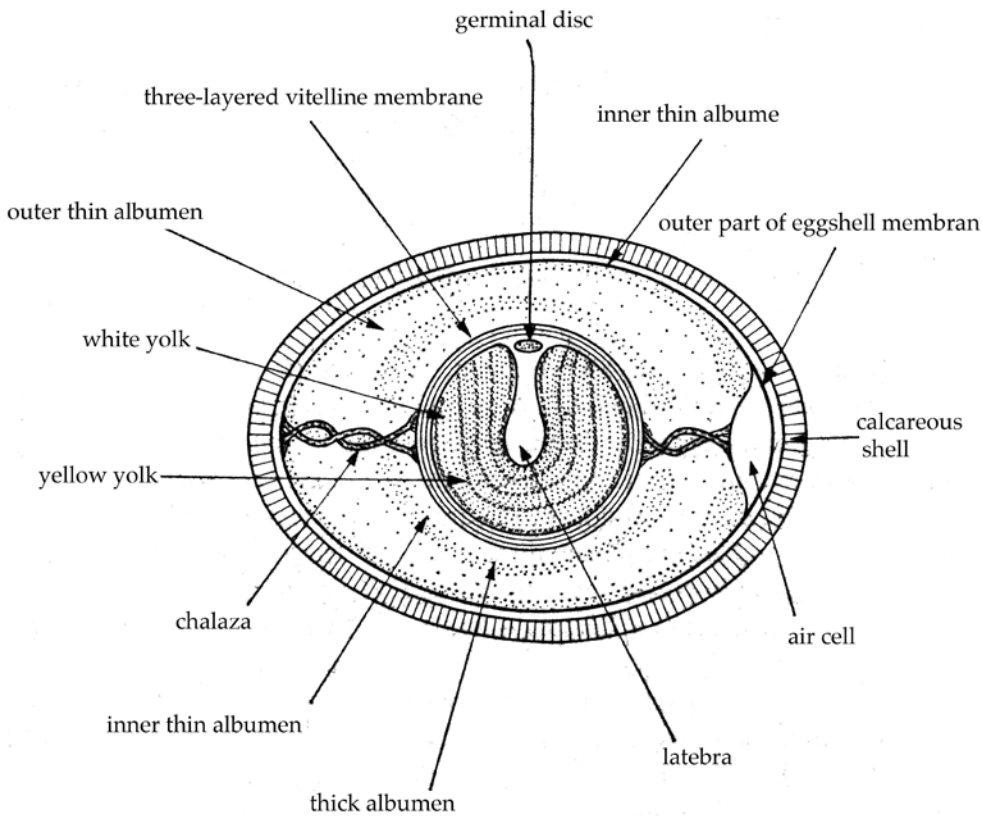

*Figure 29. T*he structure of the egg modified after Péczely (1987)

Albumen (egg-white) is the thickest tertiary oocyte sheath. It is mostly produced by the magnum of the oviduct, however, a substantial amount of water and protein components are also incorportated into the developing egg in the isthmus and the uterus (as discussed in details when describing the functioning oviduct). Three layers of albumen can be distinguished in a freshly laid egg.

Even though the components of these three layers are deposited into the albumen in the oviduct at different times, these layers only separate in the lowest segment of the oviduct, i.e., the characteristic separation is not yet visible when the developing egg leaves the magnum.

The outermost layer of the albumen, located under the shell membrane is thin and more liquid, as the ovomucin-lysozyme fibrous framework is not yet formed. This layer makes up about 23% of the albumen. The middle, thickest layer of the albumen is the protein sac, which consists of the ovomucin-lysozyme framework and liquid protein components deposited into it. The protein sac is slightly gel like, having a thicker consistency compared to the surface layer, and it makes up about 57% of the albumen. The components of this layer are mostly incorporated in the lower parts of the magnum. Similar to the outer layer, the inner thin protein layer is also mostly liquid, and lacks the ovomucin-lysozyme framework. In domestic hen, this layer forms 17% of the albumen and it is mostly produced by the transition zone between the infundibulum and the magnum, as well as by the upper section of the magnum.

In domestic hen, the albumen contains about 88.5% water, 10.5% proteins, 0.5% carbohydrates and 0.5% minerals, vitamins, as well as a small amount of hormones. The protein component consist of 10–15 proteins, however, recent gel-electrophoretic studies separated as many as 50. There are some quantitative, and smaller qualitative differences among different species.

The most important component of albumen is **ovalbumin**, which represents 54%. Ovalbumin is a 46 kDa protein with pH 4.5-4.8 isoelectric point and it contains a small amount of D-mannose and N-acetyl-glucosamine bound to aspartic acid. Based on its phosphate content, it can be separated into three fractions: ovalbumin A1 contains two phosphates per molecule, A2 contains one, while A3 lacks phosphates. Two ovalbumin derivates are known, but it is possible that they only form during the storage of the egg. S-ovalbumin is formed by the creation of a disulphide bridge, while plakalbumin is formed by the detachment of a heptapeptide. Physiologically, similar to the albumin fraction of the plasma, ovalbumin is one of the most important transport matrices of water, carbohydrates, minerals, vitamins and hormones. It plays a crucial role by providing structural proteins for the developing embryo. There is genetic polyporhism in regard to ovalbumin among bird species.

Ovotransferrin (conalbumin) is a glycoprotein with 76.6 KDa molecular weight and isoelectric point of 6.0, which has similar composition and characteristics to plasma transferrin. It makes up about 12% of the albumen. It consists of one amino acid chain, with alanine at the N terminal. Its carbohydrate content is 0.8% hexose and 1.4% hexosamine. It has two specific binding sites per molecule, which makes it possible to bind Fe^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+} and Zn^{2+} ions. Ovotransferrin has a strong bactericidal effect, as a result of high-affinity binding of free ferrous ions from the environment.

Ovomucoid is a 28 KDa molecular weight, rather heat stable glycoprotein. Its isoelectric point is between 3.9 and 4.3, which suggests it is inhomogeneous. It makes up about 12% of the albumin. Ovomucoid has a high carbohydrate content, it contains 12-14% glycosamin, 4-6% mannose and 1.5% galactose. It binds bicarbonates with a high affinity, and therefore strongly inhibits the activity of proteases. The ovomucoid of particular species has a very specific inhibitory activity: the ovomucoid from domestic chicken, ostrich, emu and rhea (*Rhea americana*) strongly inhibits bovine tripsin, ovimucoid from quail egg inhibits bovine and human tripsin, while pheasant ovimucoid inhibits bovine chemotripsin and subtilisin, but does not have an effect on human or bovine tripsin.

Lysozymes make up about 3.4% of the albumen, with a molecular weight of 14.3 KDa. Their isoelectric point is 10.5-11.0, which suggests heterogeneity. The strong glycosidase and esterase activity of lysosim makes it bactericidal. It also creates viscous fibres and therefore the framework of albumen by forming secondary bonds and polymers with itself, as well as by polymerising with ovomucin. This relatively stable molecule resists weak acids and bases, and a does not get damaged by a short exposure to boiling. Two types of lysozymes can be distinguished based on their amino acid sequence, crystal structure and enzymatic effect. Type "C" is found in the albumen of *Galliformes and Anseriformes*, while type "G" is present in the albumen of ostrich, rhea, cassowary, kiwi, tinamou, *Galliformes, Anseriformes, Podicipediformes, Charadriformes.*

Ovimucin is an acidic glycoprotein, which makes up 3.5% of the albumen. Its carbohydrate content is 18.5%, which consists of hexoseamine, glucosamine, galactosamine and hexose. It has two components: a-ovomucin (210 kDa molecular weight) and b-ovomucin (720 kDa molecular weight). This second subunit has globular elements with a molecular weight of 112.3 kDa. Together with lysozyme, it forms a meshwork as well as membranes made up from filaments, and therefore has a basic role in the formation of chalaza, the shell membrane and the protein-stabilising fibrillae.

Ovodefensins are antimicrobial peptides, which are present in very small amounts in the albumen (Gong et al., 2010). Some types have been identified recently: gallin (in domestic chicken), meleagrin (in turkey), cygnin (in black swan *Cygnus atratus*), BPS 1, BPS 2 (mallard *Anas platyrhynchus*). They consist of 41 amino acids, all with glycine in position 10, with minimal differences amoung their structure. Gallin inhibits the growth of *E. coli* in vitro in concentrations as low as 0.25 mM, proving its antimicrobial effect.

Ovoinhibitor makes up 1.5% of the albumen. This glycoprotein has low carbohydrate content (3.5% hexose and 2.7% hexosamine), 44-49 kDa molecular weight, with an isoelectric point of 5.2. It consists of one polypeptide chain and it has a relatively large spectrum, inhibiting the activity of different proteases. Ovoinhibitor probably protects the developing egg against damaging effects of sperm that move upwards in the oviduct and enter it.

Ovoglycoprotein makes up 1% of the albumen. This glycoprotein has 24.4 kDa molecular weight and 3.9 isoelectric point. Its carbohydrate content is 13.6% hexose and 13.8% glycosamine. It has a role in the formation of the tough fibres of the albumen.

Ovoflavoprotein is a 32 kDa molecular weight glycoprotein, which makes up about 0.8% of the albumen. It has an isoelectric point of 2.9-4.1, suggesting it is heterogeneous. It consists of two subunits, one larger with five, and a smaller with three cysteine bridges. It contains 14% carbohydrates, consisting of mannose, galactose and glycosamine. It has a high, 1:1 affinity for binding riboflavin.

Ovomacroglobulin has a molecular weight of 900 kDa and isoelectric point of 4.5-4.7. It makes up 0.5% of the eggwhite and it is absent in the albumen of the turkey and some quails.

Avidin has a molecular weight of 68.5 kDa and isoelectric point of 9.5. Its occurrence in albumen is highly variable among species: in domestic chicken: 0.05%, in lesser black-backed gull (*Larus fuscus*): 0.1%, while in domestic turkey: 16.2%. It is a basic protein with four subunits, each binding a molecule of biotin by the tryptophane amino acid of the subunit. Avidin also has antimicrobial characteristics.

Immunoglobulins, such as IgA and IgM are important components of albumen. Several **proteins with enzymatic activity**, such as catalase, peptidase, esterase, mannosidase, acetyl-glycosaminase, can be detected in the albumen, along with papain-inhibitor and tripsin-inhibitor proteins (Gilbert, 1971, Sturkie, 1976, Board and Hornsey, 1978, Péczely, 1987). (Figure 30)

The eggshell membrane (**membrane testacea**) is a tough, paper-thin membrane, consisting of two layers, which only separate in the first few hours following egg laying. This is when the air sac is formed between the two membranes at the blunt end of the egg. The two layers have the same composition, and they consist of an irregular network of thinner and thicker fibres, to which attach granules of different sizes. The inner layer mostly consists of thinner fibres, which form a dense network. The part of this inner zone adjacent to the albumen is infused with the outer, thinner protein of the albumen, which makes its fibrous structure less evident. However, the outer layer mostly consists of thicker fibres that form a loose network. The eggshell membrane gives its final shape to the egg. On this get deposited calcareous elements of the shell and the pores among its fibres make the diffusion of water, further liquid proteins and water soluble material into the albumen possible. On its surface, at the crossing point of the fibres, neutral polysaccharides rich is hexoses and hexosamines get deposited, forming the so-called organic mammillary knobs. On these knobs build the calcareous granules, which form the inorganic mammillary knobs, and create a tight connection between the calcareous shell and the shell membrane.

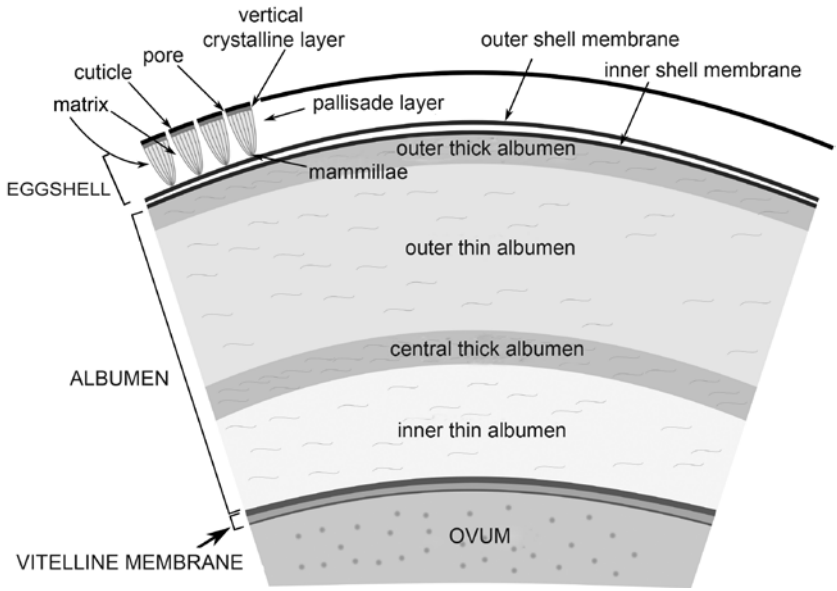

Figure 30. The structure of the oocyte sheath

The fibres of the eggshell membrane consist of sulphour-containing proteins and glycoproteins. The keratin-like material that makes up the fibres used to be called ovokeratin, but recently the composition of shell membrane has been found to be mostly hydroxyproline and hydroxylysine. This suggests that a collagen-like material is the most important component of the shell membrane. Later protein fibres with a composition similar to that of elastin were found to make up 70-75% of the shell membrane. The stability of these fibres is provided by disuphide bridges as well as desmosine and isodesmosine cross chains that originate from lysine. Besides these fibres, the eggshell membrane also contains 10% collagen and glycoprotein (Leach et al. 1981, Leach, 1982).

The **eggshell** (testa) consists of calcium carbonate crystals and matrix proteins (ovocleidin-17, C-lectins (domestic chicken), ansocalcin (goose) and osteopontin) that regulate the development of calcite polycrystal aggregates. The thickness of the eggshell is characteristic of the particular species or type, and generally three layers can be distinguished.

The inner layer is the **mammilla layer** that builds on the outer fibres of the shell membrane. It consists of calcite crystal columns that build up into spheric or conic shapes, deposited on the organic mammillary knobs or among its fibres. Mammillary knobs serve as initiator points during the development of the calcareous shell (Sturkie, 1976, Tullett and Doeming, 1984, Lakshminarayanan et al., 2002, 2005).

The calcareous mammillary knobs continue in columns that have a wider upper part, which are arranged diagonal in realation to the surface of the shell

and these form the **palisade layer**. The palisade layer is the thickest part of the eggshell, making up 65-70% of the total thickness. No sharp boundary can be identified between these two layers. Calcite crystals orienting sideways and upwards are found in the palisades, with an organic matrix forming a fibrous network among them. In the upper part of the palisade layer there is an increasing amount of smaller vesicles, which merge into each other to form an irregular channel system. These channels open to the surface of the shell with pores of different sizes and are responsible for the total gas exchange of the egg. According to Tullett and Deeming (1982), Ar and Rahn (1985) and Rahn et al., (1987), there are at least seven types of channels. The simplest system consists of simple, non-branching tubules that originate at the pore, while the most complex tubules with multiple branches that form a labyrinth. It is possible to identify avian families and even species based on the pore system and the tubules originating from it.

Very thin, vertical calcite crystals build on the palisade layer from the outside, forming the **vertical crystalline lay**er. Among the calcite needles there is a small amount of thick, also vertically organised organic matrix.

The eggshell is covered by a double-layered **cuticle**, which consists of inorganic (hydroxylapatite) and organic (a 32-kDa protein) components. The inner layer contains hydroxylapatite vesicles, which are assumed to play some part in the cessation of eggshell development. These vesicles are lacking in the outer layer of the eggshell (Fraser et al., 1999, Hincke et al, 2012). (Figure 31)

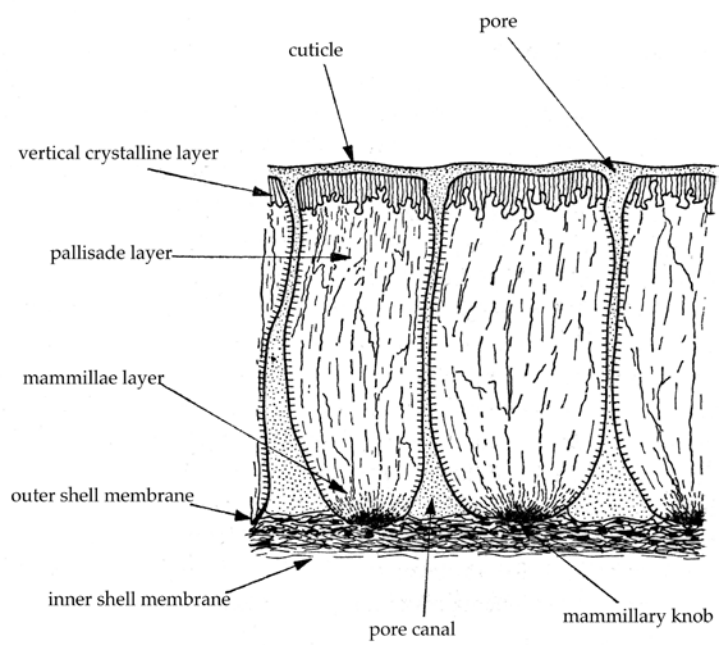

Figure 31. The structure of the calcareous shell of the egg, modified after Péczely (1987)

2.4. Changes in the calcium metabolism of the female bird during eggshell development: the role of medullary bone tissue

Eggshell development fundamentally changes the calcium metabolism of the female bird, and this change already begins before laying the egg. Functional changes affect calcium absorption of the intestine, which substantially increases, tubular bones show a structural change, with secondary bone material (medullary bone tissue) developing inside their cavity, calcium secretion decreases in the kidney tubules and changes occur in the complex hormonal system that regulates calcium metabolism. The change in calcium metabolism begins to take place at the onset of puberty, and the rearrangement settles at a new level when the first egg is laid. Eggshell development (18-22 hours in domestic hen) requires a large amount of calcium compared to the weight of the bird and the total calcium content of the body. For instance in domestic hen, it would use up the total calcium content of the blood plasma (20-30 mg) in about 10-15 minutes. A continuously working, high-capacity replacement system is necessary, the regulation of which tunes together calcium uptake, the functioning of temporary calcium depots, transport and incorporation into the eggshell (Figure 32, 33, 34.).

The elements of the complex hormonal regulation are 1,25-dihydroxy-cholecalciferol (active vitamin D_3), estrogens, androgens, parathyroid hormone, growth hormone, prolactin, thyroid hormones and calcitonin.

During puberty estrogens and androgens substantially increase the activation of cholecalciferol. The first step of this process is 25-hydroxylation in the liver, and the synthesis of 25-hydroxy-cholecalciferol, which get carried into the kidney by the circulation. Here, in the epithelial cells of the kidney tubules 25-OH-D_3-1-hydroxylase converts it into 1,25-dihydroxycholecalciferol (1,25-(OH)2-D_3 vitamin), which is active vitamin D_3 (Péczely, 1987).

Active vitamin D_3 – along with the plasma Ca^{2+} binding transport protein that gets synthesised in the liver as a result of the effect of estrogen – gets into the intestine, and binds to the 1,25-dihydroxyvitamin receptors of the cytosol of the intestinal epithelium cells (Walzem et al., 1999). These receptors contain about 420 amino acids and a peptide with a cysteine-rich amino terminal with a DNA-binding domain and a steroid-binding carboxy terminal.

Active vitamin D_3 increases tubulin synthesis by the microtubules in the epithelial cells of the tubular glands of the mucosa and the propria along the entire length of the intestine. It also increases the expression of the calcium binding proteins (formerly known as calcium-binding protein CaBP, currently **calbindin-D28K**). While mammals have a 28 and a 9 kDa calbindin, in avian tissues (so far) only the 28 kDa protein has been detected. The calbindin content

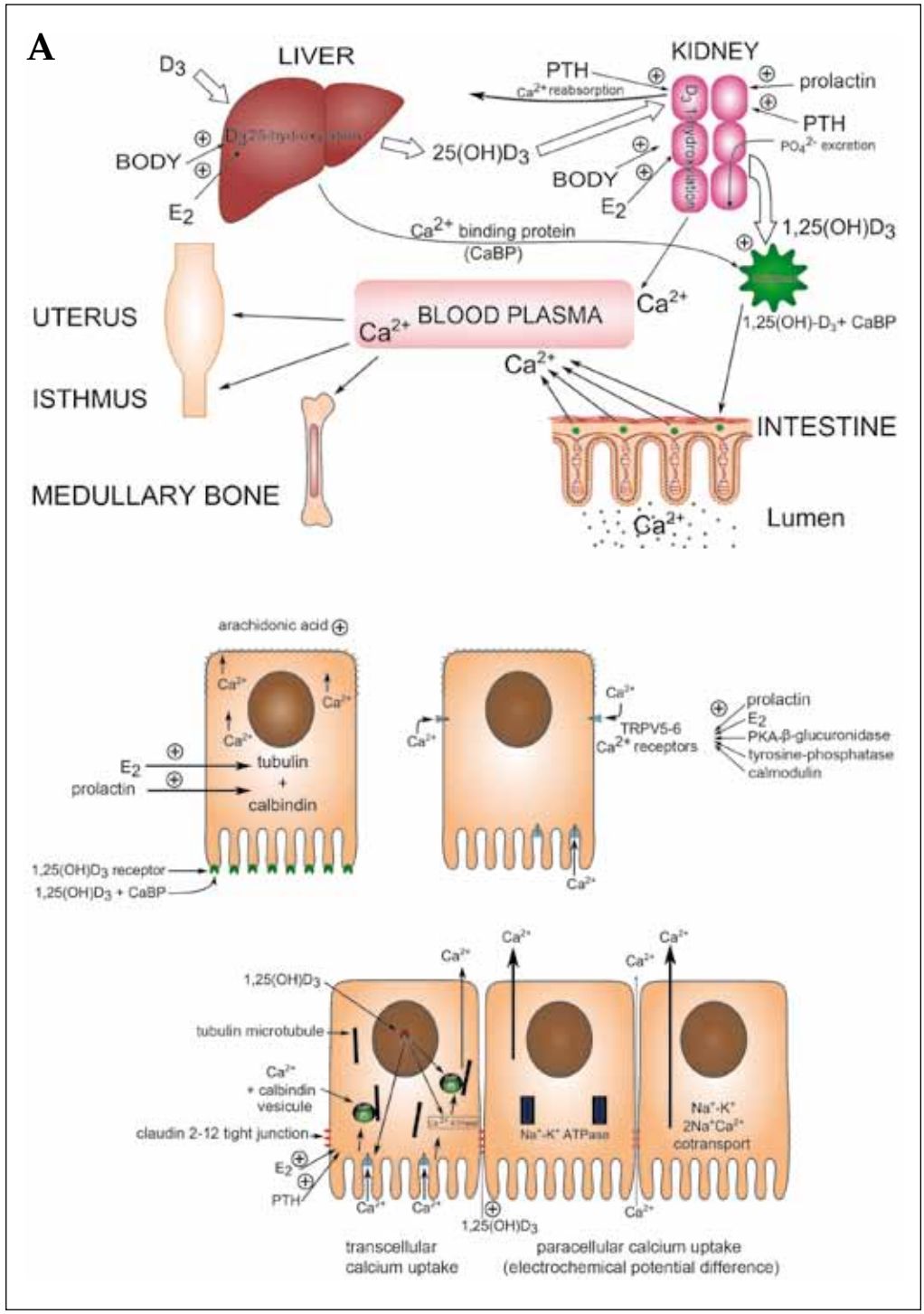

Figure 32. Calcium metabolism of the egg laying bird;
A: absorption of calcium from the intestine

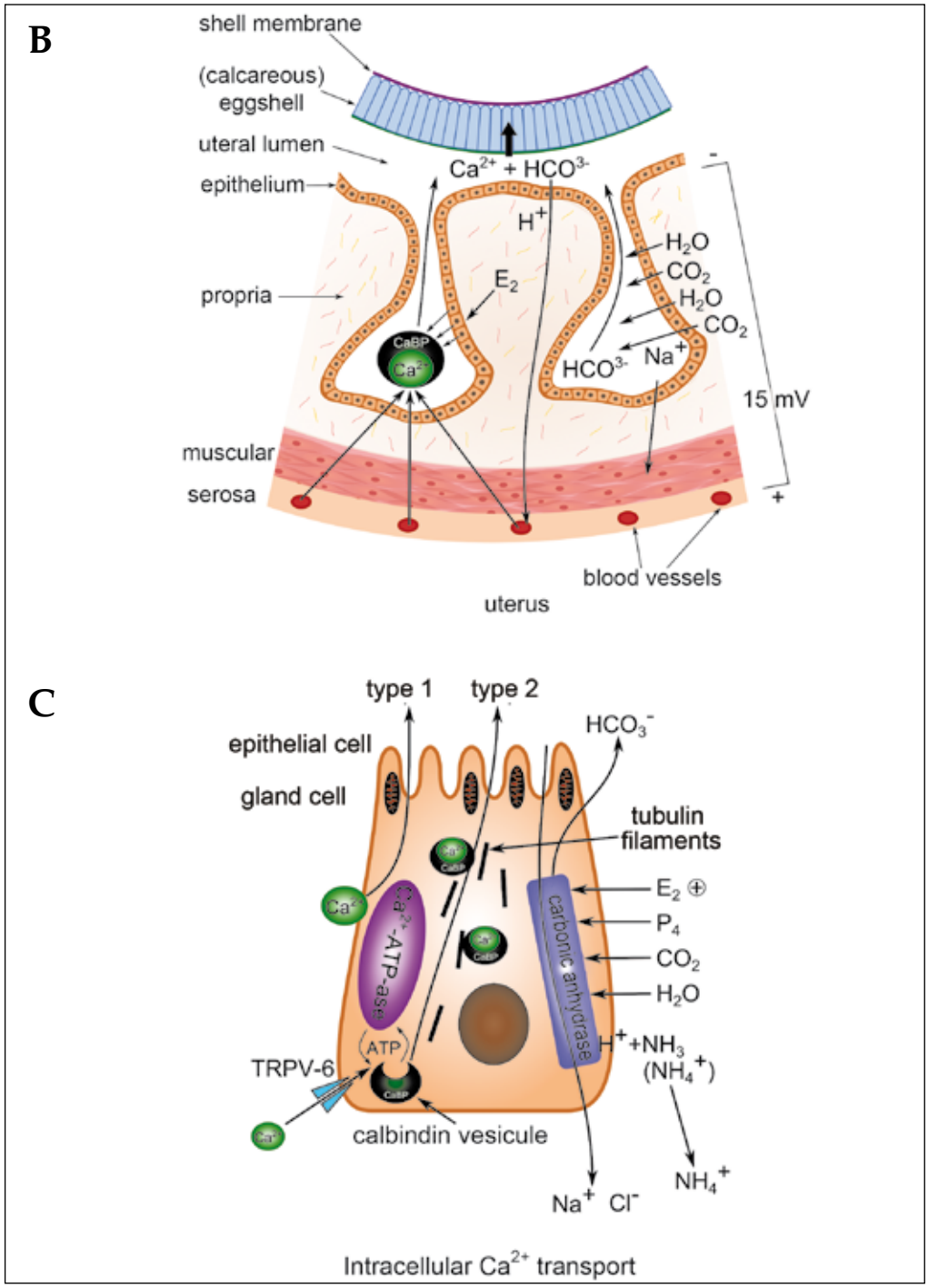

Figure 33. Calcium metabolism of the egg laying bird; B: calcium and hydrocarbonate excretion in the uterus, C: calcium and hydrocarbonate transport in the epithelial cells of the uterus

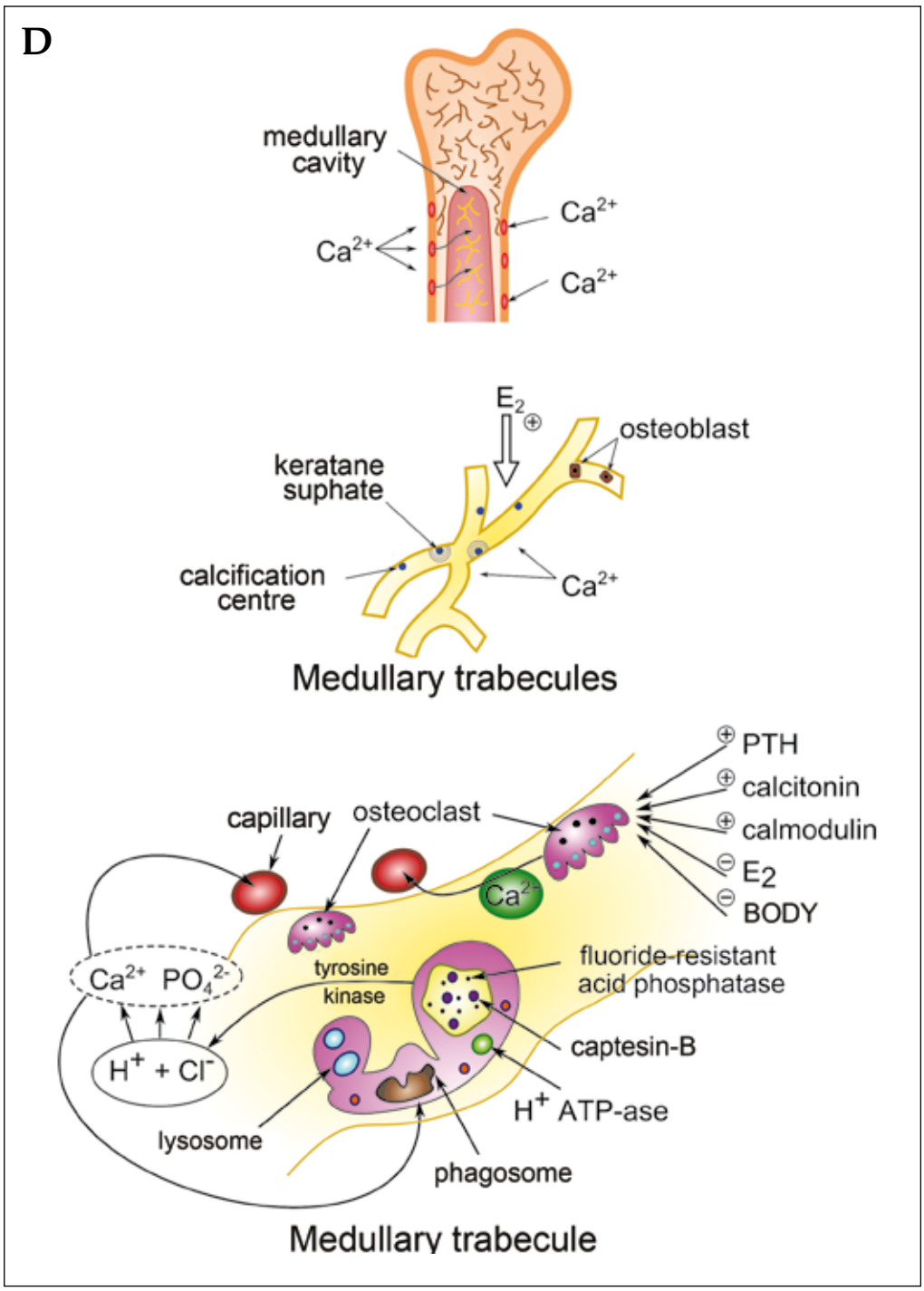

Figure 34. Calcium metabolism of the egg laying bird; D: the role of medullary bone tissue in the calcium metabolism of the female bird

of the intestine correlates with its calcium transporting ability and not with the actual amount of calcium uptaken by the cells. Also, unlike in the uterus, its expression does not show a daily fluctuation. In the organs of birds (intestine, kidney and uterus) calbindin-D28k is found, which has the same structure, containing 261 amino acids. It is characterised by six repeating alpha-helix-alpha domain sections per molecule (EF-hand), among which four bind calcium with a very high affinity: Kd = 10-8 M, while they undergo a conformational change. Calbidin gene is 19 kb long, containing 11 exons. There are vitamin D_3 response element (VDRE) and estrogen response elements (ERE) sites on its promoter (Leathers et al., 1990, Hobbs et al., 2009, Bar, 2009).

Calcium uptake in the intestine occurs through intra-, and paracellular mechanisms. Intracellular calcium uptake occurs through the heterotetramer TRPV-5 and TRPV-6 (transient receptor potential, vanilloid type-5/6), funnel-shaped calcium channels which are found in the brush border and the basolateral membrane of the intestinal epithelial cells. Calcium channels are characterised by an extensive N-terminal consisting of six rolled up ankyrins and a C-terminal that reaches deep into the cytoplasm. Depending on the calcium concentration, their efficiency is regulated by several intracellular mechanisms: through calbindin, which has four calcium binding sites, tyrosine phosphatase, and the protein kinase-A (PKA)-β-glucuronidase reaction pathway.

Increased calcium uptake can also be enhanced by the increased stability of the arachidonic acid content of the basolateral membranes, which is directly ensured by the presence of the active vitamin D_3. Calcium transport towards the base of the intestinal epithelial cells is increased by Na^+-K^+ and Na^+-Ca^{2+} cotransport processes that occur in the intestinal epithelial cells, and also by Ca^{2+}-ATPase. The second mechanism seems to be the dominant in intestinal epithelial cells (Hoenderop et al., 2005, Phelps et al., 2008, Khanal et al., 2008).

In the cytoplasm of the intestinal epithelial cells, calbindin forms vesicles by lysosomal transformation, which take up, store and transport the calcium that gets into the cell. These structures assure the faciliated transport of Ca^{2+}, which gets taken up from the food by the intestinal epithelial cells and transported into the blood vessels. Tubulin-containing microtubules play an important role in the movement of calbindin vesicules towards the base of the cell (Sugiyama et al., 2007).

Increased calcium uptake also necesitates an extracellular mechanism, the increased functioning of the paracellular pathway between intestinal epithelial cells. In this process, the electrochemical difference between the mucosa and the serosa provides the drive. At the tissue level, the process is presumably regulated by a direct effect of vitamin D_3, which also loosens the protein connections between epithelial cells, the tight juctions formed by claudin-2 and -12 (Nemere et al., 1991, Alisio et al., 1997, Hoenderop et al., 2005, Christakos et al., 2007, 2010, Fujita et al., 2008, Bar, 2009).

The Ca^{2+} absorption activity of the intestine is strongly affected by the calcium and vitamin D_3 content of the food, and slightly affected by its phosphorous content. The most important regulating factor is vitamin D and its metabolites, which determine the calcium intake by the intestine in juvenile, egg-laying and sexually inactive (moulting) birds as well (Hurwitz and Bar, 1972, Cohen et al., 1978, Bar and Hurwitz, 1984). The calcium content of the food decreases the Ca^{2+} uptake capacity of the intestine, but increases the amount of total Ca^{2+} absorbed (Bar et al., 1979). According to studies on domestic hens, the age of the individual, the intensity of egg laying and the ceasing of egg laying all affected calcium absorption: maxiumum Ca^{2+} absorption was not at the beginning of egg laying cycle, but at the beginning of the egg production period (Scott and Balnave, 1991). During egg development, 80 and 65% of the intestinal Ca^{2+} got absorbed in laying domestic chickens and Japanese quails, respectively, while in birds with an inactive uterus the values were only 36 and 18%, and in non-laying individuals it was 23 and 17% for the two species, respectively (Bar et al., 1976). The increased Ca^{2+} absorption that occurs during egg development co-occurs with a decreasing plasma Ca^{2+} content, increasing plasma PTH level, acidosis, a change in the plasma sexual steroid level, and increasing soluble calcium content in the intestine (Singh et al., 1986, Cohen and Hurwitz, 1974).

Calcium that entered the intestinal epithelial cells gets expelled from the cells into the capillaries of the propria through the basolateral membrane, against the electrochemical gradient (intestinal epithelial cells: 10^{-7} M Ca^{2+}, blood plasma: 1.25–1.5 x 10^{-3} M Ca^{2+}). This energy-consuming process occurs via two pathways: through Ca^{2+}-ATP-ase (PMCA) and Na^{+}/Ca^{2+} ion-exchange mechanisms (NCX). In the PMCA system the indirect effect of the active vitamin D_3 results in the activation of the ATP-ase, which supports the energy necessary to release calcium. Besides this, as a direct result of vitamin D_3, the 25(OH)D_3-24-hydroxylase activity of the intestinal epithelial cells and kidney tubules also increases, which increases the turnover of vitamin D_3 by intensive catabolism. Presumably, estrogens also play a part in the activation of the PMCA system (Dick et al., 2003, Bar, 2009). The NCX/ion-exchange mechanism is less known in birds. It activates in cases of calcium deficiency and is regulated by vitamin D_3 and PTH. It plays a smaller role in mammalian intestinal epithelial cells (Centeno et al., 2004).

The calcium that enters the intestinal mucosa and then the vessels of the serosa by intra-, and paracellular ways reaches the lumen of the uterus in about 11-14 hours via the blood stream. The main driving force behind it is the significantly increased activity of the carbonic anhydrase enzyme in the tissue of the uterine wall, which "sucks over" the calcium taken up by the intestine. The functioning of the the carbonic anhydrase enzyme is an energy dependent process, which shows a daily pattern of activity. The on-off regulation of the

enzyme activity is connected to the hormonal changes of the ovulation-egg production cycle. The off mechanism is probably connected to the increase in progesterone level, while on is related to not-yet-identified hormonal regulator(s), and regulatory factors of albumen production (Bar, 2009).

At the end of puberty and during egg laying, **estrogens themselves and also in synergism with androgens** increase the intensively increasing calcium absorption in the intestine, its transport and secretion into the lumen of the uterus. This has been proven experimentally, by administering androgen and estrogen together, which increased calcium absorption activity of the intestine in chicken (Common et al., 1948). Calcium absorption and transport were found to be the most intensive during the period of eggshell formation (8–12 hours before oviposition), simultaneously with the occurrence of peak values of estradiol, estrone and testosterone in blood plasma of laying hens (Péczely et al., 1980). The effect of steroids is indirect, since it occurs through increased synthesis of vitamin D_3. Increased estrogen level causes increased 25-hydroxycholecalciferol synthesis in the liver cells and increased 25-OH-D_3-1-hydroxylase activity in the cells of the kidney tubules, and therefore increase active vitamin D_3 synthesis (Tanaka et al., 1976, Sedrant et al., 1981): The calbindin content of the intestine shows a small, gradual increase in female birds during sexual maturation (Wu et al., 1994), and the same effect can be achieved in juvenile birds by administering estrogen and testosterone together (Nys et al., 1984).

On the other hand, estrogen probably has a direct effect in the intestine: it increases the amount of one of the calcium channels (TRPV6) in the epithelial cells and Ca^{2+} uptake (Hoenderop et al., 2005). Estrogens (and possibly androgens) also directly affect the increased calcium turnover, presumably through the concentration gradient between the intestinal mucosa and serosa, which can reach 25% during eggshell formation (Luck and Scanes, 1979).

In the area of the **uterus** there is also a robust change in calcium transport following maturation. Ca^{2+} and the corresponding levels of HCO_3 transport strongly fluctuate during the cycle of eggshell formation, showing a maximum value during the second part of the cycle (in domestic hen the increase of transport processes begins at 12 hours and ends at 22 hours after ovulation, reaching a maximum value at about 16-18 hours postovulation). Ca^{2+} transport in the uterus is not closely connected with the calcium balance (homeostasis) of the organism nor with vitamin D metabolism, but it is strongly influenced by HCO_3^-, Na^+, H^+ and Cl^- cotransport, and the secretion of matrix proteins of the shell (Vetter and O'Grady, 2005, Striem, 1990, Corradino et al., 1993).

During eggshell formation calbindin, as a transport protein, also plays an important role in the calcium excretion mechanism of the uterus. Calbindin-28Dk mostly expresses in the tubular propria glands of the uterus and the "red isthmus" located above it. The highest amount of calcium transport (secretion) originates from these tubular glands towards the uterine lumen. Calbindin is

synthesised according to a daily rhytm during the ovipository cycle, and is temporarily stored in the gland cells. Its mRNA content is low when the egg passes through the infundibulum, the magnum and the isthmus, but increases significantly when the egg enters the uterus. Its gene expression, however, is not influenced by the mechanical effect of the passing egg (Wasserman et al., 1991, Bar et al., 1992, Ieda et al., 1995). Calbindin secretion follows osteopontin expression – which can also be linked to the passing of the egg – in about two hours, even though there is no functional connection between the two (Lavelin et al., 2000).

Among sexual steroids, estrogens are regulatory factors of calbindin-D28k synthesis in the uterus: in the cells of the tubular glands estrogen (E2) stimulates calbindin-D28k synthesis, while the tissue concentration of active vitamin D_3 in the uterus does not affect calbindin-D28 production. Treatment with active vitamin D_3 and changing the vitamin D_3 content of the food do not affect the calbindin content of the uterus, while these treatments increase calbindin expression in the intestine and the kidney. This is a fundamental difference compared to the intestine, as here the intake of vitamin D_3 strongly stimulates calbindin-D28k synthesis and through this, indirectly increases calcium absorption and transport from the chimus (Corradino et al, 1993).

There is another active calcium transport and excretion mechanism present in the uterus and the distal isthmus: the mitochondrial Ca^{2+}-ATP-ase. Large amounts of this enzyme are present in the epithelial cells of the mucosa and tubular propria glands. Intracellularly it is mostly located in the zone of the apical microvilli. The active Na+ pump towards the propria and the serosa, which facilitates Ca^{2+} uptake from the blood by cotransport, plays an important role in this energy-dependent process. This calcium uptake probably occurs via TRPV-6 channels, which are known from the mammalian uterus, but in birds has not yet been detected (Bar, 2009).

Progesterone and glycocorticoids inhibit the calbindin-D28k mRNA synthesis in the uterus, while testosterone does not affect calbindin synthesis (Bar et al., 1996).

In addition to the active Ca^{2+} transport (excretion) in the uterus, there are probably other, paracellular mechanisms too. Passive transport occurs among the epithelial cells of the mucosa, with a mechanism similar to that in the intestine. The driving force behind it can be the electric potencial difference between the uteral lumen and the mucosa, and the fact the Ca^{2+} that gets into the luminal liquid binds immediately to the available HCO_3^-, changing the charge, and this process is (also) catalised by semi-liquid matrix proteins (Eastin and Spaziani, 1978a,b).

Recent studies suggest that HCO_3^- production by the uterus is the most important regulator of Ca^{2+} transport. A tight correlation can be shown between the active secretion of HCO_3^- into the lumen, the intensity of Na^+ and

Cl^- transport from the lumen of the uterus into the plasma and the expression of the Na^+-K^+ ATP-ase enzyme during the egg laying cycle. In case of a low Na^+ content in the lumen, if its transport is blocked, or if the carbonic anhydrase activity of the uterine wall is blocked by the administration of acetazolamide, Ca^{2+} transport significantly decreased (Bar, 2009).

During puberty increasing prolactin levels can play a role in the formation of active vitamin D_3 and by sinergistically enhancing its effect in the increasing of calcium turnover. Prolactin also increases 25-OH-D_3-1-hydroxylase activity in the kidney, and therefore active vitamin D_3 synthesis (Spanos et al., 1976). Prolactin also stimulates the mRNA expression of TRPV-6 calcium channels in the intestine, and together with the increased active D_3 tissue concentration, increases calbindin synthesis and therefore the absorption and transport activity of the intestine (Ajibade et al., 2010).

The Ca^{2+} concentration of the blood plasma and the extracellular space are regulated by the **parathyroid hormone (PTH)**. Parathyroid hormone regulates the calcium-phosphorous homeostasis in a way, that the calcium-sensitive receptors (CaSR) of the parathyroid cells register a decrease in calcium instantaneously, and can increase hormone secretion very fast. Calcium-sensitive receptors of the parathyroid have an extracellular domain of about 620 members. There are at least five calcium-binding sites on these septahelical membrane receptors, which have a G protein-binding C-terminal consisiting of 222 amino acid residues. The calmodulin-binding domain is located on this C terminal, which registers differences between intra and extracellular calcium levels (Brown et al., 1995, Huang et al., 2009, 2010).

PTH in birds, similar to mammals, consists of 84 amino acid residues, among which the 1-34 N-terminal is responsible for PTH-1R activation, calcium reabsorption and phosphate extraction in the kidney tubules, increased osteoclast activity, and 1,25 $(OH)_2$-D_3 synthesis by the kidney. The C-terminal, which consists of 35-84 elements, secretes in a large amount, partly independently and partly formed in the blood by proteolysis. It acts mostly on bone reabsorption. The other parathyroid hormone type (PTH-2H) is mostly found in certain areas of the central nervous system and does not take part in the mediation of "classic" PTH effects. PTH receptors are septahelical transmembrane type structures, which bind G protein. Type 1 is specific to both PTH and PTH-related peptide, while Type 2 is only specific to PTH (Turner et al., 1998, Murray et al., 2005). PTH-specific PTH/PTHrP, through its Type 1 receptors, initially increases Ca^{2+} reabsorption and 1α-hydroxylase activity in the kidney tubules, and therefore the synthesis of 1,25 dihydroxycholecalciferol. In the second step, active vitamin D_3, produced in the kidney, increases Ca^{2+} absorption through increasing calbindin sythensis. Additionally, PTH direcly increases calcium uptake by intestinal epithelial cells in vitro, as there are PTH/PTHrP receptors in the apical basolateral membrane (Hoenderop et al., 2005).

The effect of increased parathyroid hormone production can be observed in the regulation of calcium metabolism in female birds, especially in the kidney. The amount of PTH receptors in the kidney tubules proportionally changes with the rate of calcium reabsorption, their number increases during egg shell formation. During eggshell formation, in the proximal kidney tubules PTH directly increases Ca^{2+} reabsorption from the primary urine, while by activating the protein kinase-C and -A systems, through the Type II. Na^{+}-PO_4-co-transporter, the hormone substantially decreases PO_4^{2-} reabsorption (i.e. increases phosphate extretion). At the same time, increased 1α-hydroxylase activity in the kidney suggests a more intensive secretion of PTH-dependent active vitamin D_3 (Wideman, 1987, Elaroussi et al., 1994, Dudas et al., 2002). PTH increases the activity of adenylate cyclase in the kidney tubules, which stimulates 1α-hydroxylase and through this active vitamin D_3 synthesis. The increase in adenylate cyclase activity is an estrogen-dependent process, which occurs by increasing the expression of PTH receptors through increased plasma estrogen levels during the ovulatory-ovipositional cycle. The increased 1,25 dihydroxy-cholecalciferol production in the kindey tubules is therefore regulated by a PTH and estrogen synergism (Elaroussi et al., 1993).

According to some studies, hight PTH level in the intestine does not affect its Ca^{2+} absorption capacity (Nemere and Larsson, 2002). Recent studies on cell cultures however suggest that PTH increases Ca^{2+} uptake of the intestinal epithelial cells by activating protein kinase-A (Sterling and Nemere, 2007).

PTH receptors are also present in the uteral mucosa, and their Kd and Bmax values substantially decrease during shell formation, suggesting their functional role (Ogawa et al., 2000).

During puberty increased estrogen and androgen secretion by the ovary, increased active vitamin D_3 synthesis and the subsequent intensive calbindin production largely increase the calcium absorption capacity of the intestine. Most of the "excess" calcium that gets into the blood plasma – if there is no eggshell formation at the time – moves into the medullar cavity of the tubular bones, which are usually filled with air-sac diverticula. This leads to the formation of secondary bone tissue, **medullary bone material** with a trabecular structure. Trabecula rich in blood vessels, forming thin appendices, which grow from the outer dense cortical bone material inwards, mostly appear in the medullar cavity of the femur and the tibio-tarsus. Globular structures that consist of keratan sulphate and contain sulphur appear in the developing osteoid tissue in the matrix material of the medullary bone, which will serve as starting points for the process of calcification (Yamamoto et al., 2005). This process can increase the skeletal mass by as much as 20% (Péczely, 1987).

In species that lay early morning (domestic fowl and several wild birds with a strong diurnal rhytm) eggshell formation occurs during the night ("dark period"), when the intestine does not contain food, i.e. calcium any more.

Calcium content of the intestine gets absorbed or eliminated with the faeces 4-5 hours after feeding concludes. In this case, the female bird needs another calcium depot, supplied by the calcium in the medullary bones. Accordingly, there are typical phases of change in the medullary bone tissue.

The **calcium content of the food** (mostly in the form of $CaCO_3$ and $CaPO_4$) determines the degree to which medullary bone material is involved in eggshell formation. In domestic hen if this value is over 3.65%, Ca^{2+} absorbed by the intestine – if eggshell formation is occurring at the time – gets transported directly into the uterus and gets incorporated into the eggshell. If there is no egg with a calicifying shell in the lumen of the uterus, some of the Ca^{2+} that enters into the circulation gets stored in the medullary bones, while the rest gets eliminated from the body via the kidney. If the calcium content of the food of a laying hen is under 1.95% and there is egg formation, 30-40% of the calcareous shell originates from the medullary bone tissue. In case the laying hen is fed a calcium-poor diet, there is a constant flux of calcium from the bone system towards the uterus, which can lead to a 38% decrease of the skeletal mass. In this case, the shell of the laid eggs get thinner and eventually ovulation (egg laying) ceases. In birds that are permanently on a constant calcium poor diet, a significant reorganisation of the bone material occurs, and unexpectedly, it is the cortical bone tissue that starts to get thinner first. This phenomenon suggests that there is constant regorganisation between medullary and cortical bone materials, and the outflux of the Ca^{2+} content of the bone tissue as a result of eggshell forming occurs through structural changes in the cortical bones (Hurwitz and Bar, 1969, Simkiss, 1967).

Tyler (1954) studied calcium transport in medullary bones by marking with $Ca^{2+}45$, checking the incorporation of marked and unmarked (originating from the food) calcium into the eggshell. If there was enough dietary calcium and the bird was feeding constantly during the day (calcium uptake) and at the beginning of the night, when there was still chimus in the intestine, the absorbed calcium got straight into the uterus. The calcium storage of the medullary bone tissue only got utilised when the level of absorption got under a certain critical threshold.

In hens that were kept on a chronically calcium-poor diet (with plasma calcium concentrations under 1.0 mM) or when the calcium depots of the medullary bones got depleted, egg laying decreased and eventually stopped, to ensure the maintanence of a minimum plasma level of calcium for physiological purposes. The ceasing of egg laying is caused by a direct inhibition of GnRH (stopping of LH release) by a subphysiological calcium level. Plasma calcium level under a critical threshold blocks the functioning of the non-L type calcium channels in the LH cells, which inhibits the occurrence of the preovulatory LH peak (Davidson et al., 1987, Liu et al., 1995). This hypothesis is confirmed by the fact that treatment of these hens with LH or hypothalamus extract can

reinitiate egg laying, but the laid eggs will have thin and later soft shell (Luck and Scanes, 1979).

During eggshell formation, when plasma Ca^{2+} level decreases, the amount and size of **osteoclasts** in the medullary bone tissue substantially increases. These osteoclasts absorb inorganic bone material, have many nuclei, their surface is segmented by several sheet-like extentions and their edge is characteristically frilled. The amount of frills increases in the periphery of these enlarged osteoclasts, and a large amount of lysosomes appear in the frills. The fluoride-resistant acid phosphatase and cathepsin-B content of these lysosomes, as well as the H^+ and Cl^- that form in the peripheral cytoplasm by the the effect of H^+-ATP-ase (and create hypochlorous acid extracellularily) dissolve the inorganic material of the bone. Ion transport and excretion are regulated by tyrosine kinase. Some of the Ca^{2+} and PO_4^{2-} reabsorbed from the forming suspension gets into the phagosomes of the osteoclasts, while others move directly into the rich blood capillary system by diffusion. The dissolving of the bone material is enhanced by tartarate-resistant acid phosphatase that appears in the matrix material of the medullary bones and presumably is mostly produced by the stroma cells. When there is no eggshell formation, the frilliness of the osteoclasts decreases and even disappears, and the amount of lysosomes concentrated in the periphery strongly decline (Sturkie, 1976, Miller and Kenny, 1985, Yamamoto and Nagai, 1992, Sugiyama and Kusuhara, 1993, Pederson et al., 1997, Williams et al., 1998).

The release of Ca^{2+} from medullary bone tissue and the onset of hypercalcemia in the blood circulation are regulated by a concerted action of PTH, calcitonin, calmodulin, adrogens and estrogens and their receptors are found in the osteoclasts.

Plasma **PTH** level shows a substantial increase during eggshell formation, when osteoclasts activate in the medullary bones and there is an increase in calcium transport towards the uterus (van de Velde et al., 1984). PTH simulates the synthesis of certain cytokins in the osteoblasts and in the stroma cells, decreasing the production of osteoprotegerin, which has an antiresorptive effect. These indirect effects – which are not accompanied by the expression of PTH receptors – increase osteoclast differentiation and the reabsorption activity of mature osteoclasts. On the other hand, there is also a PTH effect that manifests through the receptors. PTH1R has been detected in the membrane of avian osteoclasts, which binds the whole (1-84 amino acids) PTH protein with a high affinity as well as the 1-34 amino acid containing N terminal. Presumably, PTH increases Ca^{2+} reabsorption activity in the medullary bones indirectly (through stimulating osteoclast differentiation), as well as directly through the mature cells (through increasing acid phosphatase activity) (Murray et al., 2005).

Calcitonin increases acid excretion in the osteoclasts indirectly, by activating their cytoskeletal system. Calcitonin causes contraction in the cytoskeletal

apparatus of the osteoclasts (actin, vimentin and tubulin) and makes them congregate centrally, while the size of the cell decreases. On the other hand, in vitro studies show that calcitonin decreases the acidity of the osteoclasts, which suggests increased excretion of H^+ and Cl^- ions from the cells (Hunter et al., 1988, 1989).

During active bone material resorption, **calmodulin** expression increases in the cytoplasm of osteoclasts. The forming calmodulin moves into the periphery of the cell, and accumulates under the lobulated surface. Calmodulin plays an important part in the intracellular acid transport by activating H^+-ATP-ase, and in the dissolution of bone material. It is activated by the intracellular Ca^{2+} concentration, which has increased through reabsorption, and it acts through calcineurin and CaMKII protein. Both have phosphatase activity, which makes it a self-reinforcing mechanism (Williams et al., 1996, Seales et al., 2006).

Estrogen decreases lysosomal enzyme production and bone resorption activity in the osteoclasts, acting through nuclear and membrane **estrogen receptors**. A 66 kDa and a 140 kDa receptor protein have been identified by using anti-human estrogen receptor antibody. The 66 kDa protein showed high consistency with the human cytosol estrogen receptor. The 140 kDa protein **membrane-bound estrogen receptor** is probably responsible for the very quick (within hours) nuclear proto-oncogen expression and the fast decrease (41%) in HCl production in the osteoclasts. Changes in plasma estrogen levels in the medullary bones therefore result in a fast and a slow bone material resorption (Oursler et al., 1991, 1993, Brubaker and Gay, 1994, Pederson et al., 1997).

Osteoclasts also posess specific cytosol-nucleus **androgen** receptors. In vitro treatment with androgen decreases catepsin-B and tartarate-resistent acid phosphatase production in the osteoclasts, and their bone material resorption activity. At the same time, osteoclast TGF-β production increased, which is a typical cellular-level effect of androgens. Some androgens can be converted into estrogens in the bone tissue by aromatisation, and in this form, bound to cytosol estrogen receptors, they can also decrease resorption (Pederson et al., 1999, Vanderschueren et al., 2004).

The bone-building **osteoblasts** mostly function when there is no eggshell formation, ie. during the daytime, when the bird is feeding, and this process is mostly regulated by estrogens. Five days of estrogen treatment triggered phosvitin synthesis in the liver, hypercalcemia, the appearance of medullary bone tissue and simultaneously the decrease of cortical bone material in male Japanese quail. The amount of osteoblasts increased in the medullary bone tissue, and they possessed a definite estrogen-binding capacity. The concurrent administration of anti-estrogen tamoxifen protects agains hypercalcemia, the formation of medullary bone tissue and the associated decrease in cortical bone material (Turner et al., 1993). In an in vitro system, in three days, estrogen causes the formation of **estrogen-α receptors** in the cytosol and the

cell membrane of isolated osteoblasts, and the alkaline phosphatase activity of the cells to increase substantially (Hiyama et al., 2009). The estrogen increasing calcium incorporation into the medullary bones can originate from androgens, which form by aromatisation in the periphery. Letrozole, which inhibits aromatase, decreases plasma estrogen levels, the concentration of alkaline phosphatase and calcium in the plasma and the expression of estrogen receptors in medullary bone cells (Deng et al., 2010). This observation is supported by finding that treatment with testosterone, as well as testosterone combined with estradiol both increase osteoblast proliferation in the medullary bone tissue (Chen et al., 2010). Androgens can also simulate bone tissue metabolism indirectly: according to human studies, there is localised **androgen receptor** expression in the cytosol and in the membrane of osteoblasts, osteoclasts and the stroma cells of medullary bones. Androgen stimulates the synthesis of cytokins and growth factors, and estrogens formed by aromatisation from DHEA in the bone tissue also have a similar effect (Adachi and Takayanagi, 2006).

References 2/2., 3., 4.

1. Adachi M, Takayanagi R (2006): Role of androgens and DHEA in bone metabolism Clin.Calcium 16, 61-66.
2. Aitken RNC (1971): Physiology and biochemistry of domestic fowl Vol.3, 1237, Academic Press, New York – London
3. Ajibade DV, Dhawan P, Fechner AJ, Meyer MB, Pike JW, Christakos S (2010): Evidence for a role of prolactin in calcium homeostasis: regulation of intestinal transient receptor potential vanilloid type 6, intestinal calcium absorption, and the 25-hydroxyvitamin D(3) 1alpha hydroxylase gene by prolactin Endocrinology 151, 2974-2984
4. Alisio A, Canas F, de Bronia DH, Pereira R, Tolosa de Talamoni N (1997): Effect of vitamin D deficiency on lipid composition and calcium transport in basolateral membrane vesicles from chick intestine Biochem.Mol.Biol.Int., 42, 339-347.
5. Ar A, Rahn H (1985): Pores in avian eggshells: gas conductance, gas exchange and embryonic growth rate Respir.Physiol., 61, 1-20.
6. Bakst MR (2011): Role of the oviduct in maintaining sustained fertility in hens In: Physiol.Endocr. Symposium, J.Anim.Sci., 89, 1323-1329.
7. Bar A (2009): Calcium transport in strongly calcifying laying birds: mechanisms and regulation Comp.Biochem.Physiol. A Mol.Integr.Physiol., 152, 447-469.
8. Bar A, Eisner U, Montecuccoli G, Hurwitz S (1976): Regulation of intestinal calcium absorption in the laying quail: independent of kidney vitamin D hydroxylation J.nutr., 106, 1336-1342.
9. Bar A, Hurwitz S (1984): Egg shell quality, medullary bone and intestinal calcium and phosphorous absoption, and calcium-binding protein in phosphorous deficient hens Poult.Sci., 63, 1975-1979.
10. Bar A, Maoz A, Hurwitz S (1979): Relationship of intestinal and plasma calcium-binding protein to intestinal calcium absoption FEBSLett., 102, 79-81.
11. Bar A, Vax E, Hunziker W, Halevy O, Striem S (1996): The role of gonadal hotmones in gene expression of calbindin (Mr 28,000): in the laying hen Gen.Comp.Endocrinol., 103, 115-122.
12. Bar A, Vax E, Striem S (1992): Relationships between calbindin (Mr 28,000) and calcium transport by the eggshell gland Comp.Biochem.Physiol.B 101, 845-848.
13. Board RG, Hornsey DJ (1978): Chemical Zoology vol.10, 37.,(eds.: M.Florkin, BT Scheer) Acad. Press New York – London

14. Brown EM, Pollak M, Hebert SC (1995): Sensing of extracellular Ca^{2+} by parathyroid and kidney cells: cloning and characterization of an extracellular $Ca(^{2+})$-sensing receptor Am.J.Kidney Dis., 25, 506-513.
15. Brubaker KD, Gay CV (1994): Specific binding of estrogen to osteoclast surfaces Biochem.Biophys. Res.Commun., 200, 899-907.
16. Brush AH (1978): Chemical Zoology, vol. 10, 141. (eds.: M.Florkin, BT Scheer) Acad. Press, New York – London
17. Centeno VA, Diaz de Barboza GE, Marchionatti AM, Alisio AE, Dallorso ME, Nasif R, Tolosa de Talamoni NG (2004): Dietary calcium deficiency increases Ca^{2+} uptake and Ca^{2+} extrusion mechanisms in chick enterocytes Comp.Biochem.Physiol. A Mol.Integr.Physiol., 139, 133-141.
18. Chen X, Deng Y, Zhou Z, Tao Q, Zhu J, Li X, ChenJ, Hou J (2010): 17beta-estradiol combined with testosterone promotes chicken osteoblast proliferation and differentiation by accelerating the cell cycle and inhibiting apoptosis in vitro Vet.Res.Commun., 34, 143-152.
19. Chien YC, Hincke MT, McKee MD (2009): Avian eggshell structure and osteopontin Cell Tissues Organs 189, 38-43.
20. Chien YC, Hincke MT, Vali H, McKee MD (2008): Ultrastructural matrix-mineral relationships in avian eggshell, and effects of osteopontin on calcite growth in vitro J.Struct.Biol., 163, 84-99.
21. Chousalkar KK, Roberts JR (2008): Ultrastructural changes in the oviduct of the laying hen during the laying cycle Cell Tissue Res., 332, 349-358.
22. Christakos S, Dhawan P, Ajibade D, Benn BS, Feng J, Joshi SS (2010): Mechanisms involved in vitamin D mediated intestinal calcium absoption and in non-classical actions of vitamin D J.Steroid Biochem.Mol.Biol., 121, 183-187.
23. Christakos S, Dhawan P, Benn B, Porta A, Hediger M, Oh GT, Jeung EB, Zhong Y, Ajibade D, Dhawan K, Newark NJ (2007): Vitamin D: molecular mechanism of action Ann.NY. Acad Sci., 1116, 340-348.
24. Cohen A, Bar A, Eisner U, Hurwitz S (1978): Calcium absorption , calcium binding protein, and egg shell quality in laying hens fed hydroxylated vitamin D derivates Poult.Sci., 57,1646-1651.
25. Cohen I, Hurwitz S (1974): Intracellular pH and electrolyte concentration in the uterine wall of the fowl in relation to shell formation and dietary minerals Comp.Biochem.Physiol. A 49, 689-696.
26. Common RH, Bolton W, Rutledge WA (1948): The influence of gonadal hormones on the composition of the blood and liver of the domestic fowl J.Endocrinol., 5, 263-273.
27. Corradino RA, Smith CA, Krook LP, Fullmer CS (1993): Tissue specific regulation of shell gland calbindin D28K biosynthesis by estradiol in precociously matured vitamin D-depleted chicks Endocrinology 132,193-198.
28. Dahm HH, Schramm U, Lange W (1980): Scanning and transmission electron microscopic observations of the cloacal epithelia of the domestic fowl Cell Tissue Res., 211, 83-93.
29. Davidson JS, King JA, Millar RP (1987): Luteinizing hormone release from chicken pituitary cells: synergism between calcium and protein kinase C and its inhibition by calmodulin antagonists Endocrinology 120, 692-699.
30. Deng YF, Chen XX, Zhou ZL, Hou JF (2010): Letrozole inhibits the osteogenesis of medullary bone in prelay pullets Poult.Sci., 89, 917-923.
31. Dick IM, Liu J, Glendenning P, Prince RJ (2003): Estrogen and androgen regulation of plasma membrane calcium pump activity in immortalized distal tubule kidney cells Mol.Cell.Endocrinol., 212, 11-18.
32. Droba M, Droba B, Bledniak D (2006): Acid glycosidases in the isthmus of the hen oviduct and egg shell membranes Reprod.Biol., 6, Suppl.2, 55-63.
33. Dudas PL, Villalobos AR, Gocek-Sutterlin G, Laverty G, Renfro JL (2002): Regulation of transepitheliai phosphate transport by PTH in chicken proximal tubule epthelium Am.J.Physiol. Regul.Integr. Comp. Physiol., 282, R139-146.
34. Eastin WC Jr, Spaziani E (1978a): On the mechanism of calcium secretion in the avian shell gland (uterus) Biol.Reprod., 19, 505-518.
35. Eastin WC, Spaziani E (1978b): On the control of calcium secretion in the avian shell gland (uterus) Biol.Reprod., 19, 493-504.

36. Elaroussi MA, Forte LR, Eber SL, Biellier HV (1993): Adaptation of the kidney during reproduction: role of estrogen in the regulation of responsiveness to parathyroid hormone Poult.Sci., 72, 1548-1556.
37. Elaroussi MA, Forte LR, Eber SL, Biellier HV (1994): Calcium homeostasis in the laying hen. 1. Age and dietary calcium effects Poult. Sci., 73, 1581-1589.
38. Fernandez MS, Escobar C, Lavelin I, Pines M, Arias JL (2003): Localization of osteopontin in oviduct tissue and eggshell during different stages of the avian egg laying cycle J.Struct. Biol., 143, 171-180.
39. Fraser AC, Bain MM, Solomon SE (1999): Transmission electron microscopy of the vertical crystal layer and cuticle of the eggshell of the domestic fowl Br.Poult.Sci., 40, 626-631.
40. Friess AE, Sinowatz F, Wrobel KH, Scklek-Winnisch R (1978): The uterovaginal sperm host glands of the quail (Coturnix coturnix japonica): an ultrastructural and ultracytochemical study Cell Tissue Res., 191, 101-114.
41. Fujita H, Sugimoto K, Inatomi S, Maeda T, Osanai M, Uchiyama Y, Yamamoto Y, Wada T, Kojima T, Yokozaki H, Yamashita T, Kato S, Sawada N, Chiba H (2008): Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca^{2+} absorption between enterocytes Mol.Biol.Cell., 19, 1912-1921.
42. Gilbert AB (1971): Physiologie and biochemistry of the domestic fowl (eds.: DJ Bell, BM Freeman) Vol. 3, acad.Press New York – London
43. Gong D, Wilson PW, Bain MM, McDade K, Kalina J, Hervé-Grépinet V, Dunn IC (2010): Gallin: an antimicrobial peptide member of a new avian defensin family, the ovodefensins, has been subject to recent gene duplication BMC Immunol., 11:12.
44. Guzsal E (1981): Háziállatok szövettana, 353-444., Mezôgazdasági Kiadó, Budapest
45. Hincke MT, Nys Y, Gautron J, Mann K, Rodriguez-Navarro AB, McKee MD (2012): The eggshell: structure, composition and mineralization Front.Biosci., 17, 1266-1280.
46. Hiyama S, Sugiyama T, Kusuhara S, Uchida T (2009): Evidence for the expression of estrogen receptors in osteogenic cells isolated from hen medullary bone Acta Histochem., 111, 501-507.
47. Hobbs CA, Deterding LJ, Perera L, Bobay BG, Thompson RJ, Darden TA, Cavanagh J, Tomer KB (2009): Structural characterization of the conformational change in calbindin-D28k upon calcium bindintg using differential surface modification analyzed by mass spetrometry Biochemistry 48, 8603-8614.
48. Hoenderop JG, Nilius B, Bindels RJ (2005): Calcium absorbcion across epithelia Physiol.Rev., 85, 373-422.
49. Huang Y, Zhou Y, Castiblanco A, Yang W, BrownEM, Yang JJ (2009): Multiple $Ca^{(2+)}$-binding sites in the extracellular domain of the $Ca^{(2+)}$-sensing receptor corresponding to cooperative $Ca^{(2+)}$ response Biochemistry 48, 388-398.
50. Huang Y, Zhou Y, Wong HC, Castiblanco A, Chen Y, Brown EM, Yang JJ (2010): Calmodulin regulates Ca^{2+}-sensing receptor-mediated Ca^{2+} signaling and its cell surface expression J.Biol. Chem., 285, 35919-35931.
51. Hunter SJ, Schraer H, Gay CV (1988): Characterization of isolated and cultured chick osteoclasts: the effects of acetazolamide, calcitonin, and parathyroid hormone o nacid production J.Bone Miner.Res., 3, 297-303.
52. Hunter SJ, Schraer H, Gay CV (1989): Characterization of the cytoskeleton of isolated chick osteoclasts: effect of calcitonin J.Histochem.Cytochem., 37, 1529-1537.
53. Hurwitz S, Bar A (1969): Intestinal calcium absoption in the laying fowl and its importance in calcium homeostasis Am.J.Clin.Nutr., 22, 391-395.
54. Hurwitz S, Bar A (1972): Site of vitamin D action in chick intestine Am.J.Physio., 222, 761-767.
55. Hurwitz S, Bar A, Cohen I (1973): Regulation of calcium absorption by fowl intestine Am.J.Physiol., 225, 150-154.
56. Ieda T, Saito N, Ono T, Shimada K (1995): Effects of presence of an egg and calcium deposition in the shell gland on levels of messenger ribonucleic acid of CaBP-D28K and of vitamin D3 receptor in the shell gland of the laying hen Gen.Comp.Endocrinol., 99, 145-151.

57. Jacob M, Bakst MR (2007): Developmental anatomy of the female reproductive tract In: Reproductive Biology and Phylogeny of Birds (ed.: BGM Jamieson), 149- 179., Science Publishers Enfield, Jersey, Plymouth
58. Khanal RC, Peters TM, Smith NM, Nemere I (2008): Membrane receptor-initiated signaling in 1,25(OH)2D3-stimulated calcium uptake in intestinal epithelial cells J.Cell Biochem., 105, 1109-1116.
59. Koyanagi F, Nishiyama H (1981): Disintegration of spermatozoa in the infundibular sperm-host glands of the fowl Cell Tissue Res., 214, 81-87.
60. Lakshminarayanan R, Joseph JS, Kini RM, Valiyaveettil S (2005): Structure-function relationship of avian eggshell matrix proteins: a comparative study of two major eggshell matrix proteins, anisocalcin and OC-17 Biomacromolecules 6, 741-751.
61. Lakshminarayanan R, Manjunatha K, Valiyaveettil S (2002): Investigation of the role of anisocalcin in the biomineralization in goose eggshell matrix Proc.Natl.Acad.Sci. USA, 99, 5155-5159.
62. Lakshminarayanan R, Manjunatha Kini R, Valiyaveettil S (2002): Investigation of the role of ansocalcin in the biomineralization in goose eggshell matrix PNAS 99, 5155-5159.
63. Lauguier C, Clastrat B, Brard E (1978): Plasma estradiol levels in quail during sexual development and after different estrogenic treatments C.R.Acad.Sci.Hebd.Seances acad.Sci.D. 287, 293-296.
64. Lavelin I, Meiri N, Pines M (2000): New insight in eggshell formation Poult.Sci., 79, 1014-1017.
65. Lavelin I, Yarden N, Ben-Bassat S, Bar A, Pines M (1998): Regulation of osteopontin gene expression during egg shell formation in the laying hen by mechanical strain Matrix Biol., 17, 615-623.
66. Leach RM Jr. (1982): Biochemistry of the organic matrix of the eggshell Poult.Sci., 61, 2040-2047.
67. Leach RM Jr., Gross JR (1983): The effect of manganese deficiency upon the ultrastructure of the eggshell Poult.Sci., 62, 499-504.
68. Leach RM Jr., Rucker RB, Van Dyke GP (1981): Egg shell membrane protein: a nonelastin desmosine/isodesmosine-containing protein Arch.Biochem.Biophys., 207, 353-359.
69. Leach RM Jr., Rucker RB, Van Dyke GP (1981): Egg shell membrane protein: a nonelastin desmosine/isodesmosine-containing protein Arch.Biochem.Biophys., 207, 353-359.
70. Leathers VL, Linse S, Forsén S, Norman AW (1990): Calbindin-D28K, a 1 alpha,25-dihydroxyvitamin D3-induced calcium-binding protein, binds five or six Ca^{2+} ions with high affinity J.Biol.Chem., 265, 9838-9841.
71. Liu RC, Lea RW, Sharp PJ (1995): Sexually differentiated role of calcium ion sin chicken GnRH-I stimulated release of LH from anterior pituitary glands from adult domestic chickens Gen.Comp. Endocrinol., 100, 267-272.
72. Lörcher K, Hodges RD (1969): Some possible mechanisms of formation of the carbonate fraction of egg shell calcium carbonate Comp.Biochem. Physiol., 28, 119-128.
73. Luck MR, Scanes CG (1979): The relatioship between reproductive activity and blood calcium in the calcium-deficient hen Br.Poult.Sci., 20, 559-564.
74. Mao KM, Sultana F, Howlider MA, Iwasawa A, Yoshizaki N (2006): The magnum – isthmus junction of the fowl oviduct participates in the formation of the avian-type shell membrane Zoolog.Sci., 23, 41-47.
75. Miller SC, Kenny AD (1985): Activation of avian medullary bone osteoclasts by oxidized synthetic parathyroid hormone Proc.Soc.Exp.Biol.Med., 179, 38-43.
76. Murray TM, Rao LG, Divieti P, Bringhurst FR (2005): Parthyroid hormone secretion and action: evidence for discrete receptors for the carboxyl-terminal region and related biological actions of carboxyl-terminal ligands Endocr.Rev.26, 78-113.
77. Nemere I, Feld C, Norman AW (1991): 1,25-Dihydroxyvitamin D3-mediated alterations in microtubule proteins isolated from chick intestinal epithelium: analyses by isoelectric focusing J.Cell.Biochem., 47, 369-379.
78. Nemere I, Larson D (2002): Does PTH have a direct effect on intestine? J.Cell Biochem., 86, 29-34.
79. Nys Y, de Laage X (1984): Effects of supression of eggshell calcification and of 1,25(OH)2D3 on MG^{2+}, Ca^{2+} and Mg^{2+}HCO-3 ATPase, alkaline phosphatase, carbonic anhydrase and CaBP levels II. The laying hen intestine Comp.Biochem.Physiol. A Comp.Physiol., 78, 839-844.
80. Nys Y, N'Guyen TM, Garabedian M (1984): Involvement of 1,25-dihydroxycholecalciferol in the short- and long-term increase of intestinal calciumabsorption in laying hens: stimulation by gonadal hormones is partly independent of 1,25-dihydroxycholecalciferol Gen.Comp.Endocrinol., 54, 59-68.

81. Ogawa H, Takahashi T, Yasuoka T, Kuwayama T, Tanaka K, Kawashima M (2000): Parathyroid hormone receptor binding property in the shell gland of oviduct of the guineafowl during an oviposition cycle Poult.Sci., 79, 575-579.
82. Oursler MJ, Osdoby P, Pyfferoen J, Riggs BL, Spelsberg TC (1991): Avian osteoclasts as estrogen target cells Proc.Natl.Acad.Sci.USA 88, 6613-6617.
83. Oursler MJ, Pederson L, Pyfferoen J, Osdoby P, Fitzpatrick L, Spelsberg TC (1993): Estrogen modulation of avian osteoclast lysosomal gene expression Endocrinology 132, 1373-1380.
84. Paczoska-Eliasiewicz H, Rzasa J, Mika M (1998): Changes of histamine concentration in chicken oviduct during the egg-laying cycle Zentralbl. Veterinarmed.A, 45, 69-73.
85. Parsons AH (1982): Structure of the eggshell Poult.Sci., 61, 2013-2021.
86. Péczely P (1987): A madarak szaporodásbiológiája, 11-110., Mezôgazdasági Kiadó, Budapest,
87. Pederson L, Kremer M, Foged NT, Winding B, Ritchie C, Fitzpatrick LA, Oursier MJ (1997): Evidence of a correlation of estrogen receptor level and avian osteoclast estrogen responsiveness J.Bone Miner.Res., 12, 742-752.
88. Pederson L, Kremer M, Judd J, Pascoe D, Spelsberg TC, Riggs BL, Oursler MJ (1999): 96, 505-510.
89. Phelps CB, Huang RJ, Lishko PV, Wang RR, Gaudet R (2008): Structural analyses of the ankyrin repeat domain of TRPV6 and related TRPV ion chanels Biochemistry 47, 2476-2484.
90. Pines M, Knopov V, Bar A (1995): Involvement of osteopontin in egg shell formation in the laying chicken Matrix Biol., 14, 765-771.
91. Rahman MA, Moriyama A, Yoshizaki N (2009): Cuticle formation in quail eggs Zoolog.Sci., 26, 496-499.
92. Rahn H, Paganelli CV, Ar A (1987): Pores and gas exchange of avian eggs: a review J.Exp.Zool.,Suppl. 1, 165-172.
93. Reyes-Grajeda JP, Moreno A, Romero A (2004): Crystal structure of ovocleidin-17, a major protein of the calcified Gallus gallus eggshell: implications in the calcite mineral growth pattern J.Biol. Chem., 279, 40876-40881.
94. Sandoz D, Boisvieux-Ulrich E, Laugier C, Brard E (1975): Interactions of oestradiol benzoate and progesterone on the development of the oviduct in quail (Coturnix coturnix japonica) II. Gen.Comp.Endocr., 26, 451-467.
95. Scott TA, Balnave D (1991): Influence of temperature, dietary energy, nutritient concentration and self-selection feedingon the retention of dietary energy protein and calcium by sexually-maturing egg-laying pullets Br.Poult.Sci., 32, 1005-1016.
96. Seales EC, Micoli KJ, McDonald JM (2006): Calmodulin is a critical regulator of osteoclastic differentation, function, and survival J.Cell Biochem., 97, 45-55.
97. Sedrant SH, Taylor TG, Akhtar M (1981): The regulation of 25-hydroxycholecalciferol metabolism in the kidney of japanese quail (Coturnix coturnix japonica) by sex hormones and by parathyroid extract Gen.Comp.Endocrinol., 44, 514-523.
98. Shahin MA (1973): Thesis, Cairo
99. Simkiss K (1967): Calcium in reproductive physiology, Chapman – Hall, London
100. Simkiss K (1975): Avian Physiology (ed.: M.Peaker) 307. Acad.Press New York – London
101. Singh R, Joyner CJ, Peddie MJ, Taylor TG (1986): Changes in the concentrations of parathyroid hormone and ionic calcium in the plasma of laying hens during the egg cycle in relation to dietary deficiencies of calcium and vitamin D Gen.Comp.Endocrinol., 61, 20-28.
102. Sterling TM, Nemere I (2007): Calcium uptake and membrane trafficking in response to PTH or 25(OH)D3 in polarized intestinal epithelial cells Steroids 72, 151-157.
103. Striem S (1990): Regulation of calcium-binding protein (calbindin28KDA) synthesis in the avian uterus Thesis, MSc.Degree, Hebrew University, Jerusalem
104. Sugiyama T, Kikuchi H, Hiyama S, Nishizawa K, Kusuhara S (2007): Expression and localisation of calbindin D28k in all intestinal segments of the laying hen Br.Poult.Sci., 48, 233-238.
105. Sugiyama T, Kusuhara S (1993): Ultrastructural changes of osteoclasts on hen medullarybone during the egg-laying cycle Br.Poult.Sci., 34, 471-477.
106. Sultana F, Yokoe A, Ito Y, Mao KM, Yoshizaki N (2003): The peri-albumen layer: a novel structure in the envelopes of an avian egg J.Anat., 203, 115-122.
107. Tanaka Y, Castillo K, DeLuca HF (1976): Control of renal vitamin D hydroxylases in birds by sex hormones Proc.Natl.Acad.Sci.USA 73, 2701-2705.

108. Tullett SG, Deeming DC (1982): The relationship between eggshell porosity and oxygen consumption of the embryo in the domestic fowl Comp.Biochem.Physiol. A Comp.Physiol., 72, 529-533.
109. Tullett SG, Lutz PL, Board RG (1975): The fine structure of the pores in the shell of the hen's egg Br.Poult.Sci., 16, 93-95.
110. Turner PR, Mefford S, Bambino T, Nissenson RA (1998): Transmembrane residues together with the amino terminus limit the response of the parathyroid hormone (PTH) 2 receptor to PTH-related peptide J.Biological Chemistry 273, 3830-3837.
111. Turner RT, Bell NH, Gay CV (1993): Evidence that estrogen binding sites are present in bone cells and mediate medullary bone formation in japanese quail Poult.Sci., 72, 728-740.
112. Tyler C (1954): Studies on egg shellsIV. the site of deposition of radioactive calcium and phosphorous J.Sci.Food Agri., 5, 335.
113. van de Velde JP, Loveridge N, Vermeiden JP (1984): Parathyroid hormone responses to calcium stress during eggshell calcification Endocrinology 115, 1901-1904.
114. Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C (2004): Androgens and bone Endocr.Rev., 25, 389-425.
115. Vetter AE, O'Grady SM (2005): Sodium and anion transport across the avian uterine (shell gland) epithelium J.Exp.Biol., 208, 479-486.
116. Walzem RL, Hansen RJ, Williams DL, Hamilton RL (1999): Estrogen induction of VLDLy assembly in egg-laying hens J.Nutr., 129, 4675-4725.
117. Wasserman RH, Smith CA, Smith CM, Brindak ME, Fullmer CS, Krook L, Penniston JT, Kumar R (1991): Immunohistochemical localization of a calcium pump and calbindin-D28k in the oviduct of the laying hen Histochemistry 96, 413-418.
118. Wideman RF Jr (1987): Renal regulation of avian calcium and phosphorus metabolism J.Nutr., 117, 808-815.
119. Williams JP, Blair HC, McKenna MA, Jordan SE, McDonald JM (1996): Regulation of avian osteoclastic H+ -ATPase and bone resorption by tamoxifen and calmodulin antagonists. Effects independent of steroid receptors J.Biol.Chem., 271, 12488-12495.
120. Williams JP, Jordan SE, Barnes S, Blair HC (1998): Tyrosine kinase inhibitor effects on avian osteoclastic acid transport Am.J.Clin.Nutr., 68, 1369S-1374S.
121. Wu JCY, Smith MW, Turvey A, Keable SJ, Colston KW (1994): Differential regulation of vitamin D receptor and intestinal calcium transport occurring during sexual maturation in the fowl (Gallus domesticus) Comp.Biochem.Physiol. A 109, 713-720.
122. WyburnGM,JohnstonHS,DraperMH,DavidsonMF(1973):Theultrastructureoftheshellformingregion of the oviduct and the development of the shell of Gallus domesticus Q.J.Exp.Physiol.Cogn.Med. Sci., 58, 143-151.
123. Yamamoto T, Nagai H (1992): A histochemical study of acid phosphatases in medullary bone matrix and osteoclasts in laying japanese quail J.Bone Miner.Res., 7, 1267-1273.
124. Yamamoto T, Nagaoka N, Hirata A, Nakamura H, Inoue M, Kawai M, Ikegame M (2005): Ultrastructural and immunohistochemical studies of medullary bone calcification, with special reference to sulphated glycosaminoglycans J.Electron.Microsc (To9kyo), 54, 29-34.
125. Yamamoto T, Ozawa H, Nagai H (1985): Histochemical studies of Ca-ATPase, succinate and NAD+ -dependent isocitrate dehydrogenases in the shell gland of laying Japanese quails: with special reference to calcium-transporting cells Histochemistry 83, 221-226.
126. Yu WC, el Halawani M, Fehrer SC, Leung BS (1987): Cytosolic progesterone receptors in the oviducts of reproductively active and quiscent turkeys (Meleagris gallopavo) Biol.Reprod., 37, 823-831.

3. Male reproductive organs

3.1. The testis

3.1.1. Postembryonic development of the testis

Around hatching (in domestic chicken on Day 19 of incubation and the first 7-10 days after hatching) histolytic processes start in the central part of the the thickened seminiferous tubules and lumen start to develop in the contorted seminiferous tubules. At this time dividing spermatogonia of 2–3 types are attached to the basal membrane of the wall of seminiferous tubules. Sertoli cells are typically hypertrophied and are mitotically dividing. During these days, the stroma material and the number of interstitial (peritoneal) cells among the seminiferous tubules also increase substantially. This period marks the onset of prepuberty.

3.1.1.1. Prepuberty

In young roosters, during the first month after hatching, the tescticular mass is intensively increasing, partly as the result of division of spermatogonia, which initially are not only present in one or two layers in the wall of the seminiferous tubules, but also from the intensive proliferation of Sertoli cells, which lasts for 8-9 weeks (Bozkurt et al., 2007). The cells with dark cytoplasm that are organised along the basal membrane are **stem** cell-like (Type A or Ad) spermatogonia, which for a while create very similar A1 (or Ap1) cells by mitotic division . After about a week or two, the division of spermatogonia becomes organised. The mitotic division of each spermatogonium A results in another A and an A1 spermatogonium. The A1 spermatogonium mitotically divides to create two Ap2 spermatogonia. Electromicroscopically, these latter show lighter cytoplasm. As a result of the increased number of spermatogenic cells, the wall of the tubules thickens, and the relative amount of interstitial (stroma) material decreases. Later, at the end of the first month – beginning of the second month, A2 spermatogonia mitotically divide to form Type B (transitional) spermatogonia, which have lighter nucleus and cytoplasm, but are richer in cellular organelles. B spermatogonia continue to divide mitotically and in a few weeks produce primary (diploid) spermatocytes, which are larger, have a lighter cytoplasm and a relatively large, compact nucleus (Jones and Lin, 1993).

After the onset of puberty, seminiferous tubules produce characteristic waves of cell population generations. This means that the group of spermatocytes and spermatids that originate from one spermatogonium forms a so-called cellular association, i.e., 32 spermatids associated with one Sertoli cell**.** In addition to their special connection to the Sertoli cell, these spermatids are also connected to each other by direct cytoplasmic bridges. Therefore the cellular association can be considered one syncytium, which is already noticeable during prepuberty as spermatogonia are connected to each other.

In the regulation of spermatogenic processes during prepuberty the role of local (paracrine and autocrine) factors is probable. Growth hormone mRNA expresses in the spermatogonia and in the primary spermatocytes, but does not express in the secondary spermatocytes and spermatids that appear during puberty (Harvey et al., 2004). The effect of a cytokine hormone (nicotinamide phosphoribosyltransferase or NAMPT) has been shown to play a part in the regulation of the metabolism of Sertoli cells during prepuberty being a regulating factor of NAD production (Ocon-Grove et al., 2010). The role of certain acid glycosidases is also probable in the developing testis. The amount of β-N-acetyl hexosaminidase (β-HEX) and β-galactosidase (β-GAL) is continuously increasing in Japanese quail (*Coturnix japonica*) testis until the appearance of primer spermatocytes (Droba et al., 2007).

In 2-3 months old roosters, the rate of testicular growth slows down significantly, as a result of the termination of spermatogenic cell differentiation and Sertoli cell division. The number of Sertoli cells does not increase any more from the end of the second month (not even during puberty or in a mature state), and by this time they reach final amount typical of a mature individual (Bozkurt et al., 2007). Nevertheless, the differentiation of Sertoli cells continues (they show a definite hyperthrophy) and by supplying growth factors and adhesion-increasing materials that support the metabolism of spermatogenic cells, they sustain spermatogonia that have avoided apoptosis (Farini et al., 2005). At this time, there is no membrane specialisation among the Sertoli cells that have enlarged and are gradually widening at the base. These membrane specialisations will appear during puberty to create the blood-testis barrier (Bergmann and Schindelmeisaer, 1987).

A characteristic change is that the division of spermatogonia slows down during prepuberty, and several of them degenerate. During this process (as suggested in the chapter about the differentiation of testis), the apoptosis-regulating Fas-ligand – Fas-receptor system of the Sertoli cells and the spermatogonia and appearance of prohibitin (mitotic division-inhibiting factor) in the spermatogonia play an important role (Thurston and Korn, 2000, Han et al., 2009).

Furthermore, a meiosis silencing mechanism begins to function in the primary spermatocytes, which inactivates sex chromosomes and temporarily blocks meiosis. ZZ chromosomes do not form synapses in the pre-leptotene

phase, at the beginning of prophase I of meiosis. Therefore, at this stage, meiotic cell division stops. Unlike in mammals, this process occurs before the detachment of DNA bundles and chromosome pairing, therefore we can consider it a meiosis-silencing mechanism, which preceeds synapse formation (Schoenmakers et al., 2010). Therefore, primary spermatocytes stay in the pre-leptotene-leptotene phase until increased gonadotropin hormone secretion begins that initiates puberty. In roosters it occurs in individuals up to 6 months of age, at 24-26 weeks.

Meanwhile the gradually compressed peritoneal cells of the stroma become more elongated and triangular, obtaining the shape of Leydig cells.

3.1.1.2. Puberty (Maturation)

Increased levels of GnRH-gonadotrop hormone secretion trigger puberty. FSH plays a primary role in this process, initiating intensive division of spermatogenic cells through the functional differentiation of Sertoli cells. There is a strong correlation between testicular mass, the increase in the amount of seminiferous tubules, the hypertrophy of Sertoli cells and the amount of increase in FSH levels. This process was first studied by Follett (1976) in Japanese quail after gonadal stimulation by increased light levels, and in recent decades his results have been corroborated in several bird species. Follett (1976) showed that plasma levels of FSH in quails after photo-gonadal stimulation increased 12-fold by the end of Day 9, and remained at this level for one week. After this, FSH concentration gradually decreased and by the time complete sexual maturity was reached – indicated by maximum testosterone levels – gonadotropin levels decreased to a third of the earlier maximum level. In birds undergoing photo-gonado stimulation, there is a similarly rapid increase in LH plasma concentration, which increases testosterone production in the testes. On Day 4 of treatment there was a five-fold increase compared to juvenile levels, and its levels remained high throughout maturation.

Testicular mass increases very rapidly during Weeks 1-3 of puberty. In domestic birds this increase can be 10-100 fold, while in wild birds it can be several hundred fold, for instance in house sparrow (*Passer domesticus*), it is over 300-fold.

During puberty two processes occur in the seminiferous tubules. One, the primary (still) diploid spermatocytes undergo the first division of meiosis, during which their chromosome assembly undergoes the pre-leptotene, leptotene, zygotene, pachytene, diplotene and diakinesis phase of prophase I. The stages of prophase are relatively slow, while metaphase, anaphase and telophase that follow it are faster. The resulting two secondary spermatocytes are haploid cells, which are smaller and have smaller nuclei. They have a well-formed nuclear envelope and aggregated chromatin material. In the second step of meiosis,

each of these haploid cells creates two daughter cells by mitotic division, in total resulting in four round spermatids. All of these cells are still united by plasma bridges in a syncytium-like unit.

The second process is when type A1 spermatogonia, located along the basal membrane, start a new, intensive division cycle to create new generations of primary spermatocytes. These two simultaneous processes result in the thickening of the cell-lining of the expanding seminiferous tubules, causing the cell layer to appear more irregular compared to its state during prepuberty.

A typical process of puberty is the development of four round spermatids out of each primary spermatocyte. These spermatids first appear at the end of the prepubertal stage and after that – in the sexually active phase – they are continuously formed. This means that from one stem-cell type A spermatogonium, 32 spermatids develop (one A spermatogonium – one A1 spermatogonium – two A2 spermatogonia – four B spermatogonia – eight primary spermatocyte – 16 secondary spermatocyte – 32 round spermatids).

The round, cytoplasm-rich spermatids, containing a small, round nucleus, undergo a significant morphological change to form elongated spermatids, and later long mature sperm with an elongated tail. This process is called spermatomorphogenesis, or spermiogenesis. At the end of spermiogenesis sperm are formed, indicating the end of puberty. At this this time, sperm detach from the apical end of the Sertoli cells in a very quick process, called spermiation. The "classic" taxonomy based on features visible by light-microscope, considers only the development of acrosome and distinguishes four phases of spermatomorphogenesis: (1) Golgi phase, (2) head cap phase, (3) acrosome phase and (4) maturation phase (Leblond and Clermont, 1952). Based on ultrastructural studies – primarily based on results obtained from quail and turkey – 12 developmental phases can be distinguished (Aire, 2007).

3.1.2. The structure and spermatogenic function of active testis

The paired testes of a sexually mature bird are yellowish-white, bean-shaped organs found in the abdominal air sac, above the cranial pole of the kidneys (in *Apodidae* (swifts) it is elongated, vermiform). In most bird species, the left testis is bigger than the right. Testes are attached medially to the wall of the aorta and the vertebrae by strong connective tissue mesorchium that is connected to the serosa. The cranial end of the testes lays against the ventral surface of the lung, and their caudal end is connected to the cranial kidney lobe. The relatively small epididymis is connected to their dorso-medial surface, and from the caudal end of the epididymis starts the deferent duct that runs to the central part of the cloaca (Figure 35, 36, 37, 38, 39, 40, 41, 42, 43).

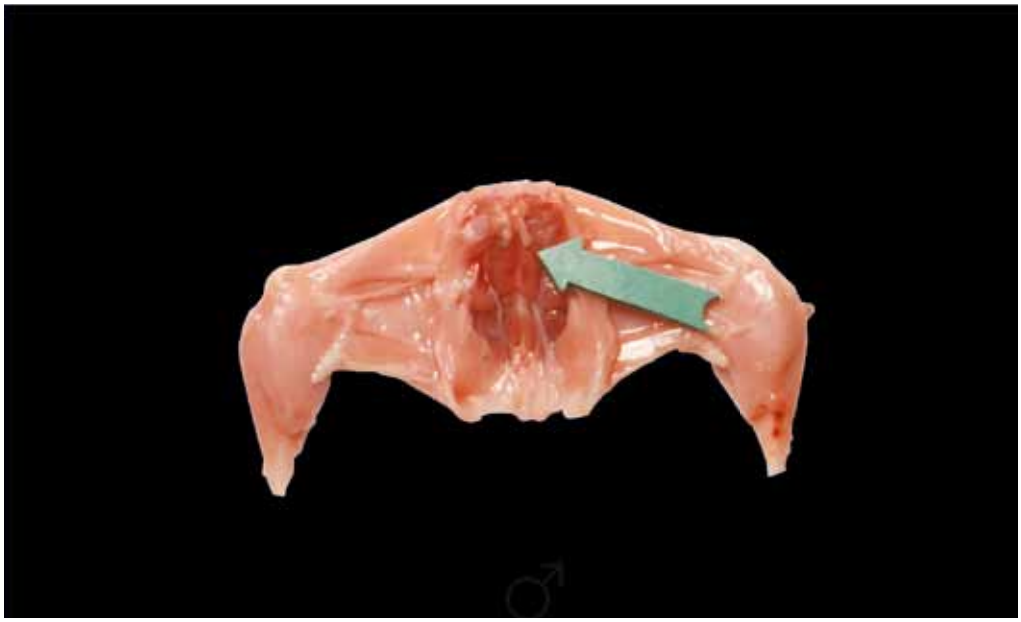

Figure 35. Pelvic region of a one-day old male dometistic turkey, the arrow indicating the testis (prepared by István Gyôrvári)

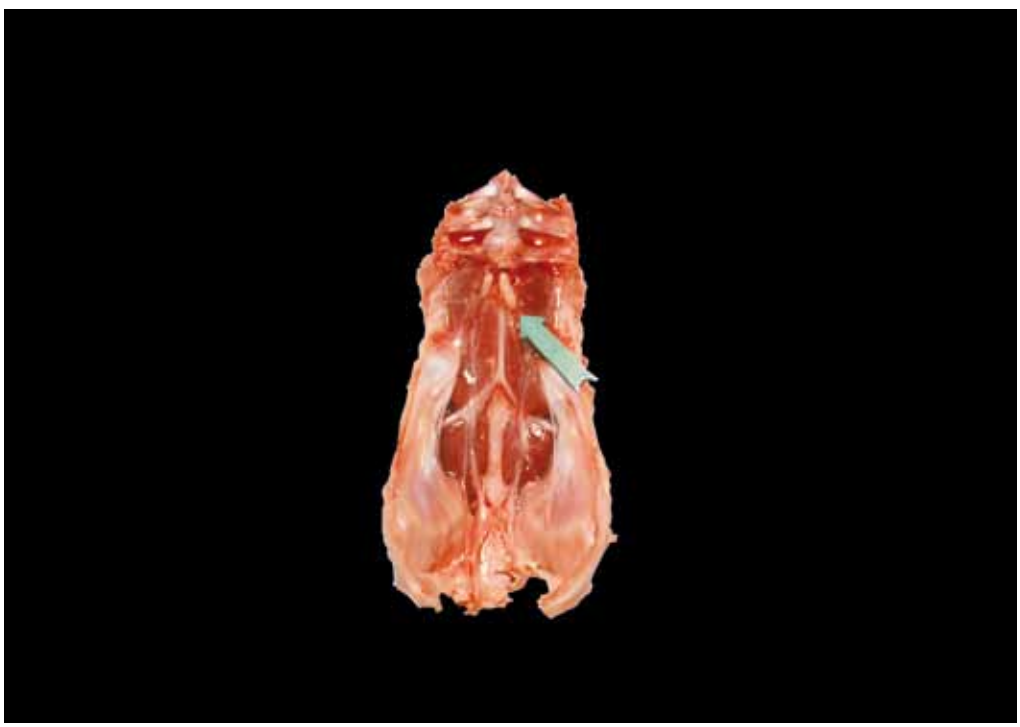

Figure 36. Pelvic region of a 10-week old male dometistic turkey, the arrow indicating the testis (prepared by István Gyôrvári)

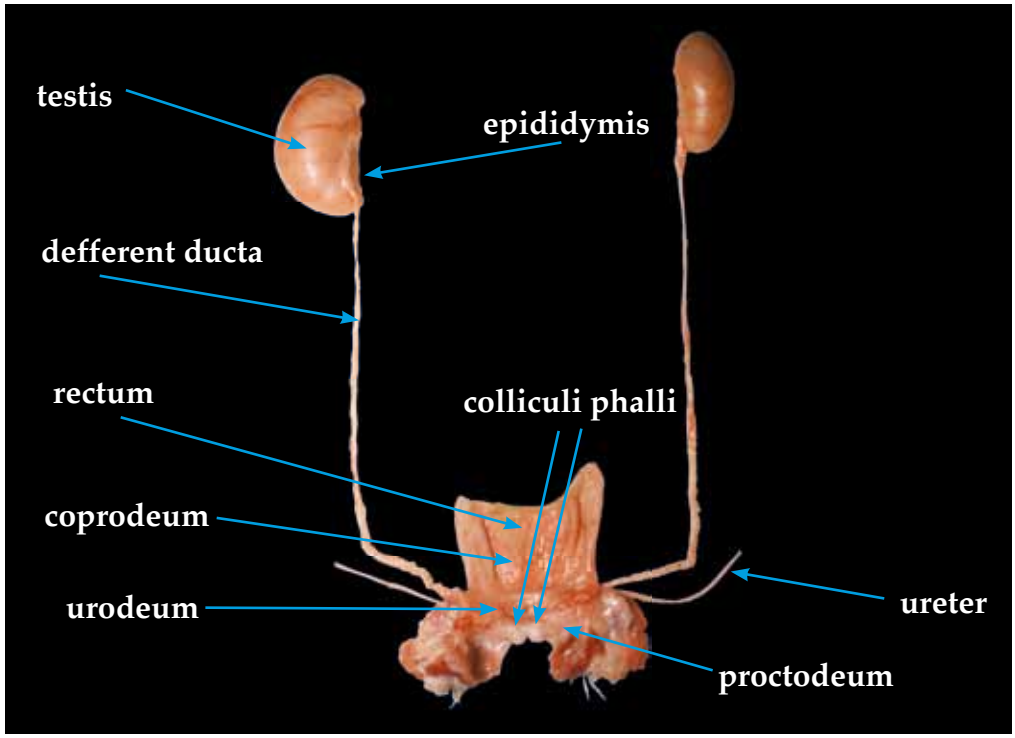

Figure 37. Pelvic region of a 56-week old male dometistic turkey, with dissected sex organs (prepared by István Győrvári)

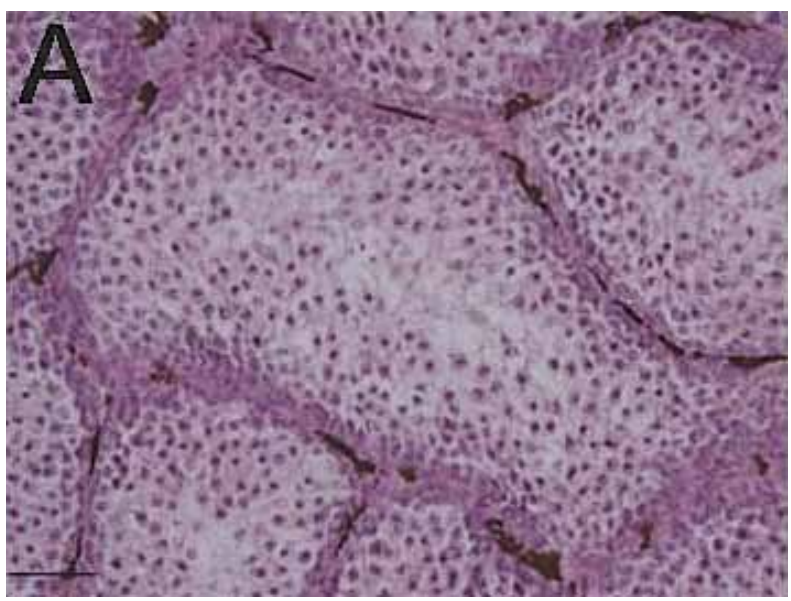

Figure 38. Testicular tubules of Starling. A: In March, during the photosensitive stage, spermatids are present, but sperm cells are still absent (photo by Ottó Pintér)

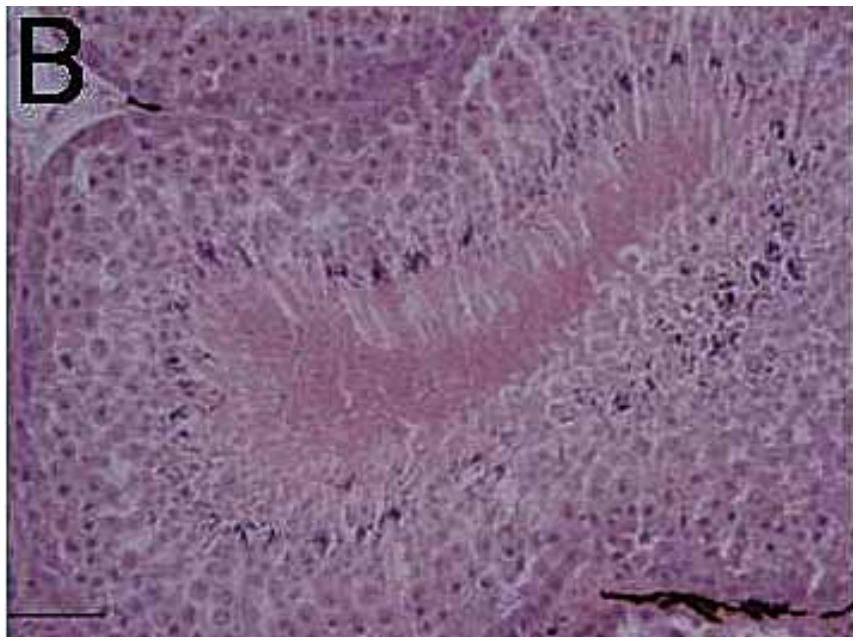

*Figure 39. T*esticular tubules of Starling. B: In the first part of April, during the photosensitive stage. Sperm cells appear (photo by Ottó Pintér)

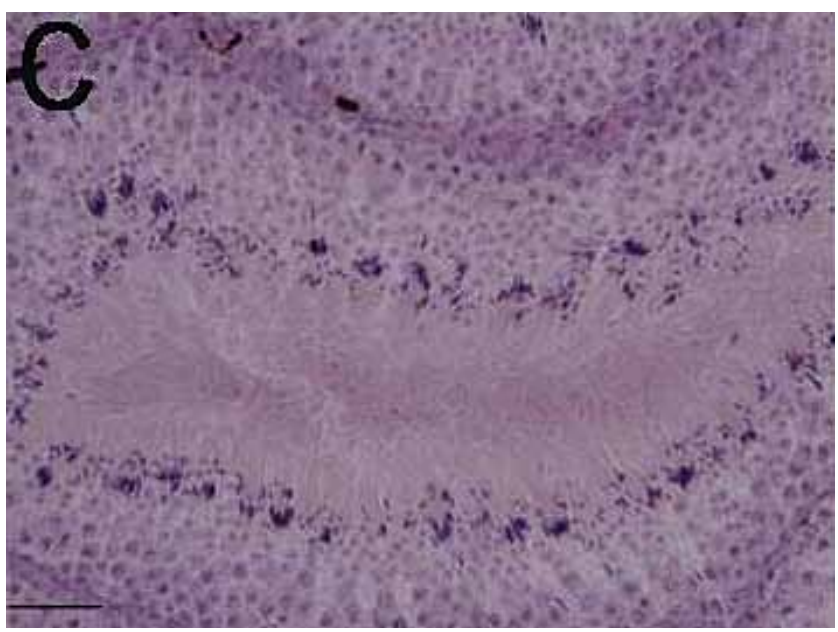

Figure 40. Testicular tubules of Starling. C: In the second part of April, during the photosensitive stage. A large amount of sperm visible, organised in bundles (photo by Ottó Pintér)

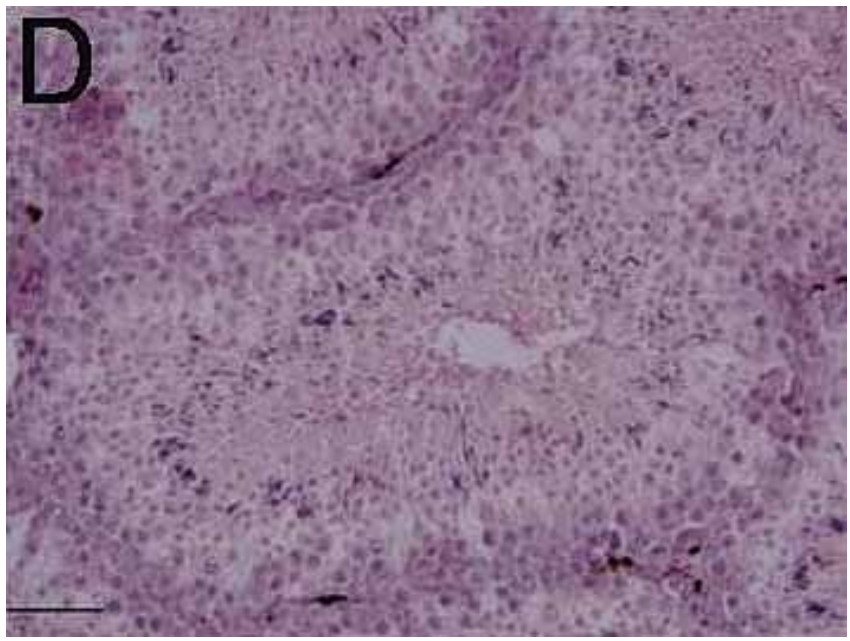

Figure 41. Testicular tubules of Starling. D: In the first part of May, during the photosensitive stage. The amount of sperm substantially decreases (photo by Ottó Pintér)

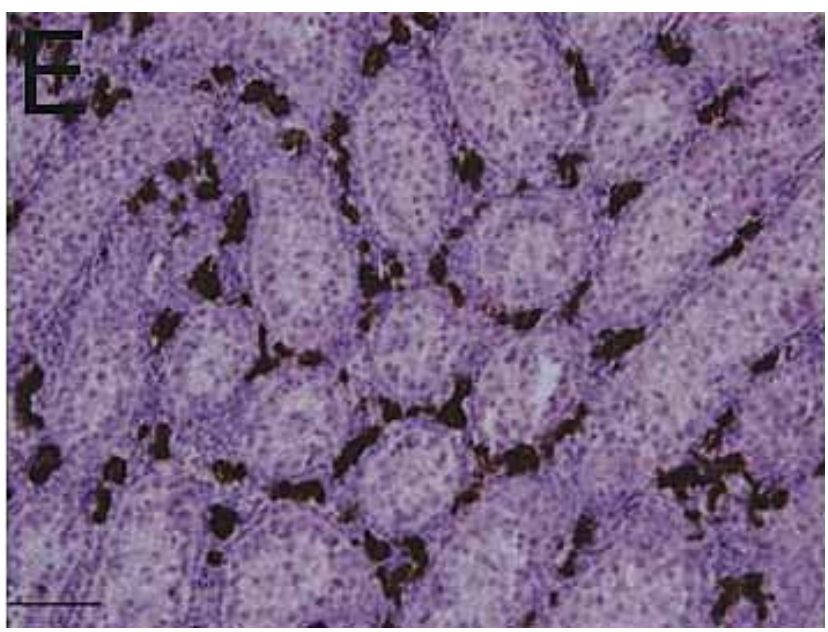

Figure 42. Testicular tubules of Starling. E: In the second part of August, during the photorefracter stage. The wall of tubules mostly consists of spermatogonia. There is an intensive accumulation of melanin-containing cells in the interstitium (photo by Ottó Pintér)

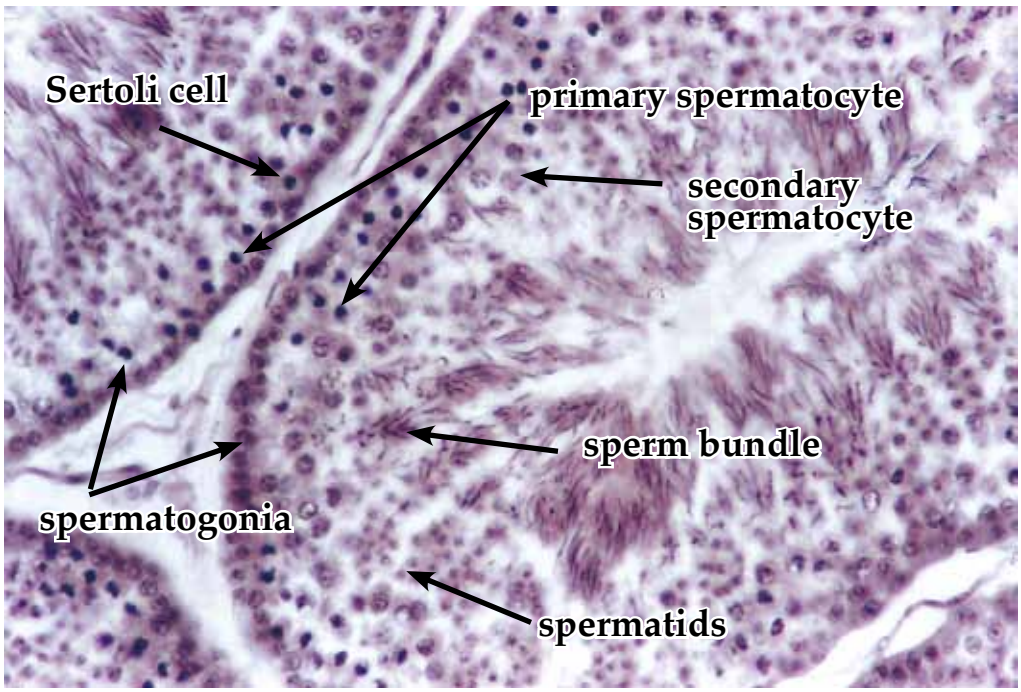

Figure 43. Testicular tubules in sexually mature goose.

Previously, an assumed function of the abdominal and post-thoracic air sacs was to work as an "air conditioning" system, cooling the tissue of the testes by a couple of degrees Celsius (which, in the case of mammals, with testes located in the scrotum, is a physiological requirement of normal spermatogenesis). Other studies, however, found the temperature of the testes in birds to be the same as that of the organs around it (Williams, 1958).

Arterial blood to the testis is supplied by the testicular artery, which originates from the renal cranial artery. It reaches the testis on the medial side, in the testicular hilum. Besides this, one or two short, thin arteries that originate directly form the abdominal aorta, penetrate the testis. The bundles of arterial branches further divide in the tissue of the testis. Larger veins run in the testicular capsule, and these receive branches of different sizes that run along the arteries. Several shorter venous branches orginate from the testicular vein, which open into the posterior vena cava. Its spinal innervation comes from the lumbo-sacral area, which sends sympathetic (adrenerg) and parasympathetic (cholinerg) fibres through the mesorchium.

The **testicular capsule (capsula testicularis**)is a less than 100 μm wide, thin structure, which is only thicker in the area next to the epididymis. In this area, thick bundles originate from it, which encapsulate the epididymis. Hystologically, it can be divided in three layers: its outer, thin element is the tunica serosa, followed by the thick tunica albuinea, and the inner layer is the thin tunica

vasculosa. The tunica serosa consists of mesothel cells, which are separated by the thick tunica albuginea (containing collagen, elastic fibres and fibroblasts) by a basal membrane. There are several smooth muscle cells visible among the fibres, which, similar to mammals, can play a role in the expulsion of sperm not yet capable of moving.

The tunica albuginea layer of the testicular capsule is relatively thin in young birds, but in several year old males, where it has been through several seasonal reactivation, it is considerably thicker during the inactive period, and even shows pronounced layering. Before seasonal reactivation, or even during the regression phase after the mating cycle, a new connective tissue capsule develops under the old tunica albuginea that covers the testis, which will replace the degenerating old one by the time it gets reactivated in the following year. The temporarily observable double capsule is therefore a good sign to distinguish first year and several-year-old males during the sexually inactive period (Péczely, 1987).

The testicular capsule in birds fundamentally differs from that of mammals by lacking the septums that divide the testicular tissue into lobules. Only connective tissue bundles run irregularly from the capsule into the intertubular area, along with blood vessels and nerves. Most of the testicular mass consists of contorted seminiferous tubules and interstitial connective tissue. A fundamental difference from mammals is that in birds the tubules are not blind, but form a continuous, anastomising tubular system. The lack of lobules and connective tissue septums probably play a role in this structural appearance. Basically there are two type of cells in the tubules: the fixed somatic (Sertoli) cells and the temporally visible, dividing spermatogenic cells that move along the Sertoli cells (Figure 44).

Sertoli cells develop from the germinal epithelium. These large, strongly differentiated somatic cells fulfill different functions. The cells that sometimes rise up from the thick germinal epithelium have characteristically electron-dense plasma, and the triangular shaped cells have a relatively large, elongated, eucromatic nucleus. They show a definite polarity, cell organelles are mostly located in the supranuclear area. In the cytoplasm there is a small amount of smooth endoplasmic reticulum, as well as granulated endoplasmic reticulum, several free ribosomes, polyribosomes, well-developed Golgi complex and micropinocytotic vesicule, microtubules and lipid drops. The microtubules located in the appendages embracing the spermatogenic cells are characteristic. Some of the mitochondria are crista type, the others have a tubular structure.

Compared to mammals, in birds the widening basal area of the Sertoli cells have fewer contact points with the basal membrane. The cell membrane, on the other hand, forms plasma appendages (arborisation), which are in contact with the differentiating spermatogenic cells. To their part closest to the basal membrane attach the primary spermatocytes, which will continue to develop

in close contact with the Sertoli cells. Spermato-morphogenesis can only occur in primary spermatocytes that are in close, cell-to-cell contact with the Sertoli cells. Their function is basically the same as that of the structures described in details in the mammalian testes, however the tubulo-bulbar structures consisting of filaments, microtubules and membrane formations, which are typical of mammals, are not developed in birds.

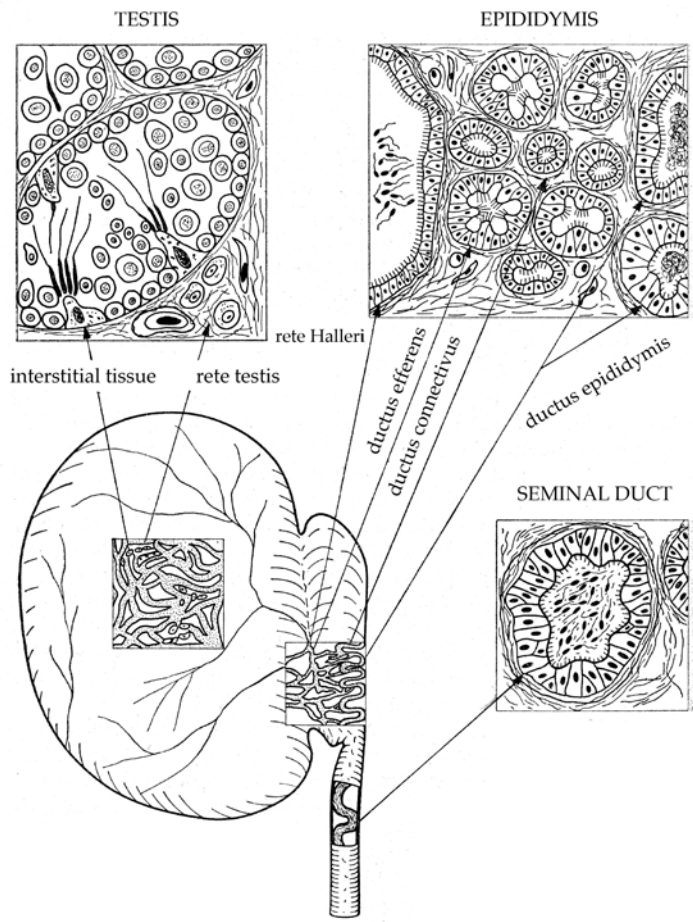

Figure 44. The structure of the testis, epididymis and the deferent duct in birds modified after Péczely (1987)

The appendages of the more basally located Sertoli cells are connected to each other by occluding and adherent zonules, as well as gap junctions. The occluding zonule system makes up the intratesticular blood-testis barrier, which separates diploid (spermatogonia and primary spermatocyte) and haploid spermatogenic cells from each other and the haploid cells from the killer cells of the immune system that leave the blood circulation. Gap junctions form

a separation between the occluding zones and the adherent zonules. Their structure is similar to that in mammals, but the filament bundles under the membrane in the occluding zonule, typical in mammals, are lacking in birds. These connections between Sertoli cells are not only present in the active Sertoli cells, but also in those that show seasonal (functional) regression (Pelletier, 1990, Aire, 2007).

The nurse-cell function of Sertoli cells is indicated by their ability to synthesise glycogen, organic acids and lipids and to produce transferrin and coeruloplasmin. On the other hand, the phagocytic-pinocytic activity can remove primary semen, proteins and corpuscular material (residual bodies) form the lumen of the testicular tubules.

The primary regulating factor of the functioning of Sertoli cells in hyphyseal FSH production, as their membrane contains FSH receptors. Their inhibin and activin production suggests that they have a feed-back regulation on gonadotrop functions. Their steroid production is (primarily?) aimed at the conversion of steroids: they can synthesise testosterone from progesterone and androstenedione. The androgen-binding protein (ABP) synthesised by Sertoli calls can play a role in the intracellular transport of steroids, by moving the synthesised steroids to the developing spermatogenic cells.

Spermatogenic cells are located in the contorted seminiferous tubules, organised around the Sertoli cells and in close contact with them. They form a cellular associational unit, and show a definite layering. The wall of the tubules consists of peritubular tissue with an outer fibrous and an inner basal membrane. Spermatogonia attach to the inner basal lamina, forming the inner layer of the spermatogenic tissue. Four types (A, A1, A2 and B) of spermatogonia can be distinguished in the functioning testis. Types A and A1 are located on the basal lamina, while type B spermatogonia, which have lighter cytoplasm, are above them. Primary spermatocytes that develop from type B spermatogonia are the largest spermatogenic cells. These cells have polymorph nucleus, as they are in certain phases of the first meiotic prophase, and in the following metaphase, anaphase or telophase.

In the leptotene phase, the chromatin material is densely aggregated because of the condensation of the chromosomes. The nucleolus is not visible in the zygotene, and the chromatin material aggregates on one side of the nucleus, indicating the pairing of the chromosomes. Mitochondria, the Golgi complex and the centrioles aggregate on the same part of the cell where the chromatin material condenses inside the nuclear membrane. In the pachytene phase the paired chromosomes evenly fill out the nucleus, and at the same time the aggregation of the organelles stops in the cytoplasm. The smooth endoplasmic reticulum becomes stronger, and the mitochondria align along the long canals formed by them. In the diplotene and diakinesis phases the chromosomes are again evenly distributed inside the nucleus (and they become unpaired). In the

metaphase, the chromosomes line up in the middle of the cell, and in the anaphase the nuclear material divides.

The size of the secondary spermatocytes is smaller and their nucleus already contains "n" set of chromatin. Generally they are located above or among the primary spermatocytes. They have a short lifespan, and their cytoplasm contains annulate lamella and chromatoid body. This type basically represents the short interphase of meiosis, and by mitotic division they soon form the substantially smaller, round spermatids.

Spermatids attach to the apical part of the Sertoli cells, and this is where they undergo a characteristic morphological change, the spermato-morphogenesis. This process has 12 steps in suboscines and six in oscines. As a result, the lumen of the testicular tubules is filled with a large amount of aggregated sperm. The morphogenesis of spermatids was studied in details in turkey (Aire, 2003).

Type 1. The spermatid is a round cell with an oval nucleus that contains chromatin aggregations, its Golgi complex is well developed, with few proacrosomal granules around it, and the diplosomes are located close to each other, free in the cytoplasm.

Type 2. In this stage, the chromatin material is decondensed, and is evenly distributed in the nucleus, there is one large acrosomal granule (acrosome phase) in the Golgi area and the distal centriole connects to the cytoplasm with a ring-shaped, dense structure (which will later form the tail).

Type 3. The chromatin material is found in the spherical nucleus as evenly distributed fine granules, the Golgi complex is broken up, the acrosomal granule attaches to the nuclear membrane (thickening it at the site of contact), and the diplosome approaches the nucleus and then attaches to it near the acrosomal granule.

Type 4. Granular-fibrous strucures develop from the finely dispersed chromatin material of the elongated nucleus, the homogenous density acrosomal granule lengthens, and its part attached to the nucleus causes an indentation on the nuclear membrane, which thickens at the contact surface, the appearing microtubules arrange themselves into a bundle at the rostral end of the nucleus, and the diplosoma structure that arranges longitudinally in the spermatid attaches to the nuclear membrane by the proximal centriole.

Type 5. The nucleus becomes pear shaped, the nucleoplasma is finely granulated, the acrosome is elongated, its thickened part attached to the nuclear membrane invaginates into the nucleus (creating a perforatorium-like structure), and the cytoplasm contains few smooth and granular endoplasmic reticulums, mitochondria and lysosomes.

Type 6. The nucleus of the spermatid is elongated, it has fine granules, the acrosome is attached to only about a third of the rostral surface of the nucleus, with its apical part oriented towards the Sertoli cell. Microtubules are visible around the nucleus (which in several suboscine species, such as in the ostrich

(*Struthio camelus*), are arranged in a circular way, forming a circular manchette structure, which plays a role in shaping of the nucleus), mitochondria migrate to the caudal area of the cytoplasm, but are not arranged yet.

Type 7. The shape of the spermatid starts to become elongated, its width is the same as that of the continuously lengthening nucleus, the acrosome is of the same width as the nucleus, the nucleoplasm begins the get denser, the caudally arranged cytoplasm causes deep indentations in the Sertoli cell, there is a regularly organised microtubular circular manchette around the nucleus, mitochondria start to get an elongated shape, their cristae arrange longitudinally, the flagellum increases (its formation is very similar in suboscines and mammals), the centrioles organise along the longitudinal axis of the spermatid, diagonal to each other, the proximal centriole is arranged diagonally to the axis, while the distal is parallel to the longitudinal axis (the one known exception is the Guinea fowl (*Numida meleagris*), in which, similar to invertebrates, both centrioles are organised parallel to the longitudinal axis of the spermatid), the distal centriole is gradually lengthening, but does not show structural changes, the dense basal plate at the base of the nucleus and the attached nine striated columns, which are present in mammals, do not form in birds.

Type 8. The spermatid continues to lengthen and its diameter continues to decrease, the chromatin material of the nucleus is condensed, there is a dense chromatoid body (as described in several mammals) under the nucleus (it has condensed from cytoplasmic fibrous material, it disappears in the end of spermiogenesis and its role is unknown), there is a cufflink around the nucleus, consisting of longitudinally and circularly organised microtubules.

Type 9. The longitudinal circular manchette reaches into the central part.

Type 10. The nucleus continues to lengthen, its material condenses, the acrosome widens and covers the nucleus as a cap, the subacrosomal area that indentates into the nucleus forms a dense perforatorium, mitochondria continue to lengthen and the density of their matrix increases.

Type 11. The elongated spermatid becomes slightly bowed, the acrosome and the perforatorium (which consists of longitudinal actin filament bundles that reach into the endonuclear channel) form a structural unit, the chromatin granules of the nucleus unite into larger pieces, the mitochondria continue to lengthen and strengthen, and the longitudinal circular manchette tubules break up and get eliminated.

Type 12. The longitudinal tubule bundles that form the circular manchette completely disappear, the elongated mitochondria arrange in a helical pattern in the central area, glycogen granules appear in the cytoplasm, the amount of endoplasmic reticulum and multivesicular bodies decreases, spermatids are connected to the Sertoli cell only by a thin cytosplasmic ribbon and are ready to separate (spermiation). The detaching sperm, unlike in mammals, does not release a cytoplasmic droplet.

Changes similar to the ones described above are present in the spermatomorphogenesis of *Columbidae*, *Anatidae* and *Ratitae* (Péczely, 1987, Aire. 2007).

Nevertheless, in Passeriformes the development and structure of mature sperm show several differences, their structure is typically helical, screwdriver like. In the spermatomorphogenesis of **Passerines** six phases can be distinguished. The process was studied in details in house sparrow (*Passer domesticus*), Bengalese finch (*Lonchura striata*) and zebra finch (*Taeniopygia guttata*) (Aire, 2007). In the early and late acrosome phase (Type 2) the pro-acrosome created by the Golgi complex develops in a concave pit of the nucleus, it does not form a real cap-like structure. When the acrosome lengthens, it becomes lighter at the edges, but the internal parts become electron dense. Passerines lack the perforatorium. First, the content of the nucleus consists of fine granules, then the granules become larger, similar to the process observed in turkey. Microtubular circular manchette do not form in mannekin spermatid, but in sparrow the microtubular bundles appear during the early developmental phase, nearly along the whole length of the spermatid.

The circular manchette however, only becomes pronounced in Type 3, when the shape of the nucleus becomes helical. At this time, the tubules are arranged spirally, following the shape of the nucleus, also in the area of the growing tail. The helically arranged microtubules do not extend onto the screwdriver-shaped acrosome, and do not contribute to the forming of its spiral shape. Mitochondria, which appear postnuclearly, fuse into long bundles, and surround the axoneme.

The development of the diplosome complex starts similar to the process in mammals and suboscines: the proximal centriole (located diagonally to the longitudinal axis of the spermatid) lays into the groove formed at the basal part of the nucleus, while it gets embedded into the stongly electron dense material, and later it will not be possible to distinguish it from the distal centriole (Nicander, 1970). Contradicting this earlier observation, Góes and Dolder (2000) found that during spermatid development in house sparrow the proximal centriole remains visible the entire time, located diagonally to the longitudinal axis. In contrast, according to Jamieson (2007), only distal centrioles are found in the mature sperm in all oscines.

In Passerines, a striated pericentriolar structure (nine columns) is visible the neck area, from which nine periferial axoneme fibres originate at the base of the head.

Spermiation can occur in two different ways in birds. In suboscines, the cytoplasm mass forming in the apical part of the Sertoli cell (which can be considered syncytial mass) contains a large amount of irregularly organised Type 12 spermatids, and from this swarm out the developing sperm cells. On the other hand, in oscines, spermatids and sperm embedded in the syncytial mass arrage in regular bundles and sperm move into the tubular lumen in organised

rows. The development and motility of sperm differ between the two groups: sperm from suboscines (e.g. *Piciformes*) are motile in vitro in a 0.95% NaCl solution, while sperm from oscines ar not.

The kinetic base of spermatogenesis is the **cellular association**, the development of spermatogenic cell groups that are connected to each other and to Sertoli cells by plasma bridges. The stages of cellular association create a cycle in spermatogenic cell production. The period necessary for the spermatogenic cells to cover all stages of development can be considered a cycle. In mammals, a cycle consists of 14 stages, while in birds the number of stages is ten (Aire, 2007).

Lin et al., (1990) studied the ten stages that form the spermatogenic cycle in Japanese quail, mostly based on the morphogenesis of spermatid types, considering acrosome developmental stages and morphological characteristics of the spermatid nucleus. Similar to mammals, these stages are indicated by roman numbers. The described characteristics are very similar in duck and Guinea fowl and similar to the developmental changes found in mammals (mostly Primates**).**

Stage I. Type 1 spermatids of a new generation appear, Type 11 spermatids get incorporated in groups into the cytoplasm of the Sertoli cells, primary spermatocytes in the early pachyten phase are visible among the spermatids and sperm. There are A1, A2 and B spermatogonia located along the basal membrane.

Stage II. Besides the cell types of the previous stage, Type 2 spermatids also appear.

Stage III. In addition to the cell types of the previous two stages, Type 3 spermatids appear, B spermatogonia are intensively dividing and some primary spermatocytes are in the pre-leptotene phase.

Stage IV. Type 4 spermatids appear and Type 12 spermatids obtain the characteristic residual body that has separated from the cytoplasm, besides the primary spermatocytes in the pachytene phase that have appeared earlier, spermatocytes in pre-leptotene and leptotene spermatocytes appear and A1 and A2 spermatogonia are located on the basal membrane.

Stage V. Type 5 spermatids appear and the ones in earlier stages detach from the apical part of the Sertoli cell (spermiation), the recently appeared primary spermatocytes are in the zygotene phase, the earlier generation is in the pachytene phase and A1 and A2 spermatogonia are visible.

Stage VI. Type 6 spermatids appear, the recently formed primary spermatocytes are in the early pachytene, and their earlier generation is the late pachytene and early diplotene phase. There is no change in A1 spermatogonia, but A2 are in the mitotic metaphase.

Stage VII. Type 7 spermatids appear, characterised by elongated nucleus and a substantial amount of cytoplasm around it. Primary spermatocytes enter

the diplotene phase and secondary spermatocytes are in the pachytene phase. Dark Type A spermatogonia and B spermatogonia are visible.

Stage VIII. Type 8 spermatids are characteristically aggregated, the centripetal migration of the cytoplasm in the spermatids indicates the development of the residual body. The earlier formed primary spermatocytes are in the stage of diakinesis, while the more recently formed ones show a state typical to Stage VII.

Stage IX. Type 9 spermatids appear, with a characteristic detachment of the residual body. The earlier formed primary spermatocytes are in the meta-, and anaphase of the first meiotic division, while the more recent spermatocytes and spermatogonia are similar to those in the previous stage.

Stage X. With the continuing elongation of the spermatids Type 10 is formed, characterised by the cytoplasm residue located in post nuclear position. Secondary spermatocytes are formed and primary spermatocytes are in the pachytene stage.

The kinetics of the ten stages of the cycle was studied in Japanese quail by the incorporation of H^3-thymidine. The results show the length of a cycle to be around 2.7 days, which is substantially shorter than the approximately 11 days described for mammals. In Japanese quail the length of each stage from I to X is: 7.7, 9.5, 15.5, 6.6, 5.3, 4.1, 6.1, 3.6, 2.5, and 3.5 hours. In regard to the lifespan of certain spermatogenic cell types, for B spermatogonia it is 2.01 days, for primary spermatocytes 3.86 days, secondary spermatocytes 0.15 day and for spermatids 4.54 days.

In birds it is difficult to study spermatogenesis in the seminiferous tubules, as the contortous seminiferous tubules form a continuous tubular system in the testis, and the surface of the cellular associations is much smaller (18,000 μm) than in mammals, and the number of Sertoli cells that determine a cellular association is only about 13. Furthermore, there are several irregular cycles intercalated among the regular cycles. In spite of the technical difficulties, Lin and Jones (1990) and Lin et al., (1990) were able to identify that sperm formation during spermatogenesis occurs as helical waves in the epithelium of the tubules.

The structure of avian **sperm** is basically similar to reptilian, but also just slightly differs from mammalian. The light- and electron-microscopic structure of avian sperm is well known and has been described for a large number of species. Early descriptions of sperm based on light microscopy have been done by Ballowitz (in 1886, 1888 and 1913) for 32 species, by Retzius (in 1909, 1911 and 1912) for 37 species, and by McFarlane (in 1963) for 39 species. Jamieson (2007) decribed sperm of 79 species based on electron microscopic studies. Sass et al., (1992) described great bustard (*Otus tarda*) sperm based on light and electron microscopic observations. Here I present three types of avian sperm based on descriptions by Jamieson (2007).

Ostrich sperm is a 70-80 μm long, filiform cell, with a 16 μm large (0.8 μm diameter) cylindrical head, the neck and the thightly fused midpiece of the tail are 3 μm long, the principal piece of the tail is 40 μm and the end section is 1 μm long.

The **head** is slightly bowed, with a 2 μm long acrosomal vesicle filled with fine, homogeneous material located at its apex. There is a 30 nm large subarcrosomal gap between the inner acrosomal membrane and the nuclear membrane, which extents coat-like up to the denser ring that indicates the end of the acrosomal vesicle. This forms a homologous structure in mammals with the so-called posterior ring (a subacrosomal actin sheath) forming a tight contact between the cell membrane, the acrosomal membrane and the nuclear membrane. The subacrosomal space is connected to the endonuclear channel at the apical part of the nucleus. The endonuclear channel intrudes into the nucleus (up to about 1/4 of it) and is formed by the invagination of the nuclear membrane. It is filled with material that contains filaments, which is equivalent to the perforatorium more markedly visible in other species. The compact chromatin material of the nucleus is strongly electron dense. There is a centrally located dip at the base of the nucleus, bounded by amorphous material. This is where the proximal certriole fits.

The **neck and the midpiece**. The proximal centriole fits into the basal dip, in a position diagonal to the longitudinal axis of the sperm. It is about 0.3 μm thick, and consists of a nine-microtubule triplet. The inside of the centriole is filled with fine granular material. Microtubules are embedded in a ring of dense, amorphous material, which continues in elongated columns at the outer surface of the two centrioles. This dense material also fills the basal dip of the nucleus. The distal centriole is located along the longitudinal axis of the sperm perpendicular to the proximale centriole and extrends through the midpiece. It consists of nine peripherial and two central microtubules, and continues in the axoneme of tail**.** Dynein arms extend from the peripherial tubules of the distal centriole to the central elements. There is often a stick-like structure visible excentrically around the central microtubules, made out of dense material, which extends to the ring at the end of the central section. From here towards the end of the tail the structure of the central microtubule pair is typical in the axoneme. The outside of the central section (proximal and distal centriole) is covered in a helical mitochondrial sheath, which consists of 20-25 members. Between the last mitochondrial gyres and the cell membrane an electron-dense ring indicates the end of the midpiece.

The **main piece** is the longest segment of the **tail** part of the sperm. Its central axis has the structure of a 9+2 axoneme, covered by a ribbed, fibrous sheath under the cell membrane, which consists of semi-circular shaped bodies. The peripherial microtubule pairs are connected to each other and to the central microtubules by dynein arms. One element (A) of the pairs is filled

with dense material, while the other (B) is light. The gradually thinning main piece can be divided into three sections. The upper section starts right under the ring, and it becomes thinner (0.5 μm) abruptly. The fibrous sheath consists of two longitudinal columns, connected by dense, circular bundles. The two longitudinal columns align with the central microtubules. In this section, there are nine accessory filaments between the fibrous sheath and the peripherial microtubules. This section has the thickest layer of cytoplasm between the cell membrane and the fibrous layer. The second section is thinner (0.4 μm), the accessory filaments disappear and the ribs on the fibrous sheat are less obvious. In the third section, the thickness of the tail decreases to 0.3-0.2 μm. The two longitudinal columns reduce to a thin dense ribbon, which surrounds the axoneme. The cell membrane tightly attaches to this structure.

In the end piece the fibrous sheath disappears, the dynein arms and the periferial pairs of the axoneme separate and the dense material is no longer present in the A elements. Therefore, in the end piece 20, irregularly arranged microtubules are visible (Figure 45).

Domestic cockerel. The thread-like sperm is about 90-100 μm long with a slightly bowed, S-shaped head, which is about 14 μm long and 0.5 μm widw.

The area of the **head** contains a cap-like 2.2 μm large acrosomal vesicle. Its basal part extends over the nucleus that becomes slightly thinner apically. There is a relatively large, cup-like subacrosomal space at the base of the acrosome, into which extends the stick-shaped, about 1.2 μm long, strongly electron-dense perforatorium. The lower part of the perforatum is attached to the groove-like endonuclear channel (fossa nuclearis).

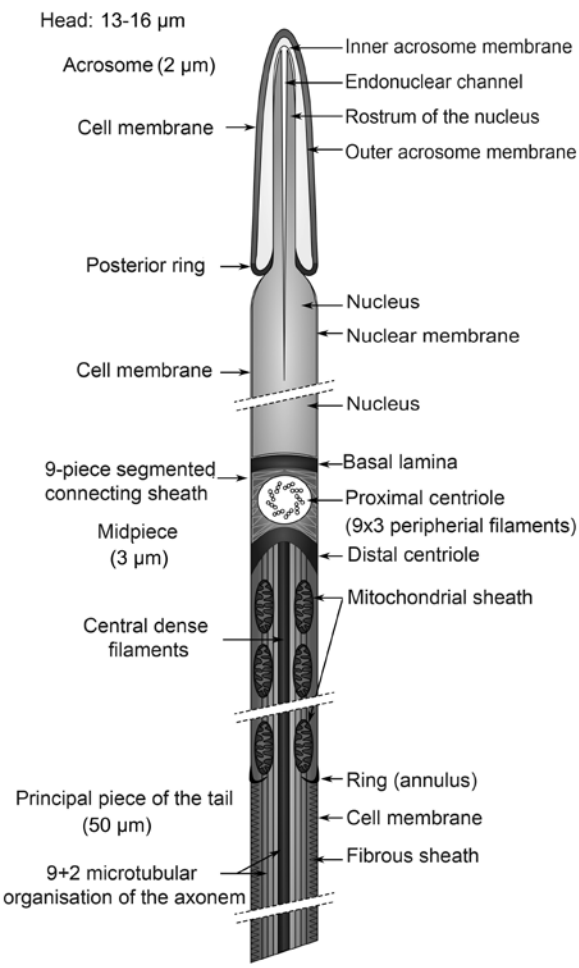

Figure 45. Schematic electron microscopic representation of ostrich sperm

At the basal end of the acrosomal vesicle, the base of the subacrosomal space between the cell membrane, the acrosomal membrane and the nuclear membrane is filled with finely granulated, weakly electron dense material, called the subacrosomal ring. The nucleus is an elongated, cylindrical, strongly electron dense structure. There is a flat indentation at its basal part, the implantational fossa.

Centrioles are found in the **neck** region. The proximal centriole is located diagonally to the longitudinal axis of the cell. It consists of nine microtubular triplets, embedded in a dense ring. This structure is attached to the implantational fossa by a connecting sheet. The 1.8 μm distal centriole (consisting of nine microtubule triplets) is located in the longitudinal axis of the cell, tightly attached to the proximal element, and the axoneme originates from here. Unlike in Struthionidae, the central tubule does not reach the proximal end of the centriole, but originates on its distal end.

The **midpiece** is about 3.7 μm long, surrounded by a helical sheath that consists of 7-8 mitochondria. The axoneme has nine dense, tightly attached peripherial microtubule pairs and two central microtubules. These dense, periferial fibres do not reach into the principal piece. The end of the midpiece is indicated by a thin, compact ring. The **principal piece** is about 70 μm long, with a characteristic fibrous sheath that lacks rings and ribs. The axoneme consists of nine peripherial microtubule pairs (one dense and one light element) and two central tubules. Each peripherial pair is attached to one of the central tubules by dynein arms. In the **end piece** there is no fibrous sheath. Characteristically, the nine peripherial tubules maintain their organisation, but the two central tubules merge into a large, dense, apical granule (Figure 46).

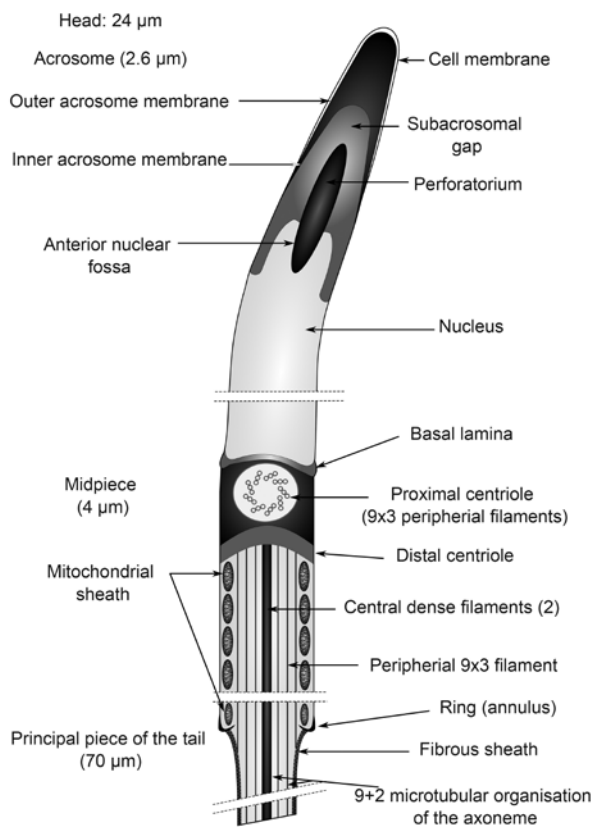

Figure 46. Schematic electron microscopic representation of the sperm of the domestic cockerel

Passerine (**Oscines**) sperm differs from other avian orders and from suboscines in several

morphological characteristics. The head, the neck and midpiece show a helical structure, and are embedded in a so-called **undulating membrane**. The acrosome is usually much longer than the head.

The **head** is elongated (the only known exception is the Eurasian bullfinch *Pyrrhula pyrrhula*), with 2.3-4.6 gyres. In some cases, the corkscrew-shaped acrosome contains a helical axis, which is not connected to the similar element of the nucleus. In some phylogenetically distantly related species (i.e. southern anteater-chat *Myrmecocichla formicivora* and sociable weaver *Philetarius socius*) the **acrosome** has two parts (bipartite acrosome). The upper acrosome crest has lower electron density, and it sits on the lower, denser acrosome core. Both parts contain helically-arranged spirals. In Passerines, the perforatorium and subacrosomal body do not form. The nucleus is strongly electron dense, has a helical cylinder shape, and it is 3-5 μm long, which is substantially shorter than the size of the nucleus in other species, for instance 6-10 μm in parrots, 12 μm in ostrich and 10-21 μm in turkey.

In Passeriformes, the area of the **neck** contains only the distal centriole. In most species, an electron dense ring forms in this area that reaches the mitochondrial sheath of the midpiece. This structure is lacking in the sociable weaver.

There is no ring at the distal end of the **midpiece**, therefore there is no sharp boundary between the mid and the end pieces. The mitochondrial sheath does not form tighly organised rings, but one helical ribbon. Its length varies among species, it is short in *Vireo*, *Corvus*, *Oriolus* and *Lanius* species, while in zebra finch it extends to nearly two thirds of the tail. In bullfinch the length of the straightened helical mitrochondrium is 1.9 μm, but in song trush (*Turdus philomelos*) it is 54 μm and in linnet (*Carduelis cannabina*) 139 μm. The number of gyres also varies among species, one in rook (*Corvus frugilegus*) and carrion crow (*Corvus corone*), 13.5 in song thrush and 29.4 in moustached warber (*Acrocephalus melanopogon*). Typically, there is also a large within species variability in the length of the (mitochondrial-sheath bound) midpiece, for instance in zebra finch. The genetical background to this is not yet know. In some species, such as the Italian sparrow (*Passer italiae*) the short, anterior section of the mitochondrial sheath is covered by a fibrous sheath.

Three structures make up the helical undulating membrane in the area of the midpiece: the nine (x2, x3) peripherial bundles of the axoneme, the mitochondrial sheath and the outmost helical membrane under the cell membrane that consists of microtubules. Several studies show that this helical membrane, which consists of microtubular bundles and is found under the cell membrane along the whole length of the sperm, is only present temprorarily, in the developmental stage associated with the contorted seminiferous tubules. When sperm leave the contorted seminipherous tubule system of the testis, in the efferent tubules they lose this outer microtubular helix.

In some sparrow, finch, treecreeper, starling and new world blackbird species, granular, compact cytoplasmic components appear in the anterior part of the midpiece and helically surround the mitochondrial sheath forming a spiral **granular mass or granular body.** This component of the midpiece forms a cytoplasmic anchoring structure.

In the area of the **main piece** the axoneme is covered by a dense fibrous sheath, and in the **end piece** the organised 9+2 structure of the axoneme disintegrates into a disordered structure (Figure 47).

Two main components can be distinguished in the **interstitial tissue of the testis**. One is the peritubular tissue, which surrounds the contorted seminiferous tubules tightly. This is dense connective tissue, containing myofibroblasts. The other type is loose connective tissue, which fills the space between the tubules, i.e. the actual interstitium.

The peritubular tissue separates into two layers, the inner fibrous layer that tightly attaches to the basal lamina of the seminiferous tubules, and the outer cellular layer that contains myofibroblasts. The fibrous layer consists of regularly arranged collagen fibres, which are embedded in an amorphous matrix. Myofibroblasts, which have characteristics of both smooth muscle cells and fibroblasts, are arranged in the cellular layer forming several concentric sheeth. Naked fibres and nerve fibres with sheath of Schwann are both visible in the peritubular area. Rhythmical peristaltic contractions of the myofibroblasts move the sperm that have detached by spermiation towards the epididymis and the deferent duct. The fibrous layer is separated from the looser-structured interstitium by a network of blood and lymphatic capillaries.

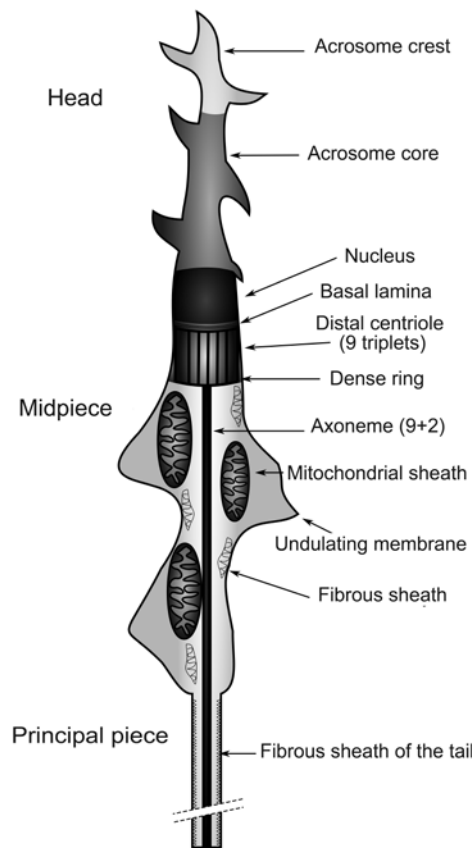

Figure 47. Schematic electron microscopic representation of the sperm of a sparrow species

The interstitium is the loose connective tissue that fills the space among the seminiferous tubules and it contains larger arterial and venous

blood vessels. Between the fibres and the capillaries there are fibroblasts, a small amount of macrophages, lymphocytes, plasmocytes and adipocytes. In the interstitium of the active testis, the melanin-containing modified connective tissue cells (possibly macrophages) are less visible because of their large dispersion. During testicular atrophy and seasonal regression however, they aggregate among the tubules, painting the tissue and the whole testicle a characteristic greyish-black colour. Their role is not known. **Leydig cells** are modified connective tissue cells of this area. They produce steroids, mostly testosterone. Leydig cells are large and electron dense, aggregated in groups in the interstitium. Their amount in birds is not too high. Their nucleus is oval or polygonal, often elongated euchromatic type. Their Golgi complex is medium developed, and they posess a large amount of oval or elongated tubular-type mitochondria. Their granular endoplasmic reticulum is poorly developed, but there is a large amount of smooth endoplasmic reticulum, free ribosomes and polyribosomes in their cytoplasm. Depending on their functioning, there is a varible amount of lipid drops of different sizes in their cytoplasm. There are adrenergic fibre endings on the Leydig cells.

The plasma membrane of the Leydig cells contains a large amount of LH receptors, and according to in vitro and in vivo studies, their testosterone secretion is stimulated by LH. Using PCR, GnIH and GnIH receptor mRNA were detected In the testicular tissue of house sparrow (*Passer domesticus*), white-crowned sparrow (*Zonotrichia leucophrys*), common starling (*Sturnus vulgaris*) and Japanese quail. GnIH mostly expresses in the interstitium (possibly in the Leydig cells), while GnIH receptors were detected in the interstitium and on spermatocytes. In vitro, a small amount (1-10 μMol) of GnIH significantly decreased gonadotropin-stimulated testosterone secretion. The GnIH-GnIH-R system probably serves as a local inhibitory mechanism of testosterone secretion in the testis (McGuire and Bentley, 2010).

3.1.3. Steroid hormone biosynthesis in the testis and their peripherial effects

Most steroid biosynthesis occurs in two cell types of the avian testis: in the interstitial, Leydig cells and the Sertoli cells. Nevertheless, developing and mature germ cells are also capable of steroid production. The functional aspects of steroid production between the two cell types are considerably different. In Leydig cells, steroids (mostly androgens and mainly testosterone) are produced in an endocrine fashion, hormones express their effect in the periphery, getting there by systemic circulation. Steroid production by Sertoli cells, however, demands autocrine and paracrine mechanisms. The enzymes of the δ-4 and δ-5 steroid biosynthetic pathways are found in both cell types, androgen biosynthesis mostly starts from progesterone and 17α-hydroxyprogesterone,

but dehydroepiandrosterone, which forms from 17α-hydroxypregnenolon is also a biologically important compound (Lake and Furr, 1971, Lofts and Murton, 1973, Sturkie, 1976, Péczely, 1985).

Through the δ-4 biosynthesis pathway **androstenedione (A)** is produced form 17α-hydroxyprogesterone by the effect of P450,17α-(17,20 lyase), which is not only the intermediate product of further androgen biosynthesis and metabolism, but is also secreted in substantial amounts into the blood plasma as a secretory end product. In young (9-12 weeks old) roosters the plasma concentration of androstenedione is 5-7 times higher than the concentration of testosterone, and even though it decreases towards the end of the prepuberty (16-20 weeks), it is still 1.5 times higher. Its value only decreases (to 3.5–5.0 ng/ml) in sexually mature roosters, when testosterone reaches its maximal value (4–9 ng/ml) (Culbert et al., 1977). According to Williams and DeReviers (1981), in male Japanese quail, androstenedione plasma level approaches that of testosterone, and its biological activity is around 70% of the activity of testosterone, therefore the amount of hormone in the circulation represents substantial androgen potential. Carrié-Lemoine et al., (1983) obtained a similar results in young roosters, as they found that during the first few weeks androstenedione plasma concentration was higher than that of testosterone, this ratio reached a maximum between days 30 and 40, and after this turned negative. In adult roosters kept under long photoperiod, plasma testosterone and dihydrotestosterone levels could be characterised by the daily rhytm that appeared in the end of the dark period, and at the same time adrostenedione plasma level lacked diurnal fluctuation (Bachman et al., 1987).

Dehydroepiandrosterone (DHEA) is also produced by P450-17α-(17,20 lyase) through the δ-5 biosynthesis pathway. This steroid is an important intermediate metabolite of androgen biosynthesis, as 3β-hydroxysteroid dehydrogenase can convert it into androstenedione, but it is also a secretory end product, that gets into the blood plasma (Lake and Furr, 1971). In male birds, only part of the DHEA that is found in the blood circulation is produced in the testis, the rest originates from the adrenal cortex. Its seasonal plasma levels are similar between the two sexes in geese, but in sexually mature males the DHEA concentration is somewhat higher than in females. It shows a seasonally high value in both sexes right after the breeding season (during postnuptial molt or the beginning of photorefractoriness), in autum (during prenuptial breeding or the beginning of postrefractory period) and right before the spring breeding season. Its amount in the blood plasma of geese, as a yearly average, is about a 1/5-1/20 of the amount of testosterone. The absolute yearly maximum value in male geese is 240 pg/ml (end of June), while the minimum is 65 pg/ml (end of September) (Do thi Dong Xuan et al., 2005, Péczely et al., 2011).

DHEA decreases lipid deposition in birds, decreasing the amount of adipocytes. A target organ of this peripherial effect is the liver. The action mechanism

suggests cAMP/PKA signal mechanism. DHEA treatment increases intracellular cAMP accumulation in the hepatocytes, inhibits cAMP-specific phosphodiesterase activity, and stimulates cAMP-dependent PKA. This latest leads to the supression of the sterol regulatory element-binding protein-1 (Tang et al., 2009). DHEA increases lipid catabolising activity in the liver, and this effect shows some sexual dimorphism. DHEA treatment of broiler chickens decreased the triglyceride content of the liver and increased the concentration of non-esterified fatty acids in both sexes, but only increased lipase activity in males. Additionally, DHEA treatment increased the expression of peroxisome proliferator-activated receptor α, carnitine-palmitoyltransferase and acyl-coenzyme-A-oxidase-1 mRNA. At the same time, the amount of acetyl-CoA-carboxylase mRNA only decreased in females (Tang et al., 2007). DHEA increases mitochondrial oxygen consumption and peroxisome catalase activity, but decreases malate and glycerol-3-phosphate dehydrogenase activity, which suggest it affects basal metabolism of the liver (Bobolyeva et al., 1993).

DHEA does not affect secondary sexual characteristics: DHEA treatment of sexually inactive male song sparrow (*Melospiza melodia*) did not increase the amount of cloacal protuberance. It also lacks immunosuppressive effects, which, in case of probable regulatory effects (in case of aggressive or reproductive behaviour) would decrease the amount of investment from the organism (Soma, 2006).

Testis produces **testosterone** (**T**) in the largest amount, by 17β-hydroxysteroid dehydrogenase converts androstenedione. In the testis (Leydig cells), in the blood plasma and in certain other tissues, testosterone can be easily converted back to androstenedione. Increased testosterone production by the Leydig cells is accomplanied by a proportional hypertrophy, which mostly comes from the increasing amount of smooth endoplasmic reticulums, and to a smaller degree of the tubular mitochondria.

The intensity of testosterone production is primarly regulated by LH, and somewhat by FSH, which act through the membrane receptors of the Leydig cells by increasing cAMP production. Testosterone implant in photorefracter young male Japanese quails increases the amount of LH receptors in testis tissue membrane preparation, but does not affect the numbers of FSH receptors, even though their number is highest in young males. Therefore, the expression of FSH receptors is regulated by other factors (Ottinger et al., 2002). The production of testosterone is regulated by intracellular and extracellular (negative) feedback mechanism. Intracellular regulation means a direct inhibitory feedback of testosterone produced in Leydig cells on 17β-hydroxysteroid dehydrogenase that halts biosynthesis. On the other hand, the produced testosterone binds to the cytoplasmic androgen receptors in the Leydig cells, which by attaching to the genome acceptor sites block the expression of 3β-HSD and CYP17, which would supply androgen production from pregnenolone. Nuclear

androgen receptors have been detected in the testicular tissue of domestic cockerel and duck, with immunopositive activity present in the nucleus of Leydig, Sertoli and some myoid cells. The amount of androgen receptors was highest in juvenile and prepubertal individuals and decreased in the Leydig and Sertoli cells as the birds matured, indicating the importance of the androgen effect in the process of differentiation and maturation (Gonzalez-Morán et al., 2008, Dornas et al., 2008).

Extracellular regulation occurs though hyphothalamic androgen receptors and the inhibition of GnRH secretion, decreasing the activity of the gonadotropic – gonadal axis and the amount of LH stimulating the Leydig cells. (The structure of androgen receptors was introduced in the section about androgen production in the ovary.)

The majority (96-98%) of testrosterone that leaves the Leydig cells enters the circulation, bound to proteins (CBG, prealbumin and albumin), while the rest forms the active, free fraction. The reversibly bound steroid molecules get released around the target cells, and in this free form they can attach to membrane or cytosol receptors.

The biological half-life of testosterone is about 26-28 minutes in both juvenile and sexually active Japanese quail, which means a 1.5-1.7 ml/min/kg metabolic clearance rate (MCR). The secretory rate (SR) of testosterone is 0.13 ng/min/kg in inactive Japanese quail (kept under short photoperiod). This value increases substantially to 3.96 ng/min/kg in photo-gonado-stimulated birds. According to in vivo studies with male Japanese quail, 30 minutes – 1 hour after the administration of tritiated testosterone, several radioactive metabolites appear in the plasma, such as 5α- and 5β-dihydrotestosterone, androstenedione, androstanedione, androsterone and epiandrosterone (Péczely, 1985, Thies et al., 1983). Androstenedione forms from testosterone reversibly, and the conversion rate is the same is sexually inactive and active male birds. δ4-5-reductase converts androstenedione into androstanedion, which has a substantially smaller androgene activity. As a result of further reduction on the C-atom in position 3, in addition to epiandrosterone active androsterone is formed, which has a very low biological activity. The conversion rate of these last two metabolites is larger in juvenile and sexually (seasonally) inactive male birds, and it increases with higher plasma level of thyroxine, which stimulates basic metabolism. The typically high thyroxine level in the end of the breeding season, especially in males, plays an important role in the quick drop in plasma testosterone concentrations, increasing MCR from 1.70 ml/min/kg to 4.43 ml/min/kg (Péczely, 1985).

δ-5-reductases convert testosterone into **5-dihydrotestosterone** by reducing the C atom in position 5 in the A benzene ring. 5-dihydrotestosterone has two isomers, 5α-DHT and 5β-DHT. This process occurs in the peripherial tissue and specific areas of the central nervous system, where a substantial amount of

testosterone gets converted into a metabolite with a higher androgen potential. The biological activity of the resulting 5α-DHT is 5-10 times higher than that of testosterone, but its plasma level is substantially lower. On the other hand, 5β-DHT has hardly any androgen activity, its production can be considered inactivational shunt.

In males, androgens (mostly testosterone and the periferially produced 5α-DHT) enact their endocrine and behaviour-regulating effects by binding to the androgen receptors in the brain tissue (mostly in the area of the limbic system and the hypothalamus). This occurs as the "androgen effect" occurs and also as aromatisable androgens (testosterone and androstenedione) get converted into 17β-estradiol in the nerve cells to trigger the "estrogen effect". (The neuroendocrine effects of testosterone and 5-DHT on the central nervous system will be detailed in the chapter "Central regulation").

The majority of testosterone catabolisation occurs in the liver. This process mostly depends on the microsomal and cytosol fractions, as the nucleus and mitochondria have a considerably lower steroid activity. Following the administration of testosterone marked in the cytosol fraction, 5β-dihydrotestosterone, 5β-androstane-3α, 17β-diol, 3α-hydroxy-5βandrostane-17-one, and 5β-androstenedione apprear. Under aerob conditions, in the microsome fraction most testosterone also gets converted into androstenedione, along with a small amount of few polar metabolites. In this in vitro system 5α-reductase avtivity could not be detected (Sugimoto et al., 1990).

Testosterone (and the dihydrotestosterone, to which it gets converted) have a specific regulatory role in the epididymal area. During puberty and maturation the excurrent duct system increases by a large amount, which indicates intensive, androgen-regulated protein synthesis. The amount of androgen receptors in the epithelial cells in the tubules of the epididymis is lower in juvenile roosters and higher in sexually mature cockerels (Shanbag and Sharp, 1996). In the epithelial lining of the epididymal tubules in sexually mature domestic cockerels and drakes a large number of androgen receptors occurs. These are mostly located in the stereocilial cells of the epididymal ducts, and to a smaller degree also in the epithelium of the rete testis and in the non-ciliated cells of the distal efferent ducts. No androgen receptors were detected in the ciliated cells of the proximal and distal channels. The difference in the amount of androgen receptors can reflect differences in secretion among different active epididymal sections or cell types (Dornas et al., 2008).

The efferent duct, seminal receptacle, cloacal grands, protuding and non-protuding copulatory organs and pseudophalli are all androgen-dependent (androgen-receptor containing) organs, their optimal development and functioning depend on an increased androgen level. They enlarge during puberty or seasonally-occuring sexual maturation, when androgen (testosterone) plasma level is high. Castration has been shown to cause degeneration in these

structures, but testosterone treatment of desexed birds re-establishes the state of hypertrophy, typical of sexually active individuals (Lofts and Massa, 1980).

Androgen receptors can be detected by immunocytochemical methods in the surface epithelial cells and the connective tissue beneath in the spirally pleated mucosa of the protruding phallus of geese. The amount of these receptors changes seasonally, there are few of them in juveniles and in individuals that are in the photorefracter stage, markedly increases during sexual reactivation in the autumn, and slightly decreases during sexual activity in the spring (Fancsovits and Péczely, 1999).

The cloacal gland in quails is a typical androgen-dependent organ, an accessory sex gland, which is rare in birds. The gland reacts to the high testosterone level of photostimulated birds by increasing to several time its original size and it produces a frothy secretum. Its secretum, a product of the accessory sex gland, mixes with the sperm. The mobilisation of testosterone and the production of 5α and β-DHT changes in a characteristic way in the cloacal gland of the Japanese quail. In birds kept under short day (8L-18D) conditions, under in vitro conditions the cloacal gland converts the added testosterone into mainly 5β-DHT, while under long day conditions the formation of the biologically much more active 5α-DHT markedly increases (Lofts and Massa, 1980). Castration causes the mass of the gland to decrease, and testosterone, 5α-DHT, 5α-androstane-3,17-dion, androstenedion and 5α-androstane-3α-ol-17-one treatment of castrated quails increases the mass of the cloacal gland to that in inact, active individuals. At the same time, 5β-DHT, 5α-androstane-3β and 17β-diol have no effect. Therefore, under in vitro conditions, the cloacal gland of juvenile birds converts testosterone mostly into androstenedione, while from sexually active quails mostly 5α-DHT is produced. It is probably testosterone acting together with 5α-DHT that cause the enlargement of the gland in active birds (Massa et al., 1980).

In birds, the functioning of the uropygial gland, i.e., the production of acidic mucins, neutral lipids, lycolipids, phospholipids and particularly unsaturated fatty acids, is regulated primarily by androgens. The androgen receptors present in the tissue of the gland bind mostly testosterone, 5α-DHT and androstenedione, but DHEA also increases secretion in the gland. The large amount of estrogen receptors located in the uropygial gland suggests local aromatisation and the possibility of direct estrogen effect (Daniel, 1980, Deadhikari and Bhattacharyya, 1987, Salibian and Montalti, 2009).

The characteristic, often colourful skin appendages that represent secondary sexual characteristics, are often also androgen-dependent structures. Not only the light or dark red colour of the rooster's comb indicate changes in plasma testosterone concentration, but also its mass, as it is tightly correlated with the actual testosterone level. This observation was used to create the comb-test, a sensitive bioassay used for a long time as an empirical test of androgen level.

Testosterone treatment causes the formation of a large number of blood capillaries close to the surface of the subepithelial connective tissue, with a large amount of red blood cells in their large lumen. In the comb, androgen receptors are mostly located in the endothelial cells of the capillaries, their activisation causes dilation of the capillary lumen, and increased arborisation of the vessels (Yoshioka et al., 2010). The size of the red facial skin of the male ring-necked pheasant (*Phasianus colchicus*) starts to increase intensively at the beginning of the sexual reactivation in the autumn, as a result of increasing plasma DHEA and testosterone levels and it reaches its maxiumum size during the spring breeding period, when plasma testosterone concentration is highest (Tamás and Péczely, 1998).

Several species have sexually dimorphic plumage, and some – generally males – display nuptial plumage during the breeding season. Sexual steroids (mostly testosterone) play a determing role in this. Similarly, the hyperplasia and colouring of the keratin part of the beak (rhamphotheca) and the enlargement and colouring of the keratinous elements of the tarsus are under sexual determination. The formation of the nuptial plumage as a typical secondary sexual characteristic will be detailed in the section about ecophysiology and molt.

Some hepatic enzyme concentrations have definite sexual dimorphism. For instance, the concentration of microsomal monooxygenases is 2-4 times higher in roosters compared to hens. Testosterone treatment of hens increases the amount of monooxigenases, indicating the enzyme-expression effect of testosterone (Pampori and Shapiro, 1993). Androgens (mainly testosterone) decrease liver lipogenesis, and cause lipid accumulation in the liver and in other parts of the body. Castrating roosters increases their body mass, which mostly results from increased lipid deposition: it increases the amount of abdominal and subcutaneous fat and the lipid content of the liver. Castration increases the amount of total lipids, phospholipids and triacylglycerols in the liver and the activity of NADP-malate-dehydrogenase. On the other hand, it decresases non-esterified fatty acid content, as well as the activity of enoyl-coenzyme ahydratase and 3-ketoacyl-coenzyme A-thiolase. Testosterone treatment of castrated individuals basically restores the levels characteristic of intact roosters (Chen et al., 2007, 2009).

In mammals, androgens have an anabolic effect, increasing muscle and bone growth. In birds, however, the effect of androgens is not obvious, some results suggest catabolic effects, increasing metabolic rate, while others report weaker or more intensive stimulating effects on muscles. Testosterone and 5α-dihydroxytestosterone inhibited body growth in female and male juvenile fowl. Castrating roosters pre and post puberty causes a drastic decrease in testosterone level and results in the increase of pectoral muscle and the gastro-intestinal tract. Treatment of castrated birds with androgen implants (19-nortestosterone, 5α-DHT and testosterone) increased myofibrillar ATP-ase

activity, and 19-nortestosterone sightly increased leg muscle mass. Testosterone treatment of juvenile (2-6 weeks old) chickens decreased growth rate and body mass (Fenell and Scanes, 1992a,b, Fenell et al., 1996, Chen et al., 2010). Contradicting these results, testosterone propionate treatment of 28 and 48-day-old male and female broiler chickens increased relative muscle mass and the size of the comb, but did not effect the relative amount of bones compared to body mass (Deyhim et al., 1992). Testosterone, 5α-DHT and 19-nortestosterone increased body mass, daily body growth and the mass of the right pectoral muscle in adult female turkeys. 5α-DHT and 19-nortestosterone had similar effects in adult males. On the other hand, these androgens did not affect skeletal mass. Castration did not affect body mass, but increased adiposity, and 19-nortestosterone treatment of castrated birds increased muscle mass and decreased the amount of lipids (Fenell and Scanes, 1992). Hayashi et al., (1993) studied a different aspect of the effect of testosterone. According to them, testosterone proprionate treatment of 2-3 week old female broiler chickens did not affect body mass, but if coadministered with thyroxine, it significantly increased muscle protein catabolism. Administered alone neither testosterone nor thyroxine affected the rate of muscle protein metabolism.

The immunosupressive effect of androgens is known from adult and prepubertal birds: testosterone treatment of 2-6 week old chickens decreased the mass of the bursa of Fabricius (Fenell et al., 1996). Androgens act as an immunosuppressant in recently hatched birds, if they get into the egg yolk in super-optimal amounts. In colonially nesting birds that lay more than one egg, testosterone deposited into the egg increases the vitality of chicks but also decreases their resistance against parasites and contagious diseases. This has been shown by treating eggs with testosterone in a black-headed gull (*Larus ridibundus*) colony, where the treated chicks grew faster and were feeding more intensively, but their cellular and humoral immunity was supressed (Müller et al., 2005). Similar results were found in Eurasian jackdaw (*Corvus monedula*) chicks, where after testosterone treatment of the eggs, chicks had higher testosterone plasma levels, and these individuals had weaker cellular and humoral immune responses at the age of 1-2-3 and 4 weeks (Sandell et al., 2009).

Besides androgens, the testis also secretes estrogens and progesterone, in amounts that depend on the age of the individual and the season. The embryonal testes, like the ovary, also produce a substantial amount of estradiol, however, the plasma estrogen level is lower in male chicken embryos (Tanabe et al., 1986, Imataka et al., 1989).

Estrogens (E) are also present in the testicular tissue of mature birds, and they can be produced in detectable amounts in vitro after the administration of precursors to testicular tissue, and there are measurable changes in their amount in the plasma of males according to age, as well as annual and diurnal cycles (Lake and Furr, 1971, Sturkie, 1976, Gulati et al., 1981, Péczely, 1987).

Plasma estron and estradiol levels in juvenile and sexually inactive males are slightly lower and basically equal to the levels in juvenile and seasonally inactive females. For instance, the estron plasma concentration in the Hungarian population of Eurasian collared dove (*Streptopelia decaocto*) is 40-60 pg/ml in males, females, juvenile, as well as in inactive individuals. Its amount does not change significantly in mature birds either, remaining 40 pg/ml in males and 66 pg/ml in females. Estradiol levels are 180-210 pg/ml in juvenile and sexually inactive male and female doves. However, after sexual maturation, estradiol plasma levels show considerable sexual dimorphism, its level substantially decreases in males to 90 pg/ml, and increases in females to 337 pg/ml during the periovulatory phase and to 150-240 pg/ml between ovulations (Péczely, 1982). The situation is similar in the rook, where the estrogen production of the gonads only differs between sexually mature and active individuals. In juvenile birds and during the winter and early spring when the seasonal increase of the testes and the ovary has not yet initiated, plasma eston and estradiol levels are basically equal between male and female individuals. These studies also underline the dominance of estradiol in both male and female birds (Péczely and Pethes, 1982). In male mallards (*Anas platyrhynchos*), plasma estradiol and testosterone levels show a definite, seasonally occurring inverse relationship. Before seasonal maturation plasma estradiol level is relatively high, and it decreases sharply during sexual activation, when testosterone is rapidly increasing, but during the photorefracter stage estrogen and sharply decreasing testosterone plasma concentrations increase again in male birds (Péczely et al., 1994). Similar results were found in roosters, where at 20 weeks of age (right before puberty) the plasma estradiol level is relatively high (29 pg/ml), and it suddenly decreases during the strong increase in testosterone level that indicates sexual maturation. At 72 weeks old, both the fertility and the testosterone plasma concentration of roosters decrease substantially, and again high estradiol levels can be detected. The strong decrease in the levels of LH and testosterone and in male fertility are thought to be resulting from the negative feedback effect of plasma estradiol (Weil et al., 1999).

A part of estrogens found in the systemic circulation of male birds are synthesised in certain areas of the brain by aromatisation. From here, a large amount of hormones get into the circulation. Studies on zebra finch and brown-headed cowbird (*Molothrus ater*) found that following the injection of radioactive androstenedione into the brain, tritiated estrogen level was higher in the jugular vein compared to the carotid artery. At the same time, in vitro testicular tissue also synthesises a large amount of estradiol from the precursor applied to the medium, which proves that estrogen detected in the systemic circulation partly originates from the testis (Arnold and Gorski, 1984, Saldanha and Schlinger, 1997).

In birds (similar to mammals), estrogens that originate from the testis are synthesised by the Leydig cells, Sertoli cells, pachytene spermatocytes and even more so by the rounded spermatids, proven by the occurrence of significant aromatase activity in these cells. Estrogens originating from the testis act though autocrine and paracrine pathways. They play an important role in the regulation of spermatogenesis (by forming the haploid phase) and steroid biosynthesis by Leydig and Sertoli cells and in sperm maturation that continues in the epididymis. In mammals, estrogen receptors were detected in Leydig and Sertoli cells (Pearl et al., 2011). During maturation a reciprocal relationship can form between sperm cells and cells of the proximal and distal efferent tubes of the epididymis. This is suggested by the substantial estrogen content of the non-cilated cells in the epididymis of roosters, which plays and important role in forming a functional relationship between sperm and epididymis and suggests that estrogen plays a regulatory role in the functioning of the epididymis (Kwon et al., 1995, 1997, Hess et al., 1995). In birds, presumably similar to mammals, estrogen also plays a role in the motility of mature sperm, which is associated with the hormone produced in the tail section, referred by the localisation of aromatase, and also by the fact, that the sperm of the ejaculate also contain α-estrogen receptors (Hess et al., 1995, Carreau et al., 2003, Bois et al., 2010).

The role of estrogens in the systemic circulation of male birds is less known, they are probably important in the age-related and seasonal decrease of male fertility and in the regulation of gonadotrop hormone secretion (as suggested by studies conducted on roosters). They probably affect behaviour, such as breeding and paternal behaviour in male birds, autumn and winter flocking behaviour, autumn hyperphagia (as a component of complex hormonal effects) and sex reversal processes, through the central nervous system.

Progesterone (P4) is an important intermediate metabolite in the testis and also occurs as an end product. However, in this case, it is more difficult to measure the amount of steroids that get into the systemic circulation, as the adrenals must also secrete an extensive amount of progesterone.

In male and female chicken and duck embryos, the allantoic fluid and the blood contains a detectable amount of progesterone, which during the last few days of incubation several times supercedes the concentration of testosterone and estrogen in male duck embryo. In vitro, pregnenolone causes the testicular tissue to synthesise a substantial amount of progesterone (Guichard et al., 1979, Tanabe et al., 1983). In day-old chicken the progesterone production is equal in the testes and the ovary (Tanabe et al., 1986). In male Eurasian collared dove, the plasma progesterone level is 1.5 times higher than testosterone level, P4 = 466 and 413 pg/ml, T = 181, and 269 pg/ml, in juvenile and sexually inactive birds, respectively. The strong dominance of testosterone only appears in sexually active males, when the P4 level is technically the same as in juveniles (463 pg/ml), while the level of testosterone has considerably increased to

2092 pg/ml (Péczely and Pethes, 1980). In male red-ledged partridge (*Alectoris greca*) progesterone plasma level increases with age, and further increases after photostimulation (Creighton, 1988). In male Adélie penguins (*Pygoscelis adeliae*) the level of progesterone remains constant throughout the year, with only smaller fluctuations, while the amount of 17-a-hydroxiprogesterone changes seasonally, is relatively high during courtship, then markedly decreases during incubation and migration (McQueen et al., 1999). Crested auklet (*Aethia cristatella*) males have a characteristic lemon odour during incubation and feeding of chickes, and the emission of this odourant is strongly correlated with plasma progesterone level. Females do not have a significant odourant emission, and it does not correlate with plasma progesterone, testosterone, dihydrotestosterone or estrogen levels (Douglas et al., 2008).

The presence of progestine receptors in the cytosol fraction of young roosters' testis suggests that progesterone has autocrine or paracrine effects (Seiki et al., 1981). The amount of progesterone receptors is highest in sexually active roosters, while lower in juvenile and aged birds. In juvenile and sexually active roosters both progesterone receptor isoforms are present, while in aged individuals Type A becomes dominant (Gonzalez-Morán et al., 2008). The conclusions of studies conducted on budgerigar (*Melopsittacus undulatus*) are different, as progesterone and estrogen receptors are present in equal numbers in the testes and the epididymis of sexually inactive, pubertal and mature males (Reitemeier et al., 2011).

The role of progesterone in the systemic circulation of male birds is little known. Nevertheless, it is known to modulate behaviour during chick rearing and after breeding. Progesterone treatment of Japanese quail decreases courtship and copulatory behaviour in photogonado-stimulated males, and this anti-androgen effect can be supported by the competition of progesterone and testosterone when 5α, and 5β-reduced metabolites are forming (Bottoni et al., 1985). Progesterone is also an important regulatory factor of the functioning of feather follicles and the process of molt (Péczely et al., 2011).

3.2. The excurrent system of semen, the copulatory organ and he semen

The contorted seminiferous tubules orient towards the medial part of the testis, and around the hilum they continue as **straight short seminiferous tubules (tubuli seminiferi recti)**, which open into the network-forming rete testis. These short, straight tubules are covered in an epithelium that consists of modified Sertoli cells, which replaces the spermatogenic epithelium. The cells of this epithelium contribute to the formation of primary semen by merocrine secretion. The well-developed Golgi complex, located in the supranuclear area, produces

vesicles with a dense core. The Golgi complex is surrounded by rich granulated endoplasmic reticle. According to Aire (1982), the contortous tubules often open direcly into the rete testis, without turning into straight tubules.

Two cell types line the wider channels and the lacunae of the **rete testis**, which is located on the hilum of the testis. Most pseudostratified cuboidal and columnar epithelial cells carry only microvilli. However, on the surface of nearly all of these cells there is one large, immobile cilium-like structure (of a 9+2 stucture), which possibly has a sensory function. The supranuclear area of the microvilli-covered cells has a well-developed Golgi complex, granular endoplasmic reticulum, several lyposomes, a dense body and a vacuole. These secretory elements contribute to the formation of the seminal fluid. The microvilli-covered cells are capable of phagocytosis**,** mostly taking up disintegrated sperm fragments and some chemical compounds, but do not filter out microorganisms. The other type of surface epithelial cells are the rarer ciliated cells. There are also a few wedged macrophages among the epithelial cells. By contracting, the subepithelial layer of myofibroblasts creates an active pumping mechanism towards the epididymis (sperm spend only about 30 seconds in the area of the rete testis). The smaller, intracapsular part of the rete testis is located under the tunica albuginea, while the larger, extracapsular part forms a transition towards the efferent ducts that lead to the head area of the epididymis (Péczely, 1987, Aire, 2007).

3.2.1. The epididymis

The avian epididymis, similar to mammals, contains efferent ducts that gather from the rete testis, short, thicker conjugent ducts, which connect these with the epididymal duct and the epididymal duct, which runs along the whole length of the epididymis. The efferent and conjugent ducts develop from mesonephric tubules during embryogenesis, and the epididymal duct originates from the Wolffian duct.

Similar to mammals, the lacunae of the rete testis continue in the **efferent ducts** (**ductuli efferentes**) that lead to the epididymis. The efferent duct system can therefore be considered part of the epididymis. This duct system consists of two sections, the proximal and the distal efferent ducts. From the latter, short tubules, **conjugent ducts (ductuli conjugens**) lead into the epididymal duct. The efferent ducts are lined with ciliated columnar and pseudostratified columnar epithelium, among which there are two non-ciliated cell types.

The epithelium of the **proximal efferent ducts** and the connective tissue underneath are both strongly ridged. In this area, there are more non-ciliated, microvilli-covered cells than ciliated cells. These cells carry relatively large microvilli and one non-motile cilium. In their subapical zone there are typically endocytotic tubules, coated vesicles and dense bodies. Ciliated cells are

less electron dense, their nucleus is located higher than in non-ciliated cells, and there are fewer and shorter microvilli among the cilia.

In the area of the thinner tubules of the **distal efferent ducts** are no epithelial invaginations into the lumen to enlarge the surface area. The number of ciliated cells is higher here compared to the previous section. The non-ciliated, microvilli-covered cells belong to another type, with less stuctures in their subapical cytoplasm suggesting endocytosis and a lower amount of mitochondria compared to the cells in the proximal area.

In birds, the efferent duct system makes up about 35-62% of the volume of the epididymis, suggesting essential transport and reabsorption activities. Material uptake from the semen and their transport from the luminal space can be especially intensive in the proximal area, where epithelial ridges invaginate into the lumen substantially increase the contact surface of the tubules. In Japanese quail, 86% of the fluid leaving the testis gets reabsorbed here, in spite of the fast flow through, as labelled sperm move through this region in about 3 minutes. Estrogens can play a part in the regulation of this reabsorption function, as suggested by the high estrogen receptor content of the proximal region in roosters (Kwon et al., 1997). This area is also important for ion transport, indicated by the rich Na-K ATP-ase, carbonic anhydrase and Na-H cotransporter systems, and the presence of the transmembrane water molecule transport protein (aquaporin-2, -3 and -9) in the epithelium. Material taken up from the luminal fluid (semen) are mostly converted and metabolised in the lysosomal system of the non-ciliated epithelial cells.

Compared to the distal efferent tubules, the **conjugant tubules** (**ductuli conjugans**) are a tubular system that consist of shorter units with wider diameter, which are lined with microvilli-carrying, non-ciliated columnar epithelium. These elements open into the considerably thicker, straight epididymal duct.

The **epididymal duct** (**ductus epididymis**) and its continuation, the thicker defferent duct run along the dorso-medial surface of the epididymis. As they all originate from the Wolffian duct, the members of this tubular system posess the same epithelial lining, which are different from the two non-ciliated cell types of the efferent duct system. In both areas there are columnar epithelial cells covered in short microvilli, and their membranes are firmly connected to each other by occluding and adherent zonules in the upper third of the cells. The basally located nucleus is round or oval, slightly heterochromatic. The nucleus is often surrounded by bundles of medium-thick filaments (cytoskeleton). Cells are characterised by rich rough granular endoplasmic reticulum and many free ribosomes. There are few lipid drops, several mitochondria and a well-developed Golgi complex in the cytoplasm. The 3β- and 17β-hydroxysteroid dehydrogenase content of the epithelial lining suggests that there is steroid synthesis in these epididymal cells. On the other hand, signs of mecrorine secretion are

also visible in these cells. The produced protein-type secretum absorbes to the sperm as they pass through, and contributes to the formation of their cell coat.

Unlike in mammals, the epididymis does not play such an important role in the storage or maturation of sperm. While in mammals sperm can stay one or two weeks in the epididymis, it is much shorter in birds, for example in Japanese quail this period is only two hours (Clulow and Jones, 1982).

3.2.2. The defferent duct (ductus defferens)

As a continuation of the epididymal duct, the defferent duct leads to the central area of the cloaca over the surface of the kidney lobes. It is a yellowish-white, heavily folded tube that runs in a connective tissue sheath, gradually thickening cranio-caudally. Its distal part is straight (pars recta of the ductus deferens, ejaculatory duct) and it opens into the urodeum directly, or through sperm-storing receptacles (receptaculi of the ductus deferens, seminal glomus) of different sizes, forming the papilla of the ductus deferens on its inner surface.

The epithelial layer of the deferent duct is similar to that of the epididymal duct, non-ciliated, pseudostratified columnar epithelium covered in microvilli. The lateral plasma membranes of the cells connect to the neighbouring elements through several interdigitations. In the apico-lateral part of the cell there are well developed occluding and adherent zonules. Their basally located nucleus is round or oval, mildly heterochromatic. Few granular endoplasmic reticulum, a well-developed supranuclear Golgi complex and many microtubules are visible. Typically, there are several vesicles of different sizes in the cytoplasm, suggesting intensive merocrine secretion. The pinocytic tubules and coated vesicles of the apical membrane suggest material uptake from the lumen (Aire, 2007).

The secretum of the cells of the defferent duct contains androgen-dependent proteins and carbohydrates, which – similar to the secretum of the epididymal duct – contribute to formation of the cell coat (glycocalyx) of the sperm. The cell coat itself contains a large amount of oligosaccharide chains, which covalently bind to the integral glycoproteins and glycolipids of the cell membrane, as well as to specific proteins, the proteoglycans. Besides carbohydrate chains, the cell coat itself also contains surface-binding glycoproteins and proteoglycans. All cells have glycocalyx, but in the case of sperm it is thicker (60 nm instead of 10 nm) and instead of 3 or 4, it contains 150 different glycoprotein components.

The cell coat that covers sperm is fomed in the epididymal duct and in the efferent duct. It contains several carbohydrates and their derivates, amoung others galactose, jacalin, glucose, mannose, N-acetylglycosamine, N-acetylgalactosamine, fucose, N-acetyllactosamine and sialic acid. The carbohydrate elements of the glycocalix are covered in a relatively thick layer of sialic acid on the outside. Certain components of the glycocalix are not distributed evenly on

the surface of the sperm, but are arranged mosaic like, attached to particular areas of the membrane. The composition of sperm glycocalyx is species specific (Peláez and Long, 2007, 2008, Peláez et al., 2011).

In turkey, the epithelial lining of the defferent duct, similar to the distal efferent tubules, also produces a serine protease enzyme that consists of two protein components, one with a molecular weight of 37 kDa and the other of 61 kDa. It supposedly contributes to sperm maturation and/or have antimicrobial effects. In other galliform species, under basic pH conditions there was no similar proteinase activity. The question is, why is it only present in turkey sperm and why only in the area of the defferent duct?

Among the epithelial cells there are basal „accessory" cells and lymphocytes. Under the basal membrane, there is a relatively thick connective tissue rich in capillaries, with a thick smooth muscle layer underneath. The muscle layer receives adrenergic and cholinergic innervation, and its role is to send on the semen and expulse it into the cloaca during mating.

The semen-storing **seminal glomus (glomus seminalis)** is covered in low columnar epithelium and cuboidal epithelium, and is especially well developed in Passeriformes. In some species there are also ciliated and secretory (PAS+), glycogen secreting cells in this area. There are **cords** and large crypts in the epithelium and in the mucosa, which facilitate sperm storage. The lumen of sexually active birds contains a large number of stored sperm. The wall of the seminal glomus consists of thick connective tissue rich is smooth muscle elements. The size of the bodies embedded in connective tissue in the dorso-lateral part of the cloaca is strongly function dependent, in sexually active, mating birds there is an evident cloacal protuberance, with a yellowish-white, loose tubular system visible through the skin. The size of the cloacal protuberance changes through the breeding cycle, reaching its maximum during the peak activity of the mating season, providing a quick way to assess sexual activity.

The seminal glomus can possibly have a function similar to the mammalian scrotum. It can contribute to the optimalisation of the fertilising ability of avian sperm, by keeping it at a temperature lower than the **core** temperature of the semen. The defferent duct (and if formed, the seminal glomus) is the primary site of sperm storage in birds, at a given time about 90% of the sperm that leave the testis are stored here and only about 10% in the epididymis. In birds, mature sperm are stored for a very short time (in Japanese quail 1-2 days, as maximum), unlike in mammals (in rats the optimal storage time is around 9 days). The maximal survival time of sperm in the defferent duct of the Japanese quail is 5-9 days, but their fertilising ability sharply decreases already after two days of storage (Clulow and Jones, 1982).

The **epididymal appendix** is a blind tubular system, close to the adrenals, sometimes its cranial part even embedded into it. On the other hand, it has no connection to the rete testis. It consists of two types of elements. One is the

aberrant duct, which is the frontal part of the epididymal duct that originates from the Wolffian duct, and which has a blind cranial end. The other element consists of also blind smaller tubules of the aberrant ductules, which develop from the mesonephral tubules. These tubules are lined with columnar or cuboidal epithelium covered in low microvilli, with smooth endoplasmic reticulum and tubular mitochondria. The epididymal appendix is capable of low-level steroid synthesis (Aire, 2007).

3.2.3. The copulatory organ (phallus)

Real copulatory organs or copulatory organ-like structures are only present in a relatively small percentage of bird species. Members of most orders have no developed copulatory organs, the transfer of semen into the cloaca of the female happens by pushing the cloacal openings together. The real copulatory organ of birds (phallus) shows a homologous structure to the organ found in turtles, squamata (lizards and snakes) and crocodiles. It is probable that Theropoda dinosaurs also had a protruding phallos (Larson and Frey, 1992). The basic structure of Sauropsida phallus consists of two, more or less elongated fibroelastic bodies, formed by the protuberances of the ventral wall of the proctodeum. These stuctures merge into one erectile body. The line of the merging is marked by the ejaculatory groove (or channel) that runs along the ventral longitudinal axis of the phallus. The functioning of avian copulatory organs is summarised by Guzsal (1981), Péczely (1987) and Montgomerie and Briskie (2007).

Among birds, real phallus (protruding and non protuding) is only found in *Struthioniformes* (ostrich, rhea, emu, cassowary and tinamou), *Anseriformes* (goose, duck and screamer) and *Galliformes* (pheasant, grouse and curassow).

Some parrots and passerines have a phallus-like copulatory organ (pseudophallus), which on the other hand, cannot be deducted from the common reptilian-avian developmental line, and can be considered a structure analogous to that of reptiles.

The real **avian phallus** can be divided into two types: protruding and non protruding. The protruding phallus of the phylogenetically older Ratites and Tinamous is very similar to the Reptilian phallus. Some types used to be distinguished based on the presence or absence of the blind tubular cavity that runs in the merged fibroelastic bodies, or based on the role they play during copulation. However, recent studies show that the development of this blind tubule is the result of adaptation as opposed to different phylogenetical processes.

Roosters posess a typical form of the **non-protruding phallus (phallus non protrudens)**. The tripartite copulatory papilla is located on the ventral side of the mucosa above the cloacal sphincter. Above and next to this open the right and left defferent ducts. The two side elements of this structure are the phallic

colliculi, which enlarge during **erection**, and push towards each other to form a nearly closed ejaculatory tubule. The central member of the copulatory papilla in *Galliformes* is the phallic papilla, which unlike to two side members, is not erectile. Male turkeys lack this central member of the copulatory papilla. Erection occurs as a result of the lymphatic filtrate that formes in the vascular bodies (corpus vasculare) in the side of the cloacal wall (Figure 48).

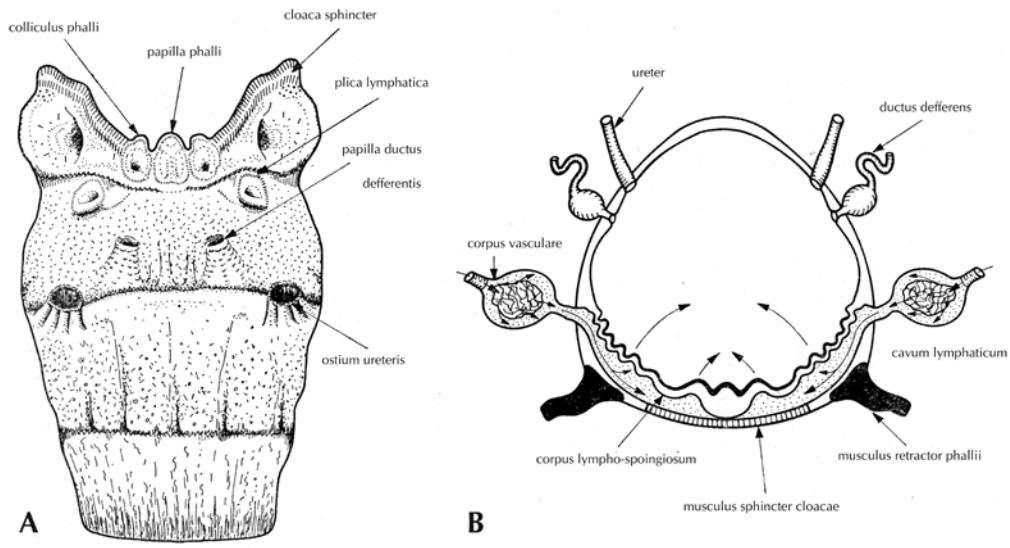

Figure 48. Non-protuding phallus in rooster. A. Dorsally opened cloaca, B. Cloaca cross section. Modified after Péczely (1987)

Before copulation, sexual neural stimuli reach the sacral parasympathetic spinal centres through sensory nervous pathways. The efferent branch of the vegetative reflex arch consists of the fibres of the pelvic nerve, which have cholinergic and vasodilatator effects. The efferent parasympathetic effect relaxes the walls of the arterioles in the vascular body, therefore high-pressure blood influxes into the glomerular capillary, plasma filtratum forms, which moves into the lymphatic cave that surrounds the erectile body. From here, a lymphatic duct carries the plasma filtrate into the colliculus of the phallus and to the base of the lymphatic folds that surround it. The influx of lymphatic fluid swells this areas, which is capable of erection. As a result, it will rise to form the ejaculatory channel, which, at most, has a slit-like opening on the top. At this time, the phallic papilla is located on the top of the tube. The closing muscles of the lympatic efferent vessels stop the lypmatic fluid from flowing away too early from the lymphatic cave. These muscles only relax after ejaculation, which results in the extension of the wall of the efferent duct. The striated closing muscles of the cloaca play an important role during copulation, as by contracting they slightly turn the copulatory mamilla outwards.

The **ejaculation** is regulated by a vegetative reflex arch. The afferent branch consists of adrenerg fibres that originate from the pericloacal sensory end-bodies, which lead ito the lumbar (symphathetic) segments of the spinal cord. The efferent branch of the relflex arch consists of the fibres of the hypogastric nerve, which gather from the lumbar segments, leading to the smooth muscle cells in the wall of the defferent duct, the ejaculatory duct and the seminal glomus. Nervous impulses cause the muscles to contract rhythmically. These muscle contractions expulse stored semen from the tubular system into the ejaculatory channel. The relatively weak muscles result in smaller semen expulsion compared to mammals, where the urethra is lined with striated muscles. In birds, typically the ejaculation of a small amount of semen is instantaneous.

The **protruding phallus (phallus protrudens**) is generally a hose-like structure that is more or less bent towards the left, as the two erectile fibrous bodies are located assymetrically. In the resting stage, the protruding phallus is similar to an inverted finger of a glove, and is located in the phallic sac, latero-dorsally on the left side of the proctodeum of the cloaca. During erection, lymph flows from the vascular body-lymphatic cavity system at the ventral part of the urodeum into the two fibrolymphatic bodies and the phallus everts as a spiral hose from the cloacal opening. The base of the phallus is connected to the ventrolateral part of the proctodeum by a widening fibrous-cartilageous base. In this basal part mucous secreting compound glands are located, which partly push into the blind tubular cavity that fills the inside of the fibro-lymphatic sacs**.** The epithelial layer is more or less keratinised, and in some species (e.g., *Anser* and *Anas*) carries spines or hooks. The protruding phallus also shows a strong seasonality, i.e., its size can increase five to tenfold in sexually active males compared to the resting stage. The number of the intraepithelial plasma cells increases drastically during the mating season.

The size and the spirality of the protruding phallus is variable among species. In kiwi it is relatively short and thick, only slightly spiral, with a nearly straight ejaculatory groove on the dorsal surface. This copulatory organ is similar to the "basic" phallus of turtles and crocodiles. In tinamou the 3-4 cm long phallus has several curves, and has a spiral ejaculatory channel running along it. In ostrich the everting protruding phallus is noticeably long, over 20-40 cm. It has well-developed retractor muscles on both sides, which pull the resting copulatory organ back after mating. In rhea, a fibrous connective tissue ribbon runs along the longitudinal axis of the phallus, which helps the retractor muscles.

Anseriform species have well developed, often quite long protruding phallus. The longest phallus, 28.5 cm, was measured in blue-billed duck (*Oxyura australis*). The right fibrous body is larger than the left, so the assymetric hose-shaped copulatory organ shows 3-4 spiralisations for instance in male mallard. In domestic goose, the base of the tube-shaped phallus is a short, fibrous cartilage that contains mucous-producing glands. In the proximal section there is

keratinised stratified squamous epithelium that carries different size papillas and spines. In the propria of the mucosa there is a lymphatic cavern system with thin connective tissue bridges. The epithelium at the distal part of the phallus is less keratinised. In this region the propria contains glands with branching tubular end chambers, and their common efferent duct, lined with simple columnar epithelium, forms the axis of the copulatory organ. Similarly to other species, erection is caused by the lymphatic fluid production of the paired vascular body – lymphatic cavity system, which is pressed into the lymphatic caverns of the phallus by the sphincter muscles of the cloaca through the lymphatic sac situated on the cartilage sheet. As a result, the copulatory organ gradually everts starting from the proximal end, in a way, that the papilla-covered mucosa gets to the surface. The distal end, as its top part is fixed by ribbons to the basal cartilage sheet, cannot fully turn inside out even during erection, and therefore forms a firm axis to the bending copulatory organ. (Figure 49)

As the period of relaxation is longer than the period of erection. In *Anseriformes* the phallus **remains** in the everted state for a few minutes after copulation. In this stage the copulatory organ is very easy to damage, therefore in animals that live (or are kept) in groups, it is exposed to picking from

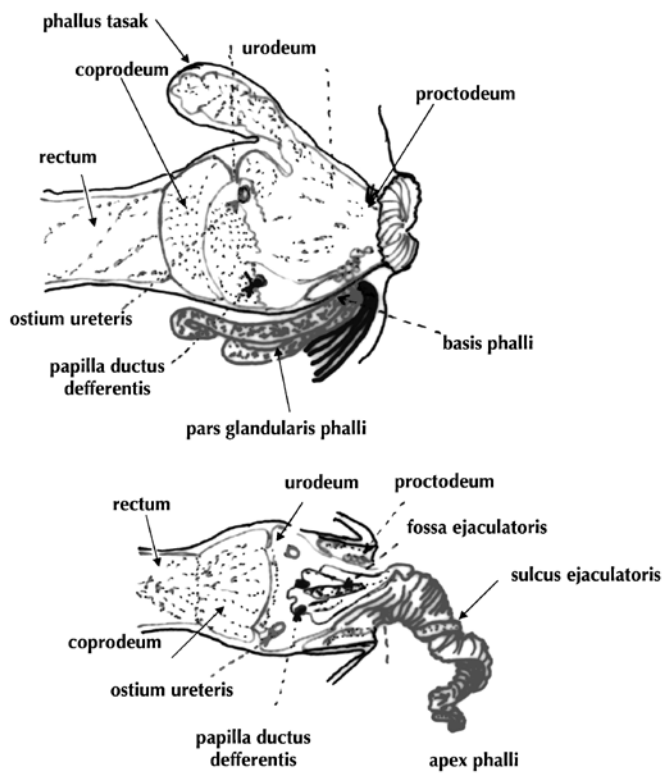

Figure 49. Protuding phallus of goose in (A) inverted and (B) everted stages. Modified after Péczely (1987)

flock-mates. Under natural conditions swans, geese and ducks always mate in the water, which prevents mechanical and picking-caused injuries of the phallus. In domestic geese kept at high densities and because of the lack of a large enough clean water body, the phallus is often injured in the flaccid state, and phallic and cloacal inflammation can occur, causing large economic losses.

In species with protruding phallus (especially in *Ratites)* females also form a cloacal structure, structurally similar to the protruding phallus. Nevertheless, the paired fibro-lymphatic fold is substantially smaller than the male copulatory organ, and it does not evert during the very moderate erection. This ventro-apically rising structure is called the clitoris.

Types of pseudophallus. In the about 9500 species of neoaves the everting "real" phallus, capable of intromission is not formed. We know few genera where a stongly vascularised cloacal protuberance, capable of intromission is formed during mating season. This sac-like organ extrudes from the cloaca up to 5 cm duing copulation in *Coracopsis* parrots, and links the mating partners together for often more than an hour and a half, after the male has already jumped off the female.

Skin protuberance on the both sides of the cloaca form an external **penis-like** or **phalloid** organ in buffalo-weavers (*Bufalornis* spp.). This organ lacks erectile apparatus and seminal duct. It is not an intromittent organ, its function is to stimulate the cloacal opening of the female during copulation and therefore assist male ejaculation.

Australian fairy-wrens (*Malurus*) have a cartilageous process at the frontal part of the cloacal protuberance, which probably plays a role during copulation, but its exact function is unknown.

In *Emberizidae*, a pair of papillae extrude from the ventral wall of the proctodeum, forming a small groove that conducts semen. It is not known if this organ is indeed an intromittent organ. Similar cloacal papillae have been described in several songbirds, but their role during copulation is not obvious. In alpine accentor (*Prunella collaris*) the papilla is longer during breeding season compared to the rest of the year. On the other hand in tree swallow (*Tachycineta bicolor*) the size of the papilla does not change seasonally.

In male *Passeriformes,* the ventral part of the cloaca forms an external cloacal protuberance during the breeding season. In most species this is caused by the substantial hypertrophy in the bilateral vascular body-lymphatic cavity system. The extended paired seminal glomus, which incorporates a large amount of sperm, also contributes to the swelling. The swollen cloacal protuberance dorsally lifts the cloacal opening, therefore assisting the touching of male and female cloacal openings, so its role during copulation is only indirect. The cloacal protuberance grows more intensively in species that copulate more often and the ones that are promiscuous. However, there is no correlation between the size of the cloacal protuberance and the length of copulation.

3.2.4. Avian seminal fluid, semen collection techniques, storage of semen and the artificial insemination (assisted reproduction techniques)

Based on the studies on roosters by Lake and Furr (1971), about two thirds of the semen stored stored in the seminal glomus and the defferent duct gets emptied daily through ejaculation. During a copulation event about 10-50 million sperm gets into the cloaca of the hen. A substantially larger amount of semen can be harvested from the males by massage compared to what empties out by natural ejaculation. According to assumptions, the amount of semen harvested by massage is close to the total (daily) amount of actually stored semen. The harvestable amount of semen is 0.5-1.2 ml from Muscovy duck (*Cairina moschata*), 0.3-0.8 ml from rooster, 0.3-0.5 ml from turkey, 0.1-0.4 ml from domestic goose, and 0.1 ml from pheasant. Among these species, sperm concentration is highest in the turkey: 3-6 million/m^3 of semen. This value is somewhat lower in rooster: 2-3.5 million, musk duck: 0.9-1.3 million and in geese only 10-600,000/m^3. Semen harvested by massage is probably thinner than semen ejaculated during natural copulation, as during the process some plasma filtrate also get pressed out of the erectile bodies.

The amount of semen obtained by artificial harvesting can be substantially increased by optimising the number of massages, which is 5-7 times weekly for roosters (De Reviers, 1986). About twice as many sperm can be obtained if collection occurs five times a week compared to twice a week.

During ejaculation a certain amount of plasma filtrate gets mixed into the semen in the area of the cloaca, which dilutes it to nearly double. The presence of this aldose-containing fluid has been detected in turkey and mallard. Functionally, the fluid secreted by the cloacal mucosa substitutes for the product of the accessory reproductive glands, which are missing in birds (Nishiyama et al., 1976., Fujihara et al., 1978, Fujihara and Nishiyama, 1984).

Avian semen is a yellowish-white, slightly opalescent fluid, which is more transparent when it contains less sperm. Its osmolality is around 300 mmol/kg, and it contains 96% water. The pH is 7.2-7.6 in roosters, but basic in goose (8.0-9.0) and budgerigar (8.2).

The chemical composition of the seminal plasma is mainly based on rooster semen (Lake, 1971). According to this, the Na/K ratio of seminal plasma is 12, and it is characterised by very low levels of citrates, phosphoryl coline, glycerol-phosphoryl coline, fructose and inositol. It also contains very little phosphorous, but a high concentration of glutamate (Figure 50).

The protein concentration of seminal plasma is 3.2 mg/ml, and it contains prealbumin and albumin α1-, 2-, β1-, 2-, 3- and γ fractions. In some poultry species (mostly turkey), the seminal plasma of some individuals is known to have a yellowish colour. This yellow semen typically has a higher, 7.03 g/100 ml

protein concentration, along with an increase in the amount of all protein fractions, except for β3-albumin. The total protein concentation is much lower in individuals with white semen: 1.84 g/100 ml.

Several compounds are known to regulate the functioning of the sperm temporarily stored in the seminal fluid. The seminal plasma of male domestic fowl, turkey, Guinea fowl, duck and goose contains a large amount of multiple-unsaturated fatty acids, which presumably play a role in the energy supply of sperm. Among these, the concentration of arachidonic acid and docosateraenoic acid is the highest. High glutation peroxidase and superoxide dismutase levels in the sperm and the seminal plasma provide protection from the unwanted peroxidation effect of the high unsaturated fatty acid content (Surai et al., 1998). Zinc can influence sperm motility and fertilisation ability. Its concentration is 1-3 mg/ml in roosters (Blesbois and Mauger, 1989). A 78 kDa protein has also been detected in rooster seminal plasma, which temporarily inhibits motility in stored sperm. This compound also has a bactericid effect, but only against Gram-negative bacteria. The neutralising factor of this inhibitor is also present in the seminal plasma, a larger, 100 kDa protein, which releases the blocking of sperm activity at the appropriate time. Nevertheless, this protein does not affect sperm motility directly, but suspends the inhibitory effect (Mohan et al., 1995).

Component	Amount
Water, %	96.40
Na^+, mEq/l	158.76
K^+, mEq/l	12.93
Ca^{2+}, mEq/1	2.55
Mg^{2+}, mEq/1	5.11
Cu^{2+}, μEq/1	10.00
Zn^{2+}, mEq/1	0.52
Fe^{2-3+} mEq/1	0.064
Cl^-, mEq/1	37.2
HCO_3^-, mEq/1	19.6
Lactic acid, mg/100 ml	34.0
Oxoglutaric acid, mg/100 ml	21.0
Ascorbic acid, mg/100 ml	3.0
Inositol, mg/100 ml	10.0
Uric acid, mg/100 ml	7.8
Creatine, mg/100 ml	92.0
N-neuraminic acid, mg/100 ml	11.5
Glutamic acid, mg/100 ml	1300.0
Aspartic acid, mg/100 ml	20.3
Threonine, mg/100 ml	3.2
Serine, mg/100 ml	5.0
Glicin, mg/100 ml	4.0
Alanine, mg/100 mi	3.3
Valine, mg/100 ml	1.5
Lysine, mg/100 ml	1.5

Figure 50. Seminal plasma composition in the rooster. Modified after Péczely (1987) and Al-Aghbari et al., (1992)

Assisted reproduction (biotechnological) processes serve to find optimal ways to harvest semen for artificial insemination, its biological evaluation, appropriate dilution and storage, as well as proper delivery into the female.

Harvesting semen most often occurs via massage, which means firmly rubbing the lumbo-sacral and ventral-pelvic areas of the male bird simultaneously,

from the front towards the back. The repeated rubbing movements result in the eversion of the cloacal opening towards the front and downwards, the relaxing of the sphincters and the outflow of the semen from the ejaculatory duct through the efferent system of proctodeum. The outflowing semen is collected in a warm glass container.

Fresh semen is quickly evaluated, first by counting the number of cells. In the past, this was done by hemocytometer (Bürker-chamber), but nowadays densitometry or cytoscaler method is used. In the next step, the ratio of live/dead sperm is determined by eosin-nigrosin or bromophenol blue staining. The mass and individual movement of sperm is determined by microscopic examination of untreated semen, which is combined with instrumental, quantitative methods. Different sperm deformities are analysed by light microscopic methods, or if necessary in preparatums by electon microscopy.

Avian semen is also diluted, regardless if it gets used immediately or after storage. The dilution rate is usually 1:2 or 1:5. Different, empirically formulated solutions are used for dilution, with 0.85% NaCl as the main component, but also solutions containing glucose, fructose and in some cases sodium glutamate or sodium citrate are also added. In other cases, the solution is egg yolk- or powdered milk -based. The diluted semen is kept at room temperature or refrigerated at +4 °C until use.

A specific way of sperm storage is deep freezing. Glycerine, dimethyl-sulphoxide, dimethyl-acetamide, ethanediol, propane-diol or methyl pyrrolidone are used as cryoprotective materials, usually in a concentrations of 5-0%. It is also possible to use a mixture, in this case polyvinylpyrrolidone is mixed with N,N dimethylacetamide in a 1:1 ratio. During the deep freezing process, the four times diluted semen is filled into artificial straw or prepared as drop granulates, and first cooled to +4 °C and stored at this temperature for 30 minutes. Next it is placed to –35 °C for 15 minutes, and then moved into liquid nitrogen for storage. Before use, the thawed semen is first quickly evaluated and then delivered deep into the vagina by a inseminating syringe (Péczely, 1987).

From the practice of poultry breeding it is known that the quality of the feed influences semen production. Protein content – as a function of age – strongly influences the quantity and quality of semen produced. In young roosters and ganders providing feed that contains 11-12% total proteins is optimal, while a feed containing less than 9% retracts maturation and the appearance of semen in harverstable amounts. In older individuals, however, lower protein content seems to be optimal. For roosters that have been showing sexual activity for weeks, 9% protein content seems to be optimal, a higher concentration decreases semen production. In regard to amino acid composition, methionine seems to be important, the addition of methionine to the feed increases the amount of semen in roosters. Among essential fatty acids the lack of linolenic acid decreases and its presence increases sperm motility and the fertilising

ability of the semen. Among vitamins, the antioxidant vitamin E increases both the amount of semen and its quality. The addition of vitamin A also has a favourable effect.

Ambiental temperature in the laying house also plays an important role in semen production. During puberty, +8 °C or lower temperatures inhibit semen production in young roosters. Similarly, +30 °C also substantially decreases the amount of harvestable semen. The inhibitory effect of high temperature can be counteracted by adding $NaHCO_3$ to the feed.

In regard to the amount of light hours, instead of the 14 hours or longer cycle applied earlier, less then 14 hours of light seems to be more effective for semen production, especially in geese. Studies suggest that the long day lighting is best achieved gradually, slowly increasing the duriation of light (Bogenfürst, pers. comm.).

References 3.

1. Aire TA (1982**):** The rete testis of birds J. Anat., 135, 97-110.
2. Aire TA (2007): Anatomy of the testis and male reproductive tract In: Reproductive Biology and Phylogeny of birds (ed.: B.G.M. Jamieson) I. 37-114. Science Publishers, Enfield, Yersey, Plymouth
3. Al-Aghbari A, Engel HN Jr., Froman DP (1992): Analysis of seminal plasma from roosters carrying the Sd (Sperm Degeneration) allele Biol.Reprod., 47, 1059-1063.
4. Arnold AP, Gorski RA (1984): Gonadal steroid induction of structural sex differences in the central nervous system Annu.Rev.Neurosci., 7, 413-442.
5. Bachman SE, Bachman JM, Mashaly MM (1987): Effect of photoperiod on the diurnal rhythm of plasma testosterone, dihydrotestosterone and androstenedione in mature male chickens Comp. Biochem.Physiol. A Comp.Physiol., 87, 775-779.
6. Ballowitz E (1886): Zur Lehre von der Struktur der Spermatozoen. Anatomische Anzeiger 1, 363-376.
7. Ballowitz E (1888): Untersuchungen über die Struktur der Spermatozoen, zugleich ein Betrag zur Lehre vom feineren Bauder contraktilen Elemente. Archive fuer Mikroskopische Anatomie 32, 401-473.
8. Ballowitz E (1913): Die Spermien der Helgolander Lumme (Uria lomvia L.) Anatomische Anzeiger 44, 305-309.
9. Bergmann M, Schindekmeiser J (1987): Development of the blood – testis barrier in the domestic fowl (Gallus domesticus) Intern.J.Andrology, 10, 481-488.
10. Blesbois E, Mauger I (1989): Zinc content of fowl seminal plasma and its effects on spermatozoa after storage at 4 degrees C Br.Poult.sci., 30, 677-685.
11. Bobolyeva V, Kneer N, Bellei M, Batelli D, Muscatello U, Lardy H (1993): Comparative studies of effects of dehydroepiandrosterone on rat and chicken liver Comp.Biochem.Physiol. B., 105, 643-647.
12. Bois C, Delalande C, Nurmio M, Parvinen M, Zanatta L, Toppari J, Carreau S (2010): Age- and cell-related gene expression of aromatase and estrogen receptors in the rat testis J.Mol.Endocrinol., 45, 147-159.
13. Bottoni L, Lucini V, Massa R (1985): Effect of progesterone on the sexual behavior of the male Japanese quail Gen.Comp.Endocrinol., 57, 345-351.
14. Bozkurt HH, Aktas A, Ulkay MB, Firat UB (2007) Sertoli cell proliferation during the posthatching period in domestic fowl J.Vet.Sci., 8, 219-222.
15. Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B, Bourguiba S (2003): Aromatase expression and role of estrogens in male gonad: a review Reprod.biol.Endocrinol., Apr 11, 1: 35.

16. Carrié-Lemoine J, Garnier DH, Richard-Yris MA (1983): Variations of plasma levels of testosterone and delta 4-androstenedione in growing Hubbard cockerels C.R.Sceances Acad.Sci. III., 296, 701-706.
17. Chen KL, Chi WT, Chu C, Chen RS, Chiou PW (2007): Effect of caponization and testosterone implantation on hepatic lipids and lipogenic enzymes in male chickens Poult.Sci., 86, 1754-1759.
18. Chen KL, Lee TY, Chen TW, Chiou PW (2009): Effect of caponization and different exogenous androgen on hepatic lipid and beta-oxidase of male chickens Poult.Sci., 88, 1033-1039.
19. Chen TT, Huang CC, Lee TY, Lin KJ, Chang CC, Chen KL (2010): Effect of caponization and exogenous androgen implantation on muscle characteristics of male chickens Poult.Sci., 89, 558-563.
20. Clulow J, Jones RC (1982): Production, transport, maturation, storage and survival of spermatozoa in the male Japanese quail, Coturnix coturnix J.Reprod.Fertil., 64, 259-266.
21. Creighton JA (1988): Thyroidectomy and the termination of juvenile refractoriness in the red-ledged partridge (Alectoris greca chucar) Gen.Comp.Endocrinol., 72, 204-208.
22. Culbert J, Sharp PJ, Wells JW (1977): Concentrations of androstenedione, testosterone and LH in the blood before and after the onset of spermatogenesis in the cockerel Reprod.Fert., 51, 153-154.
23. Daniel JY (1980): Characterization and regulation of androgen receptors in the preen gland of adult male duck In: Recent advances of Avian Endocrinology (eds.:G.Pethes, P.Péczely, P.Rudas) 153- 161., Pergamon Press – Akadémiai Kiadó, Budapest
24. De Reviers M (1986): Daily sperm production of guinea fowl 7th.Eur.Poult..Conf., Paris 916.
25. Deadhikari H, Bhattacharyya SP. (1987): Androgenic influence on the activity of the uropygial gland: the effect of dehydroepiandrosterone and androstenedione treatment on the histodynamics and the secretory lipids of the uropygial glands of pigeons Z.Mikrosk.Forsch., 101, 861-870.
26. Deyhim F, Moreng RE, Keinholtz EW (1992): The effect of testosterone propionate on growth of broiler chickens Poult.Sci., 71, 1921-1926.
27. Do thi Dong Xuan, Végi B, Szôke Zs, Péczely P (2005): Seasonal changes in plasma dehydroepiandrosterone (DHEA) levels of domestic geese Acta Biol.Hung., 56, 11-20.
28. Dornas RA, Oliveira AG, Dias MO, Mahecha GA, Oliveira CA (2008): Comparative expression of androgen receptor in the testis and epididymal region of roosters (Gallus domesticus) and drakes (Anas platyrhynchos) Gen.Comp.Endocrinol., 155, 773-779.
29. Douglas HD 3rd, Kitayski AS, Kitaiskaia EV (2008): Seasonal covariation in progesterone and odorant emissions among breeding crested auklets (Aethia cristatella) Horm.Behav., 54, 325-329.
30. Droba M, Józefczyk R, Droba B, Witkowski A (2007): Changes in the activity of acid glycosidases during posthatch development and regression after light reduction of Japanese quail testes and epididymides Comp.Biochem.Physiol. B Biochem. Mol. Biol., 146, 364-369.
31. Fancsovits P, Péczely P. (1995): Investigation of androgen receptors in phallus protrudens of the male domestic goose 1-st Egyptian – Hungarian Poultry Conf., Alexandria, Proc.1, 107-113.
32. Farini D, Scaldafeni ML, Iona S, La Sala G, De Felici M (2005): Growth factors sustain primordial germ cell survival, proliferation and entering into meiosis in the absence of somatic cells Dev. Biol., 285, 49-56.
33. Fennel MJ, Radecki SV, Proudman JA, Scanes CG (1996): The supressive effects of testosterone on growth in young chickens appears to be mediated via a peripheral androgen receptor, studies of the anti-androgen ICI 176,334 Poult.Sci., 75, 763-766.
34. Fennel MJ, Scanes CG (1992a): Effects of androgen (testosterone, 5 alpha-dihydrotestosterone, 19-nortestosterone) administration on growth in turkeys Poult. Sci., 71, 539-547.
35. Fennel MJ, Scanes CG (1992b): Inhibition of growth in chickens by testosterone, 5 alpha-dihydrotestosterone, and 19-nortestosterone Poult. Sci., 71, 357-366.
36. Follett BK (1976): Plasma follicle-stimulating hormone during photoperiodically induced sexual maturation in male japanese quail J.Endocrinol., 69, 117-126.
37. Fujihara N, Nishiyama H (1984): Addition to semen of a fluid derived from the cloacal region by male turkeys Poult.Sci., 63, 544-557.
38. Fujihara N, Nishiyama H, Nakashima N (1976): Studies on the accessory reproductive organs in the drake. 2. Macroscopic and microscopic observatorys on the cloaca of the drake with special reference to the ejacuatory groove region Poult.sci., 55, 927-935.
39. Góes RM, Dolder H (2002): Cytological steps during spermatogenesis in the hause sparrow (Passer domesticus, Linnaeus) Tissue Cell., 34, 273-282.

40. González-Morán MG, Guerra-Araiza C, Campos MG, Camacho-Arroyo I. (2008): Histological and sex steroid hormone receptor changes in testes of immature, mature and aged chickens Domest.Anim. Endocrinol., 35, 371-379.
41. Guichard A, Cedard L, Mignot TM, Scheib D, Haffen K (1979): Radioimmunoassay of steroids produced by chick embryo gonads cultured in the presence of some exogenous steroid precursors Gen.Comp. Endocrinol., 39, 9-19.
42. Gulati DP, Nakamura T, Tanabe Y (1981): Diurnal variations in plasma LH, progesterone, testosterone, estradiol and estrone in the Japanese quail Poult.Sci., 60, 668-673.
43. Guzsal E (1981): Háziállatok szövettana 395. Mezôgazdasági Kiadó, Budapest
44. Han BK, Jung JG, Nam J, Moon JK, Kim JN, Lee SI, Kim JK, Han JY (2009): Identification of the major proteins produced by cultured germline stem cells in chicken J.Androl., 30, 690-702.
45. Harvey S, Baudet ML, Murphy A, Luna M, Hull KL, Aramburo C (2004): Testicular growth hormone (GH): GH expression in spermatogonia and primary spermatocytes Gen.Comp.Endocrinol., 139, 158-167.
46. Hayashi K, Michioka M, Tomita Y (1993): Interaction of thyroxine and testosterone in stimulating muscle protein breakdown in female broiler chickens Br.Poult.Sci., 34, 1029-1034.
47. Hess RA, Bunick D, Bahr JM (1995): Sperm, a source of estrogen Environ.Health Perspect., 103. Suppl. 7, 59-62.
48. Imataka H, Suzuki K, Inano H, Kohmoto K, Tamaoki B (1989): Biosynthetic pathways of testosterone and estradiol-17 beta in slices of the embryonic ovary and testis of the chicken (Gallus domesticus) Gen.Comp.Endocrinol., 73, 69-79.
49. Jamieson BGM (2007): Avian spermatozoa: structure and Phylogeny In: Reproductive Biology and Phylogeny of Birds I. (ed.: BGM Jamieson), 349-512. Science Publishers Enfield, Jersey, Plymouth
50. Jones RC, Lin M (1993): Spermatogenesis in birds Oxf.Rev.Reprod.Biol., 15, 233-264.
51. Kwon S, Hess RA, Bunick D, Kirby JD, Bahr JM (1997): Estrogen receptors are present in the epididymis of the rooster J.Androl., 18, 378-384.
52. Kwon S, Hess RA, Bunick D, Nitta H, Janulis L, Osawa Y, Bahr JM (1995): Rooster testicular germ cells and epididymal sperm contain P450 aromatase Biol.Reprod., 53, 1259-1264.
53. Lake PE, Furr BJA (1971): Physiology and Biochemistry of Domestic Fowl (eds.: DJ Bell, BM Freeman) 3., 1469. Acad.Press New York – London
54. Larson PL, Frey E (1992): Sexual dimorphism int he abundant Upper Cretaceous theropod Thyrannosaurus rex J.Vertebrate Paleontology, 12, 38A
55. Leblond CP, Clermont Y (1952): Definition of the stages of the cycle of the seminiferous epithelium in the rat Annals of the New York Academy of Sciences 55, 548-573.
56. Lin M, Jones RC (1990): Spatial arrangement of the stages of the cycle of the seminiferous epithelium in the Japanese quail, Coturnix coturnix japonica J.Reprod.Fert., 90, 361-367.
57. Lin M, Jones RC, Blackshaw AW (1990): The cycle of the seminiferous epithelium in the Japanese quail (Coturnix coturnix japonica) and estimation of its duration J.Reprod.Fert., 88, 481-490.
58. Lofts B, Massa R. (1980): Avian endocrinology (ed.: A.Epple) 413., Acad Press New York – London
59. Lofts B, Murton RK (1973): Avian Biology (eds.: DS Farner, JR King, KC Parkes) 3.1. Acad.Press New York – London
60. Massa R, Davies DT, Bottoni L. (1980): Cloacal gland of the Japanese quail: androgen dependence and metabolism of testosterone J.Endocrinol., 84, 223-230.
61. McFarlane RW (1963): The taxonomic significance of avian sperm. In: Sibley GC /ed/ Proceedings of the XIII. International Ornithological Congress, Amer.Ornithol.Union: Ithaca , New York, 91-102.
62. McGuire NL, Bentley GE (2010): A functional neuropeptide system in vertabrate gonads: Gonadotropin-inhibitory hormone and its receptor in testes of field-caught hause sparrow (Passer domesticus) Gen.Comp.Endocrinol., 166, 565-572.
63. McQeen SM, Davis LS, YoungG (1999): Sex steroid and corticosterone levels of Adélie penguins (Pygoscelis adeliae) during courtship and incubation Ge.Comp.Endocrinol., 114, 11-18.
64. Mohan J, Saini M, Joshi P (1995): Isolation of a spermatozoa motility inhibiting factor from chicken seminal plasma with antibacterial property Biochim.Biophys.Acta 1245, 407-413.

65. Montgomerie R, Briskie J (2007): Anatomy and evolution of copulatory structures In: Reproductive biology and phylogeny of birds (ed.: BGM. Jamieson) 115-148. Science Publishers Enfield, Jersey, Plymouth
66. Müller W, Groothuis TG, Kasprzik A, Dijkstra C, Alatalo RV, Siitari H (2005): Prenatal androgen exposure modulates cellular and humoral immune function of black-headed gull chicks Proc. Biol.Sci., 272, 1971-1977.
67. Nicander L (1970): On the morfological evidence of secretion and absorption in the epididymis Morpf.Asp.Androl., 1, 12-124.
68. Nishiyama H, Nakshima N, Fujihara N (1976): Studies on the accessory reproductive organs in the drake. 1. Addition to semen on the fluid from the ejaculatory groove region Poult.Sci., 55, 234-242.
69. Ocon-Grove OM, Krzysik-Walker SM, Maddieni SR, Hendricks GL 3rd, Ramachandran R (2010): NAMPT (vistafin) in the chicken testis: influence of sexual maturation on cellular localization, plasma levels and gene and protein expression Reproduction 139, 217-226.
70. Ottinger MA, Kubakawa K, Kikuchi M, Thompson N, Ishii S. (2002): Effects of exogenous testosterone on testicular luteinizing hormone and follicle-stimulating hormone receptors during aging Exp.Biol.Med., 227, 830-836.
71. Pampori NA, Shapiro BH. (1993): Sexual dimorphism in avian hepatic monooxygenases Biochem. Pharmacol., 46, 885-890.
72. Pearl CA, Mason H, Roser JF (2011): Immunolocalization of estrogen receptor alpha, estrogen receptor beta and androgen receptor in the pre-, peri- and post-pubertal stallion testis Anim. Reprod.Sci., Mar.23 Epub.
73. Péczely P (1982): A mellékvesekéreg, a gonád és a pajzsmirigy mûködés kapcsolata madarakon DSc. Disszertáció, Budapest
74. Péczely P (1987): A hím madarak ivarszerveinek szerkezete és a hímivarsejt képzôdése In: A madarak szaporodásbiológiája, 119-135. Mezôgazdasági Kiadó, Budapest
75. Péczely P, Bogenfürst F, Kulcsár M, Polgár B (2011): Role of gonadal and adrenal steroids and thyroid hormones in the regulation of molting in domestic goose Acta Biol.Hung., 62, 1-21.
76. Péczely P, Do thi Dong Xuan, El Halawani M, Hargitai Cs. (1994): An anseriform model of the postrefractory period J.Ornithol.,Sonderheft P 129, 65.
77. Péczely P, Ladjánszky V, Biczó A, Szôke Zs, Pintér O, Kelemen K, Végi B (2004): Dehydroepiandrosterone (DHEA): it's possible role in the avian annual cycles Abstr. 8th Intern.Symp.Avian Endocrinol., Scottsdale 94.
78. Péczely P, Pethes G (1982): Seasonal cycle of gonadal, thyroid and adrenocortical function in the rook (Corvus frugilegus) Acta Physiol.Acad.Sci.Hung., 59, 59-73.
79. Peláez J, Bongalhardo DC, Long JA (2011): Characterizating the glycocalix of poultry spermatozoa: III. semen cryopreservation methods alter the carbohydrate component of rooster sperm mebrane glycoconjugates Poult.Sci., 90, 435-443.
80. Peláez J, Long JA (2007): Characterizing the glycocalix of poultry spermatozoa: I. Identification and distribution of carbohydrate residues using flow cytometry and epifluorescence microscopy J.Androl., 28, 342-352.
81. Peláez J, Long JA (2008): Characterizing the glycocalix of poultry spermatozoa: II. In vitro storage of Turkey semen and mobility phenotype affects the carbohydrate component of sperm membrane glycoconjugantes J.Androl., 29, 431-439.
82. Pelletier RM (1990): A novel perspective: the occluding zonule encircles the apex of the Sertoli cells observed in birds Am.J.Anat., 188, 87-108.
83. Reitemeier S, Hanse M, Hahn A, Schmidt V, Steinbach-Sogbirai K, Krautwald-Junghanns ME, Einspanier A (2011): Evaluating the reproductive status of the male budgeriar (Melopsittacus undulatus) Gen.Comp.Endocrinol., 171, 350-358.
84. Retzius G (1909): Die Spermien der Voegel. Biologische Untersuchungen, Neue Folge 14, 89-122. Taf. XIX- XXXVII
85. Retzius G (1911): Zur Kentniss der Spermien der Voegel. Biologische Untersuchungen, Neue Folge 16, 89-92. Taf XXVII.
86. Retzius G (1912): Weitere Beitrage zur Kentniss der Spermien der Gastropoden und Voegel. Biologische Untersuchungen, Neue Folge 17, 95-99. Taf XIV.

87. Saldanha CJ, Schlinger BA (1997): Estrogen synthesis and secretion in the brown-headed cowbird (Molothrus ater) Gen.Comp.Endocrinol., 105, 390-401.
88. Salibian A, Montalti D. (2009): Physiological and biochemical aspects of the avian uropygial gland Braz.J.Biol., 69, 437-446.
89. Sandell MI, Tobler M, Hasselquist D (2009): Yolk androgens and the development of avian immunity: an experiment in jackdaws (Corvus monedula) J.Exp.Biol., 212, 815-822.
90. Sass M, Péczely P., Mödlinger P (1998): Light and electronmicroscopic structure of the sperm cell of the great bustard 22nd Int.Ornithol.Congress, Durban, Ostrich 69/3-4, 334-335.
91. Schoenmakers S, Wassenaar E, Laven JS, Grootegoed JA, Baarends WM (2010): Meiotic silencing and fragmentation of the male germline restricted chromosome in zebra finch Chromosoma 119, 311-324.
92. Seiki K, Fujii H, Kawamura N, Enomoto T, Haruki Y, Nakano M (1981): Progestin receptors in testes from various animal species Tokai J.Exp.Clin.Med., 6, 343-351.
93. Shanbag BA, Sharp PJ. (1996): Immunocytochemical localization of androgen receptor in the comb, uropygial gland, testis and epididymis in domestic chicken Gen.Comp.Endocrinol., 101, 76-82.
94. Soma KK (2006): Testosterone and aggression: Berthold, birds and beyond J.Neuroenocrinol., 18, 543-551.
95. Sturkie PD (1976): Avian Physiology 3.rd ed. Springer, New York – Heidelberg – Berlin
96. Sugimoto Y, Ohta Y, Morikawa T, Yamashita T, Yoshida M, Tamaoki B. (1990): In vitro metabolism of testosterone on hepatic tissue of chicken (Gallus domesticus) J.Steroid.Biochem., 35, 271-279.
97. Surai PF, Wishart GJ, Maldjian A, Noble RC, Sparks NH (1998): Lipid peroxidation in avian semen: protective effect of seminal plasma Br.Poult.Sci., 39, Suppl. S57-58.
98. Tamás A, Péczely P. (1998): Seasonality of sexual steroid plasma levels in male ringnecked pheasants, Phasianus colchicus 22nd Intern.Ornithol.Congr., Durban, Ostrich 69/3-4, 229.
99. Tanabe Y, Saito N, Nakamura T (1986): Ontogenetic steroidogenesis by testes, ovary and adrenals of embryonic and postembryonic chickens (Gallus domesticus) Gen.Comp.Endocrinol., 63, 456-463.
100. Tanabe Y, Yano T, Nakamura T (1983): Steroid hormone synthesis and secretion by testes, ovary, and adrenals of embryonic and postembryonic ducks Gen.Comp.Endocrinol., 49, 144-153.
101. Tang X, Ma H, Shen Z, Zou S, Xu X, Lin C (2009): Dehydroepiandrosterone activates cyclic adenosine 3',5'-monophosphate/protein kinase-A signalingand supresses sterol regulatory element-binding protein-1 expression in cultured primary chicken hepatocytes Br.J.Nutr., 102, 680-686.
102. Tang X, Ma H, Zou S, Chen W (2007): Effect of dehydroepiandrosterone (DHEA) on hepatic lipid metabolism parameters and lipogenic gene mRNA expression in broiler chickens Lipids 42, 1025-1033.
103. Thies HU, Horst HJ, Haase E. (1983): Identification of 5 alpha-dihydrotestosterone in avian plasma Gen.Comp.Endocrinol., 51, 154-158.
104. Thurston RJ, Korn N (2000): Spermiogenesis in commercial poultry species: anatomy and control Poult.Sci., 79, 1650-1668.
105. Weil S, Rozenboim I, Degen AA, Dawson A, Friedlander M, Rosenstrauch A (1999): Ferility decline in aging roosters is related to increased testicular and plasma levels of estradiol Gen.Comp. Endocrinol., 115, 23-28.
106. Williams DD (1958): A histological study of the effects of subnormal temperature of the testis of the fowl Anat.Record, 130, 225-242.
107. Williams J, de Reviers M (1981): Variations in the plasma levels of lutrinizing hormone and androstenedione and their relationship with the adult daily sperm output in cockerels raised under different photoschedules Reprod.Nutr.Dev., 21, 1125-1135.
108. Yoshioka K, Watahiki Y, Kanie A, Tsujio M, Ikadai H, Kashimoto T, Mutoh K. (2010): Morphology of the cockerel's comb after androgen administration Br.Poult.Sci., 51, 185-194.

4. Fertilisation

Generally, in birds there is a longer time period between copulation and the fertilisation of the ovum compared to mammals. This mostly comes from the role of the avian oviduct, which has a more important function in establishing the fertilising ability of sperm (selection or capacitation) compared to mammals. In mammals, fertilisation is possible for no longer than 24-72 hours after copulation, while in birds this interval can often be over 10 days, but in some extreme cases, for example in turkey, can reach 40 days. In mammals, there is functional monospermy, which means that only one sperm enters the ovum, with a complex mechanism in place protecting against polyspermy. In birds, on the other hand, which have a telolecithal ovum, functional polyspermy occurs. This is the more common type among vertebrates, present in several fish, amphibians and reptiles.

4.1. Sperm storage and selection mechanism in the oviduct

The longevity of sperm in the avian oviduct makes it possible that a whole clutch, sometimes (e.g. in grouses) consisting of a large amount eggs, would contain fertile eggs after one or two copulations. This is probably made possible by the **sperm host glands (storage tubules)** that are located the utero-vaginal region and the infundibulum of the oviduct. At the same time, these structures also protect the upward-moving sperm from the mechanical effects of the egg that travels downwards in the oviduct, which would otherwise swipe the sperm out of the lumen. Sperm storage structures are not only characteristic of birds, they are also found in several other vertebrates, such as viviparous fish, amphibia, lizard and snake, and also among mammals, in bats. Several invertebrates, for instance pulmonate snails have sperm-storing seminal receptacles.

While the functioning of sperm host glands (also called sperm storage tubules) have been studied for decades, obvious evidence for their function is yet to be found. As most studies focus on the utero-vaginal junction (UVJ), hardly anything is known about the function of the sperm storage tubules of the infundibulum.

Sperm appear in the UVJ tubules minutes after copulation or artificial insemination, and spend a variable amount of time there. They enter assisted

by their tail movements, the upward current created by the ciliated cells of the mucosa in the area and by the antiperistaltic contractions typical of the entire oviduct. For the upward movement of sperm, or experimentally used carbon particles, in the oviduct, the timing of the entry is also important, in relation to the current events of the ovulatory-ovipositional cycle. For instance, if carbon particles enter 5-10 minutes after oviposition they appear in the infundibulum very quickly, in about 10 minutes. Entering sperm are similarly fast. On the other hand, if carbon particles are administered 50-55 minutes after oviposition, when the next egg is already in the oviduct, even hours later only few of them make it up to the infundibulum (Koyanagi and Nishiyama, 1981). Mechanical (peristalsis and the change in intensity in the movement of cilia) as well as chemical (pH) changes can also play a role in determing the speed of upward movement. According to Bakst (1980), the luminal pH of the central section in the laying hen's vagina 20 minutes after oviposition is 7.15, while in 8-12 hours it reaches 7.51, i.e., it changes from slightly acidic to slightly basic. In turkey, 10 minutes after oviposition the pH value is 7.30, which decreases to 6.95 in 12-22 hours. After egg laying and during the next ovulation higher pH levels can stimulate upward movement of the sperm (Holm et al., 1996).

The relatively high carbonic anhydrase activity of the mucosa (especially in non-ciliated epithel cells there is high membrane-bound and cytoplasmic activity) can be responsible for the pH conditions of the UVJ mucosa (and of the sperm storage tubules). On the other hand, the amino acids produced during egg development also have a similar effect, and some transmitter-type amino acids can have an additional direct effect on sperm migration (Barna and Boldizsár, 1996a,b). Low concentrations of aspartic acid and glutamic acid in the vagina and UVJ area of domestic hens does not lead to agglutination to cause sperm inactivation, but increased glutamic acid concentration and a more acidic pH causes sperm immobilisation. Sperm immobility seems to be reversible, as the administration of GABA and glycine stops it. Accoring to the above-mentioned authors, the change in Ca^{2+} concentration does not play a role in causing and ending immobility. During the hen's egg laying cycle, there is extensive amino acid secretion mostly in the magnum and the uterus: the concentration of taurine, glutamic acid, glycine and aspartic acid increases in the lumen compared to individuals with no egg development. The concentration of these amino acids, however, sharply decreases during oviposition and the following ovulation, when the migration of sperm towards the infundibulum is at its peak.

Special (enterochromaffin cell-like), serotonin-producing cells have been detected in vaginal and UVJ epithelium of the turkey. These cells can affect sperm transport, as serotonin increases tail movement in sperm, the movement frequency of cilia in the epithelial cells of the oviduct, and the muscular activity of the oviduct (Bakst, 2011).

The entry of sperm into the storage tubules is not a passive process, as only a very small amount of carbon particles of nearly identical size get from the vagina into the UVJ tubules. The supposedly active process has to be accompanied by strong selection. Studies by Brillard and Bakst (1990) and Bakst (1994) have shown that only a small amount (about 10 million) of the 50-150 million sperm that get in by insemination make it to the storage tubules of the UVJ, the majority agglutinates and drains out through the cloaca. The selection mechanism is little known. One of the conditions of entry is that the sperm possess normal motility, and they are able to swim faster than the speed of the outward current generated in the tubules. If they are unable, the outward current from the tubules prevents their entry. The outward current that is created in the tubules can be related to the water pump mechanism in the aquaporin membrane channels, which is created in apical membrane sections of epithelial cells by the effect of sperm. The intensive fluid transpont into the tubular lumen can serve as a regulatory mechanism of sperm entry (Zaniboni and Bakst, 2004). A further condition of sperm entry and temporal loss of motility (i.e., in order to remain inside), is the way the cell coat composition of their cell membrane changes and their energy production is temporarily blocked.

In male turkey, the Ca^{2+} and Zn^{2+} content of their tubular fluid can play a role in creating termporal immobility in sperm, even though these concentrations are not that different from those of the surrounding tissue, therefore are not a result of active transport. Recent studies show that current Ca^{2+} concentration can directly inhibit sperm motility, partly by influencing membrane potential, and an increased Zn^{2+} concentration can decrease basic metabolism of sperm (Holm et al., 2000).

Calcium can also play an important role in the functioning of sperm storage tubules on the level of mitochondria. Quantitative cyclic changes in Ca^{2+} level regulate phospholipase-A2 activity in the mitochondria, and therefore the amount of fatty acids that enter mitochondrial β-oxidation, i.e. the energy production. Considering the metabolism of the sperm inside the tubules, these fatty acids produced by the apical microvilli of the epithelial cells play a determining role (Bakst and Akuffo, 2007). These fatty acids detach from the microvilli enclosed in liposome-like vesicles, and they bind to the membrane of stored sperm. High glutamate content of avian semen increases Ca^{2+} uptake by mitochondria acting through N-methyl-D-aspartic acid (NMDA) receptors of the sperm. It stimulates the amount of permeability transition pores (PTP), which slows down energy production and eventually leads to decreased sperm motility. Ca^{2+} therefore indirectly increases the filling of sperm storage tubules with inactivated, non-motile sperm. The reactivation of sperm stored in the tubules that are reversibly inactivated sperm occurs by the effect of ATP, which is synthesised from phosphocreatine by the effect of creatine kinase. ATP ensures the long-lasting maintainance of the membrane potential in the inner membrane of

the mitochondria, which is necessary for the normal cyclic changes of calcium transport and for the increased energy production necessary to reinstall motility (Froman et al., 2006, Froman, 2007, Froman and Feltmann, 2010).

The entry of sperm into the storage tubules of the UVJ can define the development and maintenance of their fertilising ability. Sperm that have been artificially delivered into the upper part of the uterus, without their previous passing through the sperm storage tubules, do not have fertising ability. Lymphocytes infiltrating the mucosa can form a substantial immunological defence force against microorganisms passing upwards in the vagina. After the entrance of semen, as "foreign material", the synthesis of TGF-β isoforms and their receptors begins in the epithelial cells of the tubules, in the lymphocytes infiltrated into the walls of the tubules and into the sperm themselves. TGF-β possibly has a protective role against antisperm antibodies (Das et al., 2006). Interleukin 1-β and lipopolysaccharide-induced TNF factor can have an important role in the sperm degradation process initiated by the immune system. In the presence of sperm, their amount selectively increases in the vagina, but not in the storage tubules of the UVJ, so sperm inside are protected from harmful immune effects (Das et al., 2009). In addition to circumventing harmful immune effects, storage can also optimise the vitality and fertilising ability of sperm. In vitro, the extracts of UVJ and other sections of the oviduct are known to increase oxygen consumption, motility and life span of sperm (Ashizawa and Nishiyama, 1983). During storage, their membrane structure and acrosomal enzyme system stabilise (Bakst et al., 1994). The function of sperm storage tubules is probably activated by the sperm that enter them. There is a strong interaction between the cells of the tubules and the sperm come in contact with them. This process takes a longer time, in 48 hours following the entry of semen only a particular group of the tubule genes express, i.e. 214 genes, which is only about 1% of the total genetic material (Long et al., 2003). Progesterone-receptor regulated avidin and avidin-related protein-2, which express in the area of the UVJ, can also play an important role in the optimisation of storage, as their amount increases during sperm storage (Foye-Jackson et al., 2011).

In the mucosa of the UVJ, a considerable antioxidant capacity appears, which is substantially higher than the levels in the uteral mucosa above it and the vaginal mucosa below. This activity decreases towards the end of the reproductive period, and is also lower in older individuals. Its role is to protect the membrane of the sperm that are in storage tubules from harmful oxidative effects (Breque et al., 2006).

It is still not known what determines how long sperm are stored in the tubules of the UVJ. This process is also closely related to the mechanisms of evacuation. According to van Krey et al., (1981), storage and evacuation can be deducted to the phenomena of agglutination and de-agglutination. During ejaculation, plasma filtrate containing a fibrin-like material is mixed into the

semen, which reversibly attaches bundles of sperm to the wall of the tubules. These sperm attach to each other and to the microvilli of the epithelial cells by their head pieace, and hence become immobilised. Agglutination can only occur if the cell coat of the sperm is intact, and timed lysosomal activity would result in the release of injured or degenerated sperm. According to another hypothesis, the fluid flow from the base of the tubules towards the opening can also play a role in the selective release of stored sperm. Only the more intensively attached sperm that have a certain tail activity can resist this flow, and remain in the tubules (Froman et al., 2011).

The ability of avian sperm to get reversibly immobilised (which is lacking in mammals) can have an elementary role in the mechanism of sperm storage. This was noted during temperature changes under in vitro conditions. Phosphorylation and dephosphorylation can be behind it during the processes of storage and decapacitation in the UVJ tubules. Ashizawa et al., (2006) found that in vitro, in the presence of perivitelline membrane homogenate, Ca^{2+} triggered and increased sperm motility and initiated acrosome reaction. The motility-stimulating effect of Ca^{2+} manifests via the inhibition of protein phosphatase-1 (PP1). When protein kinase-C activator was added to the system, calcium-stimulated sperm motility decreased in a dose-dependent way. Based on these findings, the joint effect of PKC (protein phosphorylation) stimulation and PP1 (protein dephosphorylation) can be assumed to play a determining role storage-related immobility (decapacitation). The termination of immobilisation (which is the first step of capacitation) and the release of sperm from the storage tubules occur as a result of the effect of calcium.

Sperm can leave all tubules together, in a synchronised way, but also selectively, by the active operation of particular tubules. The results of Freedman et al., (2001) suggest an extracellular impulse conduction mechanism: the intensive innervation of the mucosa around the UVJ tubules can stimulate the contraction of the smooth muscle elements located there. On the other hand, the presence of a strong actin network in the apical cytoplast of individual tubule cells indicates a contraction mechanism on the level of the cell.

Others assume that the release of sperm occurs automatically, in a mechanical or hormonal way regulated by the ovulatory-ovipositional cycle. As the developing egg passes through the oviduct it can serve as a mechanical impulse to initiate release. To prove this assumption, Grigg (1957) pulled an artificial egg through the oviduct, which initiated considerable sperm release from the tubules of the infundibulum, and he assumed a similar effect in the area of the UVJ. Compton and van Krey (1979) studied UVJ tubules of egg-laying and PMSG-treated (which inhibits ovulation) hens, and found no difference between the two groups in the amount of sperm that emptied from the tubules, it was continuous in both groups. Therefore the temporal pattern of sperm release into the infundibulum is regulated by the timing of the different

steps of egg development: among the sperm that are continually released from the UVJ and moving upwards only those reach the infundibulum that leave in the first 30 minutes following oviposition, the rest are wiped away by the spirally downward moving egg.

Sperm release can also be regulated by the change is plasma sexual steroid levels and hormonal changes of the ovulatory-ovipositional cycle. In juvenile chicken, the mucosa of the UVJ contains a very low amount of estrogen-α and progesterone receptors. However, diethylstilbestrol treatment triggered substantial estrogen-α receptor activity in the epithelial cells of the storage tubules and strong progesterone receptor expression showed in the surface epithelial and storage tubule cells of the mucosa (Yoshimura et al., 2000). Studies on Japanese quail showed that maximal sperm release from the storage tubules of the UVJ occurs around the time of the ovulation, about 20 hours after oviposition. At this time, plasma progesterone level is relatively high. The sperm-ejecting effect of progesterone is supported by two observations: following progesterone injection, the storage tubules of a laying hen show maximal release in about one hour and the membrane fraction of UVJ contains a large amount of progestin-α receptors, which substantially increases 14 hours after oviposition. Through increasing membrane transduction (by an unknown protein product), progesterone causes the expansion and the contraction of sperm storage tubules, which results in the release of a large amount sperm (Ito et al., 2011).

The sperm storage tubules of the infundibulum are much less known. According to Bakst et al., (1994) and Wishart and Horrocks (2000) they are similar to the sperm storage tubules of the UVJ histologically, and their overall role is secondary sperm storage. Nevertheless, it is not known, if sperm stored in these two areas fertilise together or if the sperms responsible for fertilisation enter into the tubules of both areas. Morphological data shows that the head of sperm that get into the infundibular tubules pushes deeper among the microvilli of the tubular epithelium compared to the area of the UVJ, which suggests a tighter contact between sperm and the tubular wall (Péczely, 1987). According to a recent study by Bakst (2003) in the first 48 hours following insemination only 1-3 sperm were found in the infundibular tubules, so the function of this area is more selection, as opposed to being a storage site for viable sperm.

4.2. The capacitation of avian sperm and the acrosome reaction

The unique transformation that occurs in the membrane system of the avian sperm, i.e., the capacitation and the related acrosome reaction are still not completely understood. According to some studies, avian sperm do not go through a several-hours or several-days-long maturation process or a capacitation similar to the process in mammals, e.g., sperm delivered into the infundibulum 15

minutes after oviposition (right before the next ovulation) results in fertile eggs (Olsen and Neher, 1948). Additionally, sperm directly harvested from the testis of roosters have the same fertilising ability as sperm found in the ejaculate (Howarth, 1971, Esponda and Bedford, 1985). Sperm in the ejaculate do not have to be pretreated with special compounds with capacitation effects to have fertilising ability in vitro (Howarth, 1984, Nakanishi et al., 1990, Olszanska et al., 2002). Nevertheless, it is probable that capacitation, as a necessary prerequisite of fertilisation, is also present in birds, but occurs much quicker than in mammals, and therefore it is more difficult to study. Optimal calcium concentration can be determining in its manifestation (Lemoine et al., 2009).

Sperm in the sperm storage tubules of the UVJ are not only immobile, but also decapacitated (Bakst et al., 1994). Released sperm regain their mobility, but also, presumably in the higher segments of the oviduct, but as the latest near the perivitelline membrane, undergo a structural-functional transformation of the apical membrane system of the head, i.e. the capacitation. This transformation occurs by the effect of compounds that are found in the egg sheath and in the oviduct.

Similar to mammals, glycoproteins of the perivitelline membrane play an important role in the initiation process. Similar to the mammalian zona pellucida, these can be sorted into three types (see structure of the ovum). At least two of them, ZP3 and particularly the N-linked glycans (N-acetyl-D-glucosamine, with a fucoidin terminal) of ZP1 play an important role in initiating acrosome reaction (Robertson et al., 2000, Sasanami et al., 2007). The third zona pellucida-like compound, ZP2 is an 80 kDa glycoprotein, and its gene, based on studies in Japanese quail, expresses mostly in the oocytes of the small white follicles, and then combined with ZP3 gycoprotein gets deposites as amorph material on the surface of the ovum. This material can have a membrane stabilising role, it does not directly take part in the stimulation of the capacitation – acrosome reaction (Kinoshita et al., 2010).

The increased calcium content of the fluid in the oviduct is another initiator of capacitation and acrosome reaction. According to in vitro studies, adding a high amount (5 mM) Ca^{2+} to different incubation media initiated definite acrosome reaction, even in the absence of previtelline membrane homogenate. Between the two stimulating factors, at least in vitro, the effect of calcium seems to be determining (Lemoine et al., 2008). Capacitation and acrosome reaction are regulated by signal transduction mechanisms, which along with other reactions similar to them, also regulate current sperm motility. Membrane-linked protein kinase A (PKA), phosphatidyl-inositol-3 kinase (PIK3) and mitogen-activated protein kinase 3/1 (MAPK 3/1) definitely play a role in initiating acrosome reaction. PKA and MAPK1 also have an important regulatory role in terminating decapacication and initiating (increasing) sperm motiliy. PIK3, on the other hand, does not play a role in the regulation of motility. The phosphorylation of

AKT, MAPK1 and CREB (cAMP responsive element-binding protein), which plays a role in the functioning of signal-transduction pathway, has a fundamental role in the occurrence of the acrosome reaction. Nevertheless, it is (particular components of) the perivitelline membrane and increased Ca^{2+} concentration that initiate phosphorylation (Lemoine et al., 2009).

According to electron microscopic studies, the capacitation of avian sperm is accompanied by similar changes in membrane structures, as the ones happening in mammals. The cell membrane covering the head of the sperm closely attaches to the outer acrosome membrane at determined fusion points. This capacitation membrane transformation takes place in one or two (at most, five) minutes. In mammals, capacitation is regulated by a well-known enzyme chain reaction, in which the activation of the phospholipase and acrosin content of the acrosomal vesicle and the slicing effect of the membrane along the earlier formed fusion points play a role. In birds, the molecular chain reaction of the capacitation is not yet known.

The next step is the acrosome reaction, during which the cell membrane and the outer acrosome membrane stick to each other, and they get cut up into vesicles along the fusion points. Through this process the lumen of the acrosome opens up, and the proteolytic enzymes (acrosin and hyaluronidase) that were inside get out to the perivitelline membrane. The hydrolysing enzymes form a characteristic entry hole in the perivitelline membrane.

After the acrosome reaction, the apical part of the sperm head is only covered in the inner sheet of the acrosome membrane, which is attached to the bottom to the subacrosomal ring and through this continues to connect the bottom part of the head to the covering cell membrane (Oura and Toshimori, 1990). This newly formed membrane structure will be the "male component" in the following membrane fusion between the sperm and the ovum.

4.3. The penetration of sperm into the ovum, the formation of pronuclei and their fusion

The outer, extravitelline sheet of the vitelline membrane does not play a role in the mechanism of fertilisation. This semifluid, protein fibre-containing layer is formed in the infundibulum nearly at the same time as the upward-swimming sperm penetrate the ovum. Sperm "swim through" the forming extravitelline membrane propelled by the stong movements of their tail. This layer will later become a tough, fibrous-granular membrane, effectively blocking the entry of sperm and preventing "pathological polyspermy" (Okamura and Nishiyama, 1978a, Stepinska and Bakst, 2007).

In vitro penetration of sperm that has been through capacitation and acrosome reaction into domestic hen ovum was first studied electron microscopically by

Okamura and Nishiyama (1978a,b). As a first step, acrosin is relased from the sperm that has been through the acrosome reaction when it is near the perivitelline membrane. Acrosin makes a hole in the perivitelline membrane, and the acrosomal vesicles that wash off the sperm as it passes by get incorporated into this hole. Meanwhile a sperm covered with inner acrosome membrane at the apical part of its head penetrates the perivitelline gap.

Based on mammalian analogues, some receptor-like factors, such as β-1,4 galactosyltransferase and fertilins (ADAM1(2) and ADAM3), which are bound to the perivitelline membrane, can play a part in the penetration of sperm into the perivitelline membrane (Ikawa et al., 2008). According to other studies, a hydrophobe protein is secreted by the epithelial cells of the infundibulum, which forms about 100 μm-long structures between the perivitelline and extravitelline membranes. The outer surface of these sperm-associated bodies is covered in calcium. During fertilisation these bodies attach to the distal end of the tail of the sperm, and when the sperm moves through the perivitelline membrane, they get washed off near the penetration hole. Presumably, their presence is essential to penetrate the perivitelline membrane (Sultana et al., 2004, Rabbani et al., 2007). (Figure 51)

Birds have functional polyspermy, which means that several sperm penetrate the perivitelline membrane simultaneously. Some studies show that sperm are unevenly distributed on the surface, most enter the perivitelline gap above the germinal disc (animal pole). In chicken and in turkey about 20 times more penetration holes have been observed in the perivitelline membrane above the germinal disc than in other areas of the ovum. The penetration of sperm above the germinal disc is also unevenly distributed. Contradicting earlier data, they penetrate in higher numbers at the periphery of the area above the germinal disc, hardly touching the centre. The efficiency of polyspermy is reflected in the several hundred holes above the about 3 mm diameter disc, but because this area is minuscule compared to the whole surface of the ovum, the whole perivitelline membrane – with a much lower density of holes – is still penetrated by 50 times more sperm (Wishart, 1997). The cause of the higher density penetration by sperm above the germinal disc is not yet known. The higher density of sperm-binding sites (ZP1 and ZP3-type glycoprotein receptors) can be a reason, but it has not been confirmed yet. Others assume that the special characteristics (e.g., the receptor effect of more and longer microvilli) of the germinal disc or the covering oolemma cause the higher number of sperm to penetrate in this area. Radially arranged microvilli spead through the fibres of the perivitelline membrane, and therefore can get in direct contact with sperm reaching the perivitelline membrane (Stepinska and Bakst, 2007).

The role of the "new" membrane structures in the cover of the head part of sperm that get into the perivitelline membrane is dual: it ensures attachment of the oolemma to the microvilli (adhesion phase) and makes the fusion between

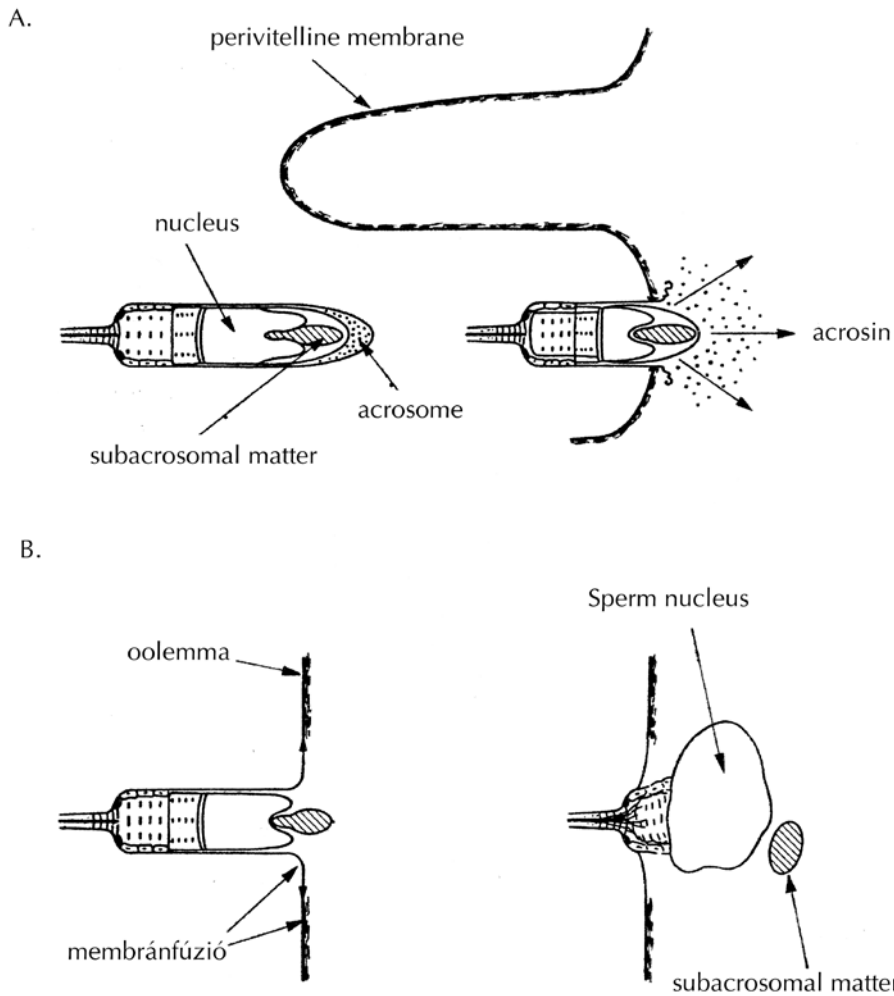

Figure 51. The penetration of sperm into the ovum.
Modified after Péczely (1987)

the cell membrane of the sperm and the oocyte possible. As the progress of the adhesion process in birds is visibly the same as in mammals, similar mechanisms can be assumed. In mammals, the mostly actin-containing subacrosomal sheath that connects the inner acrosome membrane and the cell membrane has a considerable c-lysozyme activity. As the microvilli of the oolemma show a definite receptor affinity towards c-lysozyme, the receptor-ligand-type bond assists in the attachment of the equatorial zone of the sperm head to the oolemma (Herrero et al., 2005). According to other observations, certain integral proteins of the sperm membrane, such as α-fertilin (ADAM1), β-fertilin (ADAM2) and cyritestin (ADAM3) carry a spherical disintegrin element on their outer surface, which can bind to the ligand-binding sites of the CD9-type,

heterodimer-forming integrin molecules of the oocyte membrane, which reach to the extracellular surface, thereby making the adhesive phase of the gametes possible (Evans, 2002).

The fusion of sperm cell membrane and the oolemma occurs after adhesion. Based on electron microscopic observations, Okamura and Nishiyama (1978b) described that after approaching the oolema diagonally, the apical membrane (inner acrosome membrane) of the head of the sperm attaches to the oolemma at one point, causing the lipid bilayer of both cells to open like a scissor, and the two membranes intercalate to form a singular bilayer. Sperm that approach in a different angle, touching the oolemma with the side of the head, do not go through membrane fusion, but enter the ooplasm through phagocytosis. Through this process a phagosome is formed, in which sperm maintains its membrane structure. Later the content of the phagosome (the incorporated sperm head), gets enzymatically digested by DNase, which is produced in extensive amounts. This is the fate of most sperm entering as a result of polyspermy, only one sperm head enters from the ones that arrived in an angle diagonal to the oolemma. This head enters by membrane fusion and it will form the male pronucleus.

Again, the process of membrane fusion will be introduced through the mammalian analogue, as its molecular mechanisms are not known in birds. Some proteins capable of fusion, such as equatorin, β-fertilin and cyritestin, are known from the cell membrane of mammalian sperm. In the oolemma, phosphatidylinositol-linked protein, and tetraspanin protein (CD9) have fusional ability. Zn^{2+}-dependent metalloprotease(s) also play a role in membrane fusion, as their specific inhibition prevents membrane fusion between the gametes (Talbot et al., 2003).

Considering the kinetics of the fusion, according to a hypothetical mammalian model, the fusion protein of the sperm membrane possesses a hydophobic peptide terminal, which drills into the lipid bilayer of the oolemma and linking its two outer sheets, first forms a hemifused state between the two membranes. In the next step, the inner sheets connect, while among the sections of the double membrane bent in a "U" shape fusion pores form. These fusion pores rapidly flatten, and the two membranes approach each other and eventually merge (Evans, 2002).

As a result of the demembraned sperm passing through the oolemma, the perforatorium, the nucleus and the centrioles all enter the ooplasm in unchanged form. After penetration, the nuclear membrane of the sperm disintegrates into vesicules. The nucleus decondenses, its material swells and chromosomes start to form. Based on mammalian studies, during pronuclear formation the disappearance of protamine "attaching sheets" and their replacement by histamine molecules that originate from the ooplasm initiate the decondensation of nuclear chromatin material (Bao et al., 2002).

Syngamy begins by the partial reformation of the male nuclear membrane and the division of the proximal centriole of the sperm (diplosome forming). New nuclear membrane is formed from the elements of the earlier nuclear membrane vesicules and vesicularly disintegrated endoplasmic reticules, which stay partially open by vesicules on the side facing the female pronucleus. This is the surface where the fusion of the two pronuclei will occur. The formation of the female pronucleus begins when the second meiotic division concludes, when the male pronucleus is aready formed. In the case of the female pronucleus, the nuclear membrane also gets cut up vesicularly, and it is particularly loosely organised on the sides facing the male pronucleus and the equator. The elements of the two haploid chromosome sets that formed are organised equatorially by the spindle fibres of the diplosome, and soon occurs the first mitotic division of the now diploid set (Okamura and Nishiyama, 1978b, Stepinska and Bakst, 2007).

In domestic hen, syngamy occurs 3-5 hours after ovulation, when the fertilised ovum and the forming egg are located in the distal section of the magnum (Perry, 1987, Waddington et al., 1998). As the ovum passes through the oviduct until the egg is laid, the cleavages of the ovum form the blastula stage germinal disc, which is "swimming" on the yolk, forming a disc visible with the naked eye.

References 4.

1. Ashizawa K, Nishiyama H (1983): Prolonged survival of fowl spermatozoa in the oviduct tissues in organ culture Br.Poult.Sci., 24, 27-32.
2. Ashizawa K, Wishart GJ, Katayama S, Takano D, Ranasinghe AR, Narumi K, Tsuzuki Y (2006): Regulation of acrosome reaction of fowl spermatozoa: evidence for the involvement of protein kinase C and protein phosphatase-type 1 and/or type 2A Reproduction 131, 1017-1024.
3. Bakst MR (1980): Chicken and turkey oviductal pH at known times postoviposition Poult.Sci., 59, 2793-2796.
4. Bakst MR (1994): Fate of fluorescent stained sperm following insemination: New light on oviducal sperm transport and storage in the turkey Biol.Reprod., 50, 987-992.
5. Bakst MR (2003): Oviductal sperm storage in turkeys. The infundibulum as a secondary sperm storage site, or is it? In: A Legakis, S Sfenthourakis, R Polymeni, m Thessalou-Legaki (eds) The new panorama of Animal Evolution. Proc.XVIII. Intern.Congr. of Zoology, Athens Greece, 447-450. Pensoft Publishers, Sophia –Moscow
6. Bakst MR (2011): Role of the oviduct in maintaining sustained fertility in hens, In: Physiology and Endocrinology Symposium, J.Anim.Sci., 89, 1323-1329.
7. Bakst MR, Akuffo V (2007): Alkaline phosphatase reactivity in the vagina and utero vaginal junction sperm storage tubules of turkeys in egg-production: Implications for sperm storage Br.Poult.Sci., 48, 48, 515-518.
8. Bakst MR, Wishart G, Brillard JP (1994): Oviductal sperm selection , transport and storage in poultry Poult.Sci.Reviews 5, 117-143.
9. Bao S, Obata Y, Ono Y, Futatsumata N, Niimura S, Kono T (2002): Nuclear competence for maturation and pronuclear formation in mouse oocytes Hum.Reprod., 17, 1311-1316.
10. Barna J, Boldizsár H (1996): Motility and agglutination of fowl spermatozoa in media of different amino acid content and pH value in vitro acta Vet.Hung., 44, 221-232.

11. Barna J, Boldizsár H, Zsolnai-Harczi I (1996) Amino acid secretion of the hen's oviduct during the egg formation cycle Acta Vet.Hung, 44, 233-241.
12. Breque C, Surai P,Brillard JP (2006): Antioxidant status of the lower oviduct in the chicken varies with age and dietary vitamin E supplementation Mol.Reprod.Dev., 73, 1045-1051.
13. Brillard JP, Bakst MR (1990): Quantification of spermatozoa in the sperm-storage tubules of turkey hens and the relation to sperm numbers in the perivitelline layer of eggs Biol.Reprod., 43, 271-275.
14. Compton MM, van Krey HP (1979): Emptying of the utero-vaginal sperm storage glands in the absence of ovulation and oviposition in the domestic hen Poult.Sci., 58, 187-190.
15. Das SC, Isobe N, Nishibori M, Yoshimura Y (2006): Expression of transforming growth factor-beta isoforms and their receptors in utero-vaginal junction of hen oviduct in presence or absence of resident sperm with reference to sperm storage Reproduction, 132, 781-790.
16. Das SC, Isobe N, Yoshimura Y (2009): Changes in the expression of interleukin-1beta and lipopolisacharide-induced TNF factor in the oviduct of laying hens in response to artificial insemination Reproduction 137, 527-536.
17. Esponda P, Bedford JM (1985): Surface of the rooster spermatozoon changes in passing through the Wolffian duct J.Exp.Zool., 234,441-449.
18. Evans JP (2002): The molecular basis of sperm-oocyte membrane interactions during mammalian fertilization Hum.Reprod.Update 8,297-311.
19. Foye-Jackson OT, Long JA, Bakst MR, Blomberg LA, Akuffo VG, Silva MV, Guthrie HD, McMurtry JP (2011): Oviductal expression of avidin, avidin-related protein-2, and progesterone receptor in turkey hens in relation to sperm storage: effects of oviduct tissue type, sperm presence, and turkey line Poult.Sci., 90, 1539-1547.
20. Freedman SL, Akuffo VG, Bakst MR (2001): evidence for the innervation of the sperm storage tubules in the turkey (Meleagris gallopavo) Reproduction, 121, 809-814.
21. Froman DP (2007): Sperm motility in birds: insights from fowl sperm Soc.Reprod.Fert.Suppl., 65, 293-308.
22. Froman DP, Feltmann AJ, Pendarvis K, Cooksey SM, Burgess SC, Rhoads DD (2011): A proteome-based model for sperm mobility phenotype, In: Physiology and Endocrinology Symposium J.Anim. Sci., 89, 1330-1337.
23. Froman DP, Wardell JC, Feltmann AJ (2006): Sperm mobility: deduction of a model explaining phenotypicvariation in roosters (Gallus domesticus) Biol.Reprod., 74, 487-491.
24. Grigg GW (1957): The structure of stored sperm in the hen and the nature of the release mechanism Poult.Sci., 36, 450-451.
25. Herrero MB, Mandal A, Digilio LC, Coonrod SA, Maier B, Herr JC (2005): Mouse SLLP1, a sperm lysozyme-like protein involved in sperm-egg binding and fertilization Dev.Biol., 284, 126-142.
26. Holm L, Ekwall H, Wishart GJ, Ridderstrale Y (2000): Localization of calcium and zinc in the sperm storage tubules of chicken, quail and turkey using X-ray microanalysis J.Reprod.Fertil., 118, 331-336.
27. Holm L, Ridderstrale Y, Knutsson PG (1996): Localisation of carbonic anhydrase in the sperm storing regions of the domestic hen oviduct Acta Anat.(Basel): 156, 253-260.
28. Howarth B (1971): An examination for sperm capacitacion in the fowl Biol.Reprod., 3, 338-341.
29. Howarth B (1984): Maturation of spermatozoa and mechanism of fertilization, In: FJ Cunningham, PE Lake, D Hewitt (eds) Reprod. Biol. Poultry, British Poultry Sci., Ltd, The Alden Press Ltd, Oxford
30. Ikawa M, Inouque N, Okabe M (2008): Mechanism of sperm-egg interactions emerging from gene-manipulated animals Int.J.Dev.Biol., 52, 657-664.
31. Ito T, Yoshizaki N, Tokumoto T, Ono H, Yoshimura T, Tsukada A, Kansaku N, Sasanami T (2011): Progesterone is a sperm-releasing factor from the sperm-storage tubules in birds Endocrinology 152, 3952-3962.
32. Kinoshita M, Rodler D, Sugiura K, Matsushima K, Kansaku N, Tahara K, Tsukada A, Ono H, Yoshimura T, Yoshizaki N, Tanaka R, Kohsaka T, Sasanami T (2010): Zona pellucida protein ZP2 is expressed in the oocyte of Japanese quail (Coturnix japonica) Reproduction 139, 359-371.
33. Koyanagi F, Nishiyama H (1981): Disintegration of spermatozoa in the infundibular sperm-host glands of the fowl Cell Tissue Res., 214, 81-87.

34. Lemoine M, Dupont J, Guillory V, Tesseraud S, Blesbois E (2009): Potential involvement of several signaling pathways in initiation of the chicken acrosome reaction Biol.Reprod., 81, 657-665.
35. Lemoine M, Grasseau I, Brillard JP, Blesbois E (2008): A reappraisal of the factors involved in in vitro initiation of the acrosome reaction in chicken spermatozoa Reproduction 136, 391-399.
36. Long EL, Sonstegard TS, Long JA, Van Tassell CP, Zuelke KA (2003): Serial analysis of gene expression in turkey sperm storage tubules in the presence and absence of resident sperm Biol. Reprod., 69, 469-474.
37. Nakanishi A, Utsumi K, Iritani A (1990): Early nuclear events of in vitro fertilization in the domestic fowl (Gallus domesticus) Mol.Reprod.Dev., 26, 217-221.
38. Okamura F, Nishiyama H (1976a): The passage of spermatozoa through the vitelline membrane in the domestic fowl, Gallus gallus Cell tissue Re., 188, 497-508.
39. Okamura F, Nishiyama H (1978b): Penetration of spermatozoon into the ovum and transformation of the sperm nucleus into the male pronucleus in the domestic fowl, Gallus gallus Cell Tissue Res., 190, 89-98.
40. Olsen MW, Neher BH (1948): The site of fertilization in the domestic fowl J.Exp.Zool., 109, 355-366..
41. Olszanska B, Stepinska U, Perry MM (2002): Development of embryos from in vitro ovulated and fertilized oocytes of the quail (Coturnix coturnix japonica) J.Exp.Zool., 292, 580-586.
42. Oura C, Toshimori K (1990): Ultrastructural studies on the fertilization of mammalian gametes Int.Rev.Cytol., 122, 105-151.
43. Péczely P (1987): A Madarak Szaporodás biologiája, 143- 149., Mezôgazdasági Kiadó, Budapest
44. Perry MM (1987): Nuclear events from fertilisation to the early cleavage stages in the domestic fowl (Gallus domesticus) J.Anat., 150, 99-109.
45. Rabbani MG, Sasanami T, Mori M, Yoshizaki N (2007): Characterization of the sperm-associated body and its role in the fertilization of the chicken Gallus domesticus Dev.Growth Differ., 49, 39-48.
46. Robertson L, Wishart GJ, Horrocks AJ (2000): Identification of perivitelline N-linked glycans as mediators of sperm-egg interaction in chickens J.Reprod.Fertil., 120, 397-403.
47. Sasanami T, Murata T, Ohtsuki M, Matsushima K, Hiyama G, Kansaku N, Mori M (2007): Induction of sperm acrosome reaction by perivitelline membrane glycoprotein ZP1 in Japanese quail (Coturnix japonica) Reproduction 133, 41-49.
48. Stepinska U, Bakst MR (2007): Fertilization In: Reproductive Biology and Physiology of Birds A. 533-587. BGM Jamieson (ed.) Science Publishers, Enfield, Jersey, Plymouth
49. Sultana F, Mao KM, Yoshizaki N (2004): Possible involvement of a sperm-associated body in the process of fertilization in quail Zoolog.Sci., 21, 851-858.
50. Talbot P, Shur BD, Myles DG (2003): Cell adhesion and fertilization: steps in oocyte transport, sperm-zona pellucida interactions, and sperm-egg fusion Biol.Reprod., 68, 1-9.
51. van Krey HP, Balander RJ, Compton MM (1981): Storage and evacuation of spermatozoa from the uterovaginal sperm-host glands in domestic fowl Poult.Sci., 60, 871-877.
52. Waddington G, Gribbin C, Sterling RJ, Sang HM, Perry MM (1998): Chronology of events in the first cell cycle of the polyspermic egg of the domestic fowl (Gallus domesticus): Intern. J. Dev. Biol., 42, 625-628.
53. Wishart GJ (1997): Quantitative aspects of sperm:egg interaction in chickens and turkeys Animal Reprod.Sci., 48, 81-92.
54. Wishart GJ, Horrocks AJ (2000): Fertilization in birds In: JJ Tarin, A Cano (eds), Fretilization in protozooa and Metazoan Animals: Cellular and Molecular Aspects, 193-222. Springer Verlag, Berlin
55. Yoshimura Y, Koike K, Okamoto T (2000): Immunolocalization of progesterone and estrogen receptors in the sperm storage tubules of laying and diethylstilbestrol-injected immature hens Poult.Sci., 79, 94-98.
56. Zaniboni L, Bakst MR (2004): Localization of aquaporins in the sperm storage tubules in the turkey oviduct Poult.Sci., 83, 1209-1212.

5. The neuroendocrine regulation of breeding

5.1. The structure of the hypothalamo-hypophyseal system

Several factors affect the development of diencephalon in birds: the large cerebral hemispheres, the well-developed cerebellum and the large mesencephalic optic lobes, pushed latero-ventrally by the first two parts. The specific development of all three parts is strongly connected to the organisation of the large eyes as well as the well-developed optic nerves and optic centres. As a result, a large part of the thalamus is moved into a dorso-lateral position, mixed with lateral hypothalamic elements, and the dorsal thalamic nuclei and the epithalamic habenulas are pushed on the mediodorsal hypothalamus and the forward-shifted midbrain tegmentum. The dorso-medial part of the medial hypothalamus gets in close contact with the septal area, the accumbens nucleus and the bed nucleus of the stria terminalis (BnST), which are part of the limbic system, and a part of its ventral area forms the large preoptic area, which is located in front of the optic chiasma.

The medial hypothalamus consists of smaller (8–15 μm diameter), parvocellular and larger (25–30 μm diameter), magnocellular neurons, which unlike in mammals do not form entirelly separate nuclei, but are arranged mixed inside the nucleus, showing a heterogenous pattern. Morpho-functionally the magnocellular hypothalamo-neurohypophyseal (earlier called Gomori positive, staining with chrome-hematoxylin and aldehyde fuchsin) and the parvocellular (Gomori negative) hypothalamo-adenohypophyseal systems do not separate. Another complication is, that some of the parvocellular neurons (especially in the area of the paraventricular nucleus) stain Gomori positive. There is also an overlap between the two systems in regard to the fibres, as some of the Gomori positive axons end in the anterior part of the median eminence, while others in the pars nervosa. These ambiguous results from classic histochemical studies were clarified by immunocytochemical analyses, which showed that there are also neurohyphophyseal hormones in the median eminence, i.e. arginine vasotocin-containing axon endings, which underlied that there is a substaintal morpho-functional overlap in birds between the Gomori-positive hypothalamo-neurohypophyseal and the Gomori-negative hypothalamo-adenohypophyseal systems.

Considering neuroendocrine regulatory functions, the **medial part of the hypothalamus** is particularly important. This area can be considered as a thickened wall of the third ventricle, which is bordered rostrally by the lamina terminalis, and caudally extends until the mammillary area. It shows a tripartite structure. The frontal, preoptic-supraoptic area of the medial hypothalamus is located in front of and above the optic chiasma, and its base is formed by the preoptic recess of the third ventricle, which spreads to a characteristic triangular shape. The central part of the medial hypothalamus is the paraventricular area, which raises dorsally from the base of the ventricle to the medial septum and behind, reaching the anterior commissure. The posterior part of the medial hypothalamus is the tuberal and the the mammillary area behind it, which dorsally reaches the **habenular** and **subhabenular** nuclei, with the commissure posterior and the surrounding subcommissural **ependymal expansion** (Péczely, 1969, Oksche and Farner, 1974, Kuenzel and van Tienhoven, 1982).

The nuclei of the **preoptic-supraoptic area** are the preoptic rostral and the preoptic periventricular nuclei, which are located between the terminal lamina and the preoptic recess, behind them and slightly more dorsally the extensive medial preoptic nucleus (POM) and above and slightly more laterally the anterior preoptic nucleus (POA). The **medial preoptic area** consists of a smaller, medially located and a larger, laterally located group of cells. The medial division of the suprachiasmatic nucleus (SCN) is located in a lateral angle of the preoptic recess, and at the base of the preoptic area, laterally from this is the visual division of the suprachiasmatic nucleus, both of which are important stations in the retino-hypothalamic tract. The supraoptic nucleus is located somewhat more caudo-laterally, above the chiasma. It contains mostly magnocellular elements and usually (e.g., in pigeon) it consists of anterior, prosterior and lateral divisions. In the preoptic area, between the fibre bundles that run under the recess and towards the two sides (dorsal and ventral supraoptic decussations) are located the neurons of the dorsal and ventral supraoptic decussation (Péczely, 1969, 1987, Rehák and Kostová, 1993, Panzica et al., 1995).

The medial preoptic nucleus is located in this area, and it fulfills multiple neuroendocrine functions. It shows definite sexual dimorphism, as its medial extension and the number of cells are much larger in males. It has major afferent and efferent connections, some cells form reciprocal connections with other hypothalamic nuclei, while others connect with structures in the septum, the bed nucleus of the stria terminalis (BnST), the central grey matter (susbstantia grisea), the posterior areas of the mesencephalic tegment and the Ponse. Incoming information from sensory areas are functionally important, and the nucleus sends important impulses to neuro-vegetative and motor areas (Balthazart et al., 1994, Panzica et al., 1996).

The medial, more compact paraventricular nucleus (PVN) and the lateral, looser part are located in the anterior part of the **paraventricular area**. Both

divisions contain both magno-, and parvocellular neurons. Moving towards the back, the **medial** division gradually shifts dorsally and ends behind the anterior commissure. The PVN receives afferent fibres from the limbic system, the lower and upper sections of the brainstem, the ventral tegment and the area postrema. Its efferent fibres radiate into the accumbens nucleus, the lateral septum, the habenula, the reticular formation of the brainstem and the vertebral medulla, several hypothalamic areas and organum vasculosum of the lamina terminalis. In the posterior part of the area is located the medial posterior hypothalamic nucleus, which contains parvocellular neurons and the lateral posterior hypothalamic nuclei located more laterally (Péczely, 1969, 1987, Péczely and Kiss, 1988, Kiss and Péczely, 1987, Korf, 1984).

In the central area of the **posterior hypothalamus** tuberal nuclei are located, which reach into the infundibulum, and divide into a medial and two lateral nuclei, arranged along the walls of the ventricle and the infundibular recess. They consists of parvocellular neurons. The medial, lateral and supramammillary nuclei are wedged into the periaqueductal area, and some of their fibres cross (supramammillary decussation) and lead into the epithalamus.

Surrounding the infundibular recess of the third ventricle that becomes thinner in the posterior part, the base of the central hypothalamic area continues in the infundibulum, and ends in the connecting **neurohypophysis**. The anterior area of the neurohypophysis is the **median eminence**, which becomes gradually thinner, and ends in the widening, dorso-ventrally flattened, often lobulated **pars nervosa**. This stalk-like structure with its blood vessel plexus suspends the distal part of the hypophysis. The base of the infundibular recess is thicker in the anterior (or rostral) part of the median eminence, and thinner in the posterior. Looking at the U-shaped frontal cross section, the ventral part of the median eminence is covered by the **tuberal part of the adenohypohysis (pars tuberalis)**, as a few cell-layer thick coat. The tuberal part is permeated by the two precapillary **vascular plexuses** that originate from the median eminence, the anterior and posterior bundles of the hypophyseal portal „veins". The plexuses of the portain veins lead to the cephalic and caudal lobes of the **distal part of adenohypophysis** (Mikami et al., 1970).

Several fibre bundles run in the median eminence, some of which orginate from the supraoptic-paraventricular nuclei (supraoptic-paraventricular-hypophyseal tract) and run towards the pars nervosa through the dorsally located internal zone. These axons originate from the (mostly) magnocellular, Gomori-positive cells of the supraoptic and paraventricular nuclei, and carry neurohypophyseal hormones, such as water balance-regulating arginine vasotocin and the smooth-muscle contraction-triggering mesotocine (mostly in the uterovaginal area) to the pars nervosa. In the area of the internal zone, some Gomori-positive fibres, as they continue backwards, basally deviate, arrange radially in the external zone and mix with other, Gomori-negative fibres and end basally,

in the mantel plexus of the tuberal part. Presumably, the aldehyde fuchsin-staining axons that end in the anterior median eminence originate from both Gomori-positive magno- and parvocellular neurons. Other fibre bundles that end at the base of the external zone orginate from the preoptic area, diffusely arranged cells of the lateral hypothalamus, the lateral septum, parvocellular areas of the paraventricular nucleus, the posterior hypothalamic nucleus and the tuberal nucleus. Most axons that originate from the posterior hypothalamic and the tuberal nuclei end in the Gomori-negative posterior median eminence (Wingstrand, 1951, Péczely, 1969, 1972, Péczely and Calas, 1970, Elekes and Péczely, 1972, Knapp and Silver, 1995).

The **lateral hypothalamus** is an area mixed with thalamic nuclei and is richly profused with fibres. Especially its posterior part can be considered as a **continuation of the mesencephalic formatio reticularis**. Its anterior part contains the entopeduncular nucleus and the ventro-lateral thalamic nucleus, and somewhat more posterior is the lateral hypothalamic nucleus, which contains diffuse nuclei. The backwards-reaching lateral hypothamic nucleus is in the central area of the hypothalamus, and it contains two nuclei, the reticular and the intercalated thalamic nuclei. The lateral hypothalamic nucleus gets gradually thinner towards the posterior ventral area, it shifts ventrally**,** surrounding the lateral part of **mamillary area (the lateral mammillary nucleus and the ectomammillary nucleus**). The posterior dorsal part consists of the **stratum cellulare externum**, which continues caudally in the mesencephalic tegment (Karten and Hodos, 1967, Kuenzel and van Tienhoven, 1982).

The circumventricular **ependymal organs** that form in the lining of the ventricles, form a part of the hypothalamic neuroendocrine system. They have a multi-layered ependyma and some of the neurons beneath it possess special liquor contact dendrites with a chemosensory function, while others are secretory neurons that release neuro-hormones into the liquor. Such ependymal organs are found in the preoptic recess (vascular organ of the lamina terminalis, OVLT), the paraventricular region of the third ventricle (paraventricular organ), in the vault of the third ventricle surrounding the posterior commissure (subcommissural organ, SCO), in the infundibular recess and in the lateral horns of the ventral ventricles (Péczely and Muray, 1967, Mikami, 1976, Vigh-Teichmann and Vigh, 1983, Vigh et al., 2004). (Figure 52, 53, 54, 55.)

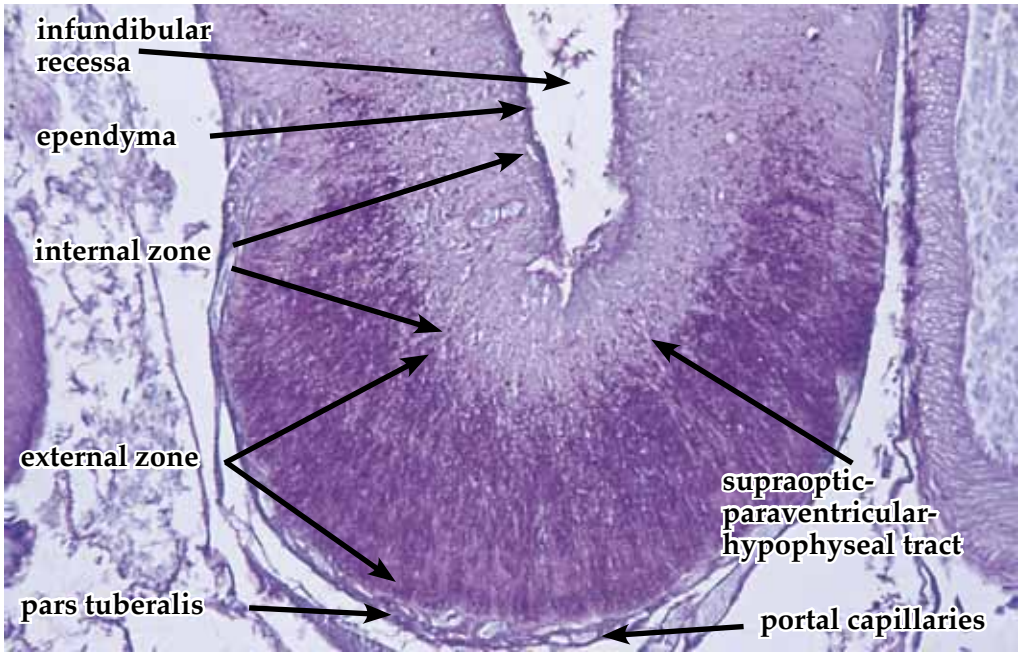

Figure 52. Anterior median eminence in domestic pigeon with radially running, aldehyde-fuchsin staining fibres in the external zone

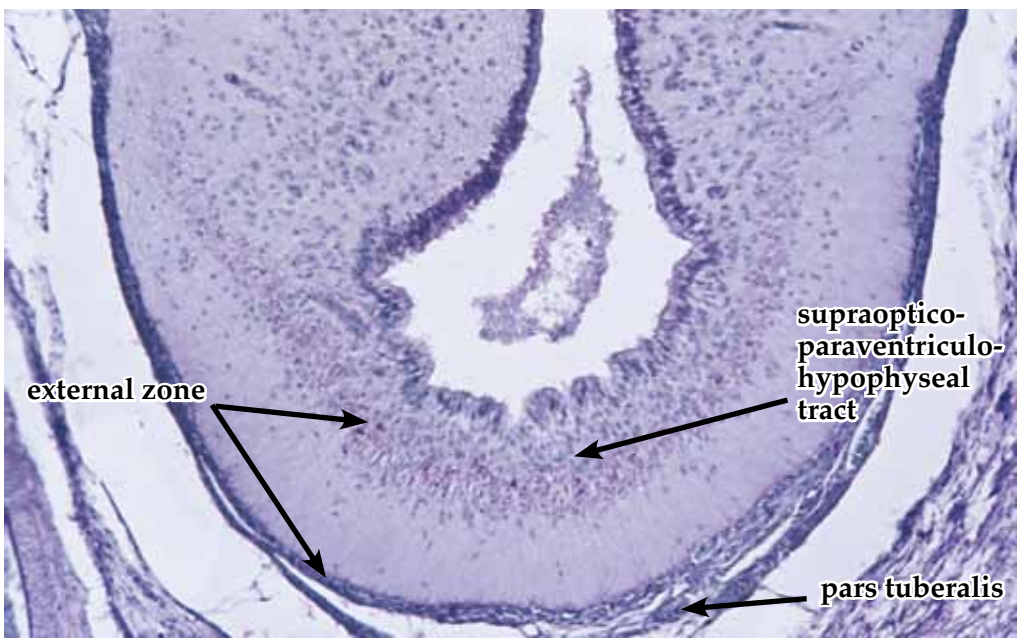

Figure 53. Posterior median eminence in domestic pigeon, the external zone lacks staining fibres, the internal zone has aldehyde-fuchsin-staining granules in the supraoptico-paraventriculo-hypophyseal tracts.

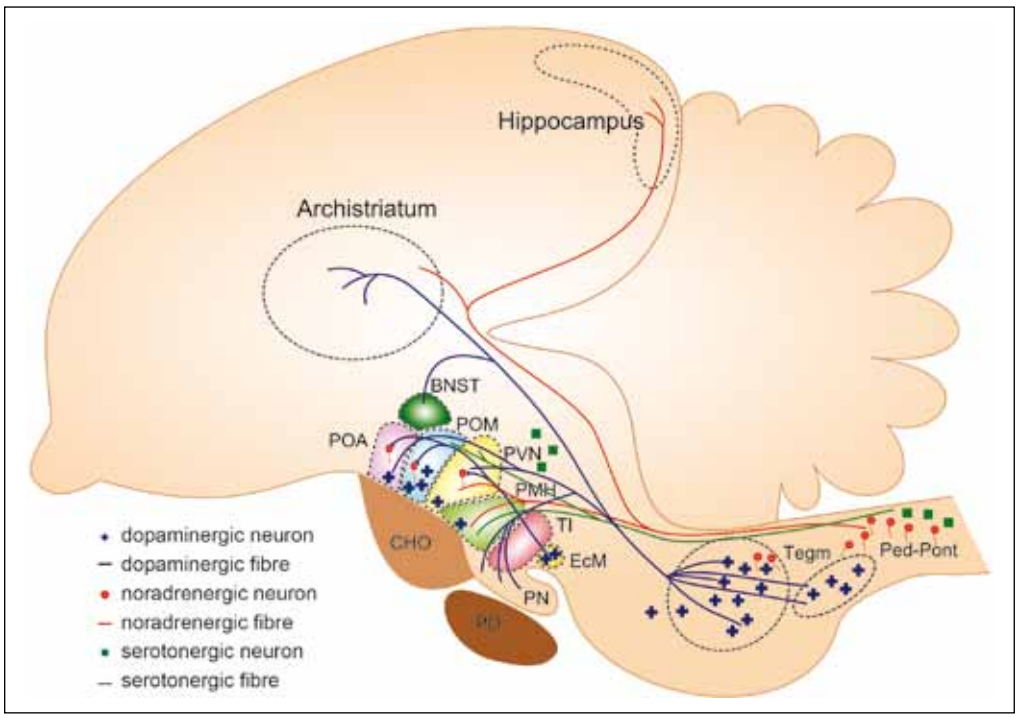

Figure 54. Biogenic amine systems of the avian brain

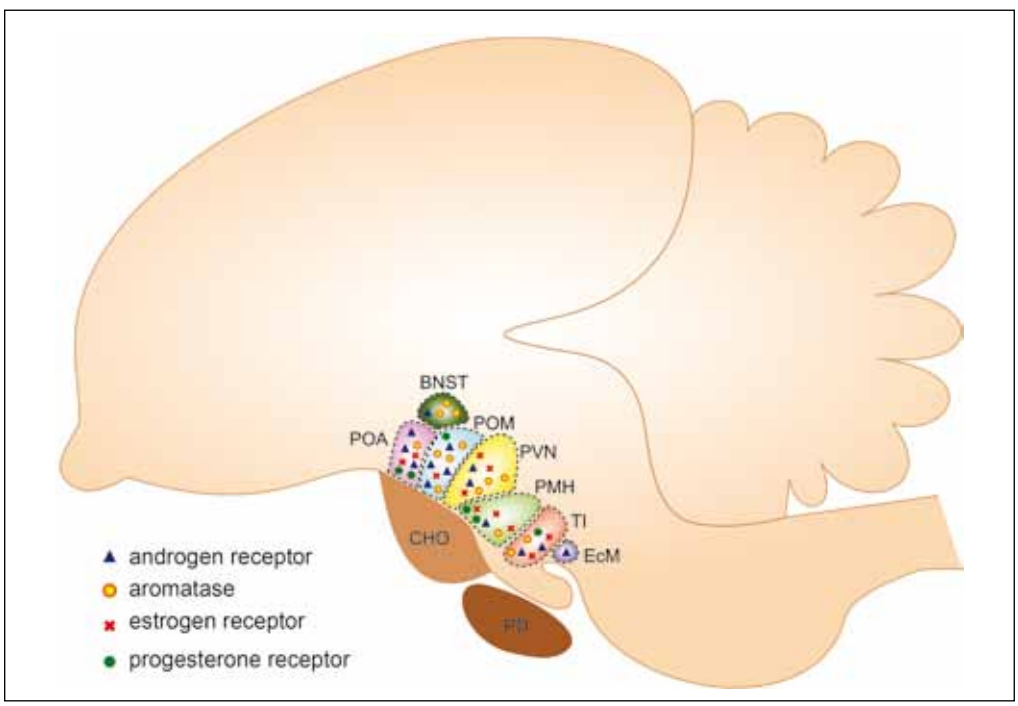

Figure 55. Sexual steroid and aromatase-containing areas of the avian brain

5.2. A neuroendocrine systems of the hypothalamus

5.2.1. Neuropeptides

Several **neuropeptides** are secreted by the neurons (neurosecretory cells) of the medial hypothalamus. These neurons regulate adenohypohyseal functions. The soma of neurons that produce corticotropin-releasing factor (CRF) have also been identified in birds, located in the medial division of the POM, in the medial part of the PVN and in the septal area, among small and medium-sized arginine-vesotocin containing neurons (Péczely and Antoni, 1984). Thyrotropin-releasing hormone (TRH) immune-positive neurons are parvocellular elements of the suprachiasmatic nucleus, the rostral preoptic nucleus and the PVN (Péczely and Kiss, 1986). Axons that contain both releasing hormones lead to the anterior and central median eminence. The PRF-acting vasointestinal peptide (VIP), which increases prolactin production, is secreted in the medial and lateral posterior hypothalamic nuclei, the medial and lateral tuberal nuclei and in the parvocellular neurons of the mammillar area (Péczely and Kiss, 1986).

The chicken growth hormone-releasing hormone (cGHRH), similar to mammals, is produced in the tuberal area of the posterior hypothalamus (Wang et al., 2007). Nevertheless, further stimuting factors also take part in the regulation of growth hormone secretion. One such factor is the Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP), which can have a role similar to that in mammals, it is secreted in the areas of the tuberal nucleus and the PVN, as well as in certain parts of the limbic system. In birds, VIP and TRH are also assumed to have a growth hormone secretion-stimulating role (Montero et al., 2000, Wang et al., 2010). Gherlin, another gastrointestinal peptide, is secreted by the parvocellular cells of the medial anterior hypothalamic nucleus and the magnocellular cells of the preoptic nucleus and preoptic division of the supraoptic nucleus. Similar to mammals, gherlin in birds is also assumed to play a role in the stimulation of growth-hormone secretion (Ahmed and Harvey, 2002). Most somatostatin neurons that produce somatotropin release-inhibiting hormones (SRIF) are located in the septal and other limbic areas, but also occur in the preoptic area, in the lateral supraoptic nucleus, the paraventricular nucleus and the tuberal area (Blasher, 1984).

Other, functionally not properly described neurons have been detected in the medial hypothalamus, which presumably produce neuromodulatory neuropeptides. An example is neurotensin, which mostly occurs in the POM area. Its amount shows sexual dimorphism, females have more immunopositive neurons and the amount of neurotensin changes during the ovulatory cycle. These neurons are presumed to be steroid-dependent elements (Absil and Balthazart, 1994). In the periventricular, infundibular and medial mammillary areas of the hypothalamus some neurons were found, which reacted to

cholecystokinin (CCK) treatment by early gene expression of cFOS, and CCK-synthesising neurons are also presumed to be present in these areas (Boswell, 1998). P-substance, which is thougth to be a neuromodulator, was detected in the medial hypothalamus and the external zone of the median eminence (Mikami and Yamada, 1984).

The extensive areas of the medial hypothalamus contain neurons with gonadotropin releasing (GnRH) and inhibiting (GnIH) hormones, which regulate pituitary cell function. These will be introduced in detail later.

In some cases, the functions of neurohypophyseal hormones and neuropeptides that regulate the adenohypophysis overlap. A "classic" hormone of pars nervosa is arginine vasotocine (AVT). As an adenohypophyseal factor, AVT has a CRF-like effect in birds. Forming extensive neuronal network, AVT is also a regulating factor of the hypothalamo-limbic system, and can play a role modulating the regulation of sexual behaviour. In the bed nucleus of the stria terminalis and in the supraoptic nucleus it is co-localised with galanin. In roosters, both neuropeptides are found in substantial amounts in the bed nucleus, while in hens both are lacking in these neuron groups. These neuropeptides can play a role in the regulation of male copulatory behaviour. In hens, compared to roosters, the supraoptic nucleus contains substantially more neurons that show co-localisation, which can play a regulatory role in the mechanism of egg laying (Klein et al., 2006). Besides the two Gomori-positive magnocellular nuclei, a large number of AVT somas is also located in the anterior preoptic nucleus, and diffusely through the lateral habenular nucleus hypothalamus. Their fibres form an extensive network in the POM area, the septum, the ventral tegmental area of Tsai of the ventral area, the lateral habenular nucleus of hypothalamus, the central substantia grisea, the intercollicular nucleus and surrounding the robust nucleus of the archistriatum, which is important in the regulation of singing (Voorhuis and de Kloet, 1992). Other studies suggest, that the rich AVT fibre network found in the POM of male quail originate from the parvocellular neurons located medially in the bed nucleus of the stria terminalis. This system shows a determined sexual dimorphism, castrating males decreases, while their testosterone-treatment increases fibre mass, and it plays an important role in the regulation of certain sexual behaviours (e.g., mate competition aggression) in male quails (Vigletti-Panzica et al., 1994, Absil et al., 2002, Kabelik et al., 2010).

5.2.2. Adenohypophyseal hormones of hypothalamic origin

Certain neurosecretory cells of the hypothalamus secrete hormones typical of the adenohypophysis. The first observation that raised this issue occurred when studying ACTH bioactivity of the basal hypothalamus (median eminence and tuberal nucleus) of pigeon in vitro (Péczely and Zboray, 1967). This

"extrahypophyseal" ACTH does not originate in the pars distalis, as it persists for a long time after adenohypophysectomy, and its amount even increases as a compensation (Péczely et al., 1970). Other parts of the hypothalamus, such as the paraventricular-preoptic area, were also soon discovered to have ACTH activity (Péczely, 1969). Later, immunocytochemical studies confirmed the existence of ACTH-secreting neurons: ACTH-immunopositive neurons were detected in the infundibular nucleus of the mallard (Péczely and Kovács, 2000b).

Further studies identified other proopiomelanocortin (POMC)-derivates in those areas of the avian brain that participate in neuroendocrine integration. It is a complex system, in which peptide hormones previously identified in the adenohyphophysis, neuromodulatory peptides, receptors of peripherally-produced compounds with hormone-like effects, and enzymes, as well as the neurons containing these all participate. For instance, met-enkephalin has been found in several nuclei of the medio-basal hypothalamus and in the external zone of the median eminence (Mikami and Yamada, 1984), α-melanocyte-stimulating hormone (α-MSH) and agouti-related peptide (AGRP) somas in the tubero-infundibular area (Strader et al., 2003), and POMC neurons in the same posterior hypothalamic area (Phillips-Singh et al., 2003). The neurons that synthesise these POMC-derivates (orexigenic and anorexigenic peptides) can play an active role in the modulation of the metabolic effects of prolactin, as well as in the regulation of nutrient uptake and energy homeostasis. Functional elements of this system are 1. the network of prepro-orexin (orexin) neurons, located in the lateral hypothalamic area and the periventricular nucleus of the hypothalamus, 2. neurons expressing the hypothalamic leptin receptor (Ob-R), 3. fatty acid synthase (FAS)-containing neurons, and the extensive (and multi-functional) excitatory-type neuropeptide-Y (NPY) network (Dridi et al., 2005). A large number of neuropeptide-Y receptors are located in the hypothalamus, medially in the area of the periventricular and paraventricular (magnocellular cells) nuclei and the medial and lateral areas of the tuberal nucleus, as well as in the lateral regions of the hypothalamus. The fibres of NPY form a rich network along the wall of the third ventricle and in the internal zone of the median eminence. The hypothalamic NPY system plays an important role in the regulation of neuroendocrine and vegetative functions as a neuromodulatory and/or neurotransmitter element (Esposito et al., 2001).

Besides the hypophyseal neurons that contain POMC, neurons expressing other adenohypophyseal hormones have also been detected in the avian hypothalamus. Immunocytochemical studies identified growth hormone- and prolactin-producing neurons in turkey and dove. Growth hormone-containing somas and axons have been identified in the periventricular nucleus of the hypothalamus, the PVN, the infudibular nucleus, the posterior hyphothalamic nucleus, the external zone of the median eminence, the lateral septum and the

hippocampus. The direct hypothalamic effect of growth hormone has been confirmed by detecting a specific growth-hormone binding (receptor) ability of chicken hypothalamic membrane preparation (Attardo and Harvey, 1990). Prolactin-containing neurons have been described from the lateral and medial septum and in the bed nucleus of the stria terminalis. Presumably, both hypophyseal hormone-secreting cell types play a role in the regulation of parental, feeding and sexual behaviour (Ramesh et al., 2000).

5.2.3. Biogenic amines

There are several nerve fibres and somas in the neuroendocrine system of the hypothalamus that contain biogenic amines. As important carriers of neurotransmitters, they form a part of the neurohormonal regulatory system. The axons of these neurons end on the somas of peptide-secreting neurons, causing excitatory or inhibitory effects, and also modulate neuropeptide release by axo-axonic connections in the external zone of the median eminence. Additionally, some secretory neurons (e.g., dopamine) can have a direct hypophysiotropic effect, by getting into the portal vessels of the median eminence.

Neurons with **dopamine** phenotype form an extensive network in the hypothalamus and in the limbic system. The thyrosine hydroxylase (TH) enzyme plays a key role in the dopamine biosynthesis in the hypothalamus. TH is present in the periventricular area of the soma and fibres of several neurons, which are located diffusely in larger cells of the lateral hypothalamus between the preoptic area and the tubero-infundibular and mammillary areas. Characteristically, TH-positive neurons are lacking in the postero-medial tuberal and the periventricular regions, which correspond to the mammalian arcuate nucleus. This suggests that the hypothalamic "dopamine inhibition" is absent in birds, or at least that it has a subordinate role.

TH-positive nuclei in the caudal part of the hypothalamus enlarge, and continue to reach into the mesencephalon, the ventral tegmental area of Tsai and the pedunculopontine tegmental nucleus. From here, a TH-positive fibre bundle projects forward paraventricularly, forming rich arborisation in the area of the paraventricular ependymal organ and the vascular organ of the lamina terminalis, and subependymally in the median eminence. Several mesenchymally-originated TH-positive fibres are located in the septal area, the bed nucleus of the stria terminalis and in the paleostriatum augmentation. Relatively few radially running TH-positive fibres are present in the external zone of the median eminence, suggesting a certain level of axo-axonic regulatory and direct hypophysiotropic effect of dopamine in birds (Kiss and Péczely, 1987, Moons et al., 1994, Sartsoongnoen et al., 2008).

Neurons that contain **noradrenalin** (or the key enzyme of its synthesis, dopamine-β-hydroxylase, DBH) are located in the caudal part of the mesencephalic tegment, in the locus coerelus and the subcoeruleus nucleus, the nucleus of the solitary tract, as well as the reticular nucleus of the myelencephalon (medulla oblongata). The number of noradrenergic fibres that innervate the periventricular areas of the hypothalamus (ventromedial hypothalamic nuclei), the preoptic area (especially in females), the septum and the hippocampus, is rather high. A particularly large number of fibre endings are located on the cells and around the neurons of the tuberal area (Bailhache and Balthazart, 1993, Moons et al., 1995, Mello et al., 1998).

Early studies conducted by Falck-Hillarp direct fluorescence method (Péczely and Elekes, 1970, unpublished data) suggested that **serotonin-**containing neurons in the avian hypothalamus were located mainly in the hypendyma layer of the paraventricular ependymal organ. This observation was later confirmed by immunocytochemical methods. Serotonin-containing cells connect with each other to form a loose network between the paraventricular organ and the infundibular nucleus (Haida et al., 2004). A large number of serotoninergic cells are located in the mesencephalon, where cells with a relatively small soma are located in the ventro-rostral area, middle-sized cells are in the dorsal mesenchephalo-pontius area, and forming a third group, ventrally in the ponto-medullar zone. A large number of serotonin-containing bundles of fibres are in the septal region, the POM area of the hypothalamus, the intercollicular nucleus and the external zone of the median eminence (Cozzi et al., 1991, Haida et al., 2004).

Glutamic acid-decarboxylase enzyme (GAD65) activity, which plays a critical role in **glutamate** (NMDA) and **γ-aminobutyric acid** (GABA) synthesis can be detected in a large number of neurons in the area of the forebrain, including the hypothalamus. The formed amino acid neurotransmitters and their timed (sequential) synthesis play a determining role in the formation of the neural networks of the developing brain, and in the timing of fuctional changes, such as puberty (Veenman and Reiner, 1994, Sun et al., 2005, Clarkson and Herbison, 2006). Large amount of GABA neuron fibres are found in the periventricular hypothalamus of pigeon and chicken, the number of unevenly distributed soma is low in the hypothalamus, but high in the dorsal thalamus, the dorsal geniculate nucleus, the surface layer of the mesencephalic optic tectum, the magnocellular nucleus of the isthmus nucleus, the intercollicular region, the central substantia grisea, the interpeduncular nucleus and in extensive zones of the cerebrum. The high amount of hypothalamic fibres suggest GABA-ergic, inhibitory regulation of the secretion of several neuropeptides (Veenman and Reiner, 1994).

5.2.4. Steroid hormones and receptors

Steroid hormones play an important part in the regulation of neuroendocrine function. This can occur in three different ways in the central nervous system, particularly in areas of the hypothalamo-limbic system. A receptor system developed to uptake steroids in the plasma circulation is located in the cytoplasm and in the nucleus, while another type is cell membrane bound. A typical way for the development of the steroid effect is the conversion of androgen hormones in the nervous tissue: (aromatisable) androgens can be converted into estrogens, or the active androgen hormone can be converted into 5α-, or 5β-dihydrotestosterone by testosterone 5-reductases. The third way to produce steroids is de novo steroid hormone synthesis, which occurs in the neurons and glia cells, starts from cholesterol, and its end products are the so-called neurosteroids. Steroid hormones, usually indirectly, can regulate the secretion of releasing hormones, but they can also function as neuromodulators. In the central nervous system they usually operate through their specific receptors.

Androgen hormones and receptors. Androgen receptors have been described in the neurons of sexually active male and female doves. These cytoplasmic-, and nuclear-steroid receptors occur in extensive areas of the telencephalon, diencephalon and mesencephalon in large numbers. In the area of the diencephalon they are located in the anterior and posterior regions of the hypothalamus. In the anterior region they are present in the neurons of the anterior preoptic nucleus, the magnocellular paraventricular preoptic nucleus, and the lateral hypothalamic nucleus. In the posterior region, they are present medially in the posterior medial hypothalamic nucleus, the tuberal nucleus, and the area of the ectomammillary nucleus, and laterally the posterior lateral hypothalamic nucleus and the lateralis mammillary nucleus contain androgen receptors. The amount of androgen-containing neurons does not show sexual dimophism in doves. Nevertheless, their number is exceptionally high during the courtship phase and radically decreases during breeding and chick-rearing. This suggests androgens play a role in regulating courtship and acceptance behaviour. Increasing daylength increases the amount of androgen receptors in both sexes (Belle and Lea, 2001).

On the other hand, the amount of androgen receptors shows a determined sexual dimorphism in Japanese quail: they mostly express in the brain of males, but in a smaller amount are also found in females. In both sexes, most androgen receptors express in the area of POM, where their absolute concentration is considerably higher in males. In contrast, in the lateral septum the concentration of androgen receptors is higher in females than males (Voigt et al., 2009).

In song sparrow (*Melospiza melodia*) most androgen receptor-containing neurons are located in the anterior preoptic area (POA) of the hypothalamus, and the number of receptors in this area increases substantially in sexually active

males. This confirms earlier results about the role of this area as the primary regulator of testosterone-dependent reproductive and aggressive behaviour in males. After the breeding season, during postnuptial moult, plasma testosterone levels and the androgen-receptor content of the POA considerably decrease and at the same time reproduction-related aggressive behaviour is low (Wacker et al., 2010).

Androgen receptors are also located in the song-regulating nuclei of white-crowned sparrow, as well as in higher regulatory brain centres, such as the ventral hyperstriatum (HVc) and the lateral division of the bed nucleus of the stria terminalis, where the number of androgen receptors increases substantially during breeding season. Nevertheless, there is no seasonal change in the number of receptors in the robust nucleus of the archistriatum and in the lateral magnocellular nucleus of the anterior nidopallium. This data supports the direct effect of testosterone on HVc, the area that regulates the functioning of efferent, song-regulating nuclei in a trophic way, and therefore creates the seasonal plasticity of song (Fraley et al., 2010). The spotted antbird (*Hylophylax naevioides*) is a tropical species with a year-round territoriality. Aside from a short period (the change in social behaviour accompanying pair formation), this bird has a low blood testosterone level all year. Therefore, territorial behaviour is not caused by testosterone. Testosterone-treatment of sexually inactive males (raising testosterone and DHT levels) leads to increased aggression and more intensive singing in these males. Aromatase-inhibitor and androgen-receptor inhibitor decreased between-male aggression and prevented them from singing. Hence in these birds male competition-linked aggression is regulated by a different factor (testosterone, and DHT and E2 forming from it), compared to territorial agression (Hau et al., 2000). In the Scottish red grouse *(Lagopus lagopus scoticus)* besides being typical in the spring, territorial behaviour also occurs in the autumn. Plasma testosterone levels in males are high in both seasons (a higher spring peak is followed by a less prominent autumn peak), which suggests that this steroid is responsibe for the development of aggressive behaviour both in spring and autumn (Mougeot et al., 2005).

The role of androgens is also detectable in the behavioural activation connected to neuroplasticity. Steroid receptor coactivator-1 plays an important role in this process. Two models are known. In male Japanese quail the amount of POM that regulates male behavioural processes, shows a defined seasonal pattern: its amount increases substantially during breeding season, castration decreases the size of the nucleus, and testosterone-treatment of castrated males causes the area to nearly double in size. The androgen (receptor)-dependent increase of POM comes from the growth of the neurons that are located here: dendrite arborisation increases, but the number of POM neurons remains the same. In male canary, the extent of the hyperstriatal area (HVc), which plays a basic role in vocal control, also increases during the period of intensive singing.

However, in this case, HVc growth is caused by the invasion of neurons that were formed in the areas around the ventricle, so the androgen effect does not increase dendrite arboriasation, but enhances mitotic division and directed transport of neuroblasts (germ cells) (Balthazart et al., 2010).

In spite of the fact that DHEA-specific receptors have not yet been identified in the central nervous system nor in the peripheral tissues of birds, the neuroendocrine and behaviour-regulating role of DHEA seems plausible. The neuroendocrine effects of changing plasma DHEA levels have mostly been studied in connection with aggressive behaviour. It can be assumed that along with or instead of testosterone, DHEA can be responsible for aggression. Most of the effects of this androgen in the central nervous system are probably caused directly by a neurosteroid-like DHEA, which is formed in the brain. However, it is also possible that DHEA originating from the gonad and the adrenal cortex also have an indirect effect, if it gets converted in the brain by 3β-HSD into other, more active androgens.

3β-HSD, which has a crucial role in the conversion of peripherally produced DHEA, has also been detected in the brain of songbirds. Its concentration is high in the diencephalon and the telencephalon, and its activity is higher in females than in males. The enzyme is stress sensitive: in males stress increases, in females it decreases its amount. In males, 3β-HSD activity is higher in non-breeding individuals compared to sexually active ones (Schlinger et al., 2008). In amphibians and lungfish the exact location of 5β-HSD is better known – it has been detected by immunocytochemical methods in the hypothalamus and (also) in the preoptic area.

In song sparrow both testosterone and DHEA can play a role in developing aggressive behaviour. Testosterone plasma level is high in sexually active and aggressive song sparrows, but low during moult, when males are less aggressive. During autumn after postnuptial moult, testosterone level of is still low in males, but their territorial agression increases again. At this time, however, DHEA plasma level increases, which can trigger agression. DHEA-treatment can trigger terrirotial singing in song sparrows in the autumn, but other forms of territorial behaviour do not develop (Soma, 2006).

In the males of the Central American spotted antbird provoked territorial behaviour and the accompanying aggressive vocalisation were associated with increased plasma DHEA level, but the level of testosterone did not change (Hau et al., 2004). In contrast, breeding-related "social pressure" did not change DHEA (or testosterone) level in male Buff-breasted Wren (*Thryothorus leucotis*), another Neotropical bird (Gill et al., 2008). Faecal steroid analysis of male great bustard (*Otis tarda)* showed that during the early phase of displaying (March), both testosterone and DHEA concentration start to increase and they rise further during April, the "main displaying season". During postnuptial moult, testosterone concentration substantially decreased, but DHEA continued to

increase. In juvenile males, the androgen balance was characterised by a higher faecal DHEA content compared to testosterone (Biczó and Péczely, 2007).

Along with several tissues in the periphery, testosterone, androstendione and DHEA – through androstenedione – can also convert into a more active androgen in the neurons. This product is the 5α- and 5β dihydrotestosterone (DHT), which forms by the effect of 5α- and 5β-reductases. The ratio of the two formed DHT is an important regulating factor of GnRH secretion in the central nervous system, by triggering or blocking a negative feedback loop. 5α-reductase activity of the anterior hypothalamus and the preoptic area is larger in breeding Wilson's phalaropes (*Phalaropus tricolor*) compared to non-breeding males (Schlinger et al., 1989). 5β-reductase activity of the midbrain and the ventromedial telencephalon of male song sparrow was high in spring and autumn, but did not increase during moult in the end of summer, when plasma testosterone, 5α-reductase and aromatase activity substantially decreased. This suggests that the inactive metabolite-forming 5β-reductase does not contribute to the mechanism of testosterone inactivation (Soma et al., 2003). On the other hand, studies conducted on Japanese quail, starling (*Sturnus vulgaris*) and partridge suggest that the activity of hypothalamic 5β-reductase is higher in juvenile, prepubertal and photorefracter birds compared to mature individuals, therefore it forms an important part of the mechanism of testosterone inactivation (Massa and Sharp, 1981, Massa and Bottoni, 1985).

Studies conducted on song sparrow also highlight the reciprocal relationship between androgens (testosterone) and aggressive behaviour. Testosterone treatment increases the level of aggression in birds, but aggressive behaviour by itself also increases plasma testosterone level (Soma, 2006).

The distribution of neurons that contain **aromatase enzyme** (ARO), which is responsible for androgen-estrogen conversion, was studied in several bird species. In Japanese quail and zebra finch the highest amount of ARO cells were located in the POM and in the tuberal area of the preoptic region of the hypothalamus, while only few immune-positive neurons were detected in the paraventricular and ventromedial nuclei. Aromatase-containing cells can be detected in the hyperstriatum accessorium, the medial area of the ventral hyperstriatum, the proximal and caudal areas of the neostriatum which are close to the ventricule, the magnocellular nucleus of the neostriatum (which plays a role in song regulation), the dorsal lamina of the archistriatum, in the area of the limbic system in the archistriatum, the septum, the bed nucleus of the stria terminalis (BnST), in the dorsal and medial areas of the hippocampus and in the area of the parahippocampus (Shen et al., 1995, Balthazart et al., 1996, Absil et al., 2001).

The degree of aromatisation is probably regulated by processes involving multiple factors. For instance, catecholamines (DA), the arginine vasotocin (AVT) system, as well as androgens and estrogens play an important role.

In Japanese quail, noradrenergic and dopamine-containing fibres have been detected in the area of the POM and the BnST. The effect of noradrenalin on aromatisation is small, but dopamine – according to biochemical measurements – strongly inhibits aromatisation. This inhibition is direct and rapid, decreasing the amount of estrogens formed in the neurons (Baillien and Balthazart, 1997). Studying the effect of dopamine on the sexual behaviour of male quail and analysing the POM showed that the DA effect is not even: this biogenic amine hyperpolarises 80% of POM cells, but depolarises about 10%. D1 and D2 antagonists did not, but the inhibition of noradrenergic receptors effectively neutralised these effects. The dopaminergic effect therefore occurs through noradrenergic (a1: excitatory and a2: inhibitory) receptors, not through dopaminergic receptors (Cornil et al., 2002).

Aromatase neurons of the POM – besides the TH-positive fibres – are also surrounded by a large amount of arginine vasotocine fibres. Furthermore, immunocytochemical studied detected localised AVT occurences (axo-somatic synapses) on these neurons. The preoptic area contains a large amount of androgen and estrogen (α and β) receptors, which do not always show co-localisation. Given that only a small percentage of neurons with aromatase activity contain estrogen or androgen receptors, but at the same time the dopaminergic as well as arginine vasotocine-producing neuros contain steroid receptors, the steroidal regulatory effect can be assumed to affect the androgen-estrogen transformation not directly, but through the AVT and DA system. Steroids have a stronger effect in males, which can be related to the different amount of steroid receptor coactivator-1. Aromatase activity is known to downregulate quickly, decreasing neural estrogen synthesis within minutes. This process is based on the increase of intracellular calcium, magnesium and ATP levels and protein phosphorylation, which are bound to membrane processes (Absil et al., 2001, Balthazart et al., 2003).

Aromatase activity (i.e., the amount of estrogen hormone produced) is strongly related to agression in male birds, but can also regulate reproductive behaviour. In song sparrow, aromatase inhibition can effectively prevent the birds from displaying aggressive behaviour that normally occurs both before and after the breeding season (Soma et al., 2003). Immunocytochemical localisation indicated that aromatase activity was high in the areas of the POA and in the bed nucleus of the stria terminalis in sexually active male song sparrows, and this aromatase activity was related to the increased E2 production, associated with male reproductive behaviour. On the other hand, aromatase activity in the ventromedial nucleus of the hypothalamus compared to non-aggressive, moulting indiividulas was not only high in sexually active males, but also in inactive birds, which means that here locally-produced estrogen can play a role in developing aggressive behaviour both during the breeding season and afterwards (Wacker et al., 2010). Similar functional aspects of the localisation of

aromatase activity have been observed in pied flycatcher (*Ficedula hypoleuca*). The amount of aromatase-containing neurons in the preoptic area of the hypothalamus showed a strong correlation with aggressive behaviour in males, but there was no correlation between agression and aromatase activity in the posterior hypothalamus or the telencephalon (Silverin et al., 2004).

The arginine vasotocin-containing neurons in the bed nucleus of the stria terminalis, which also contain aromatase enzyme, stimulate competitive aggression in male zebra finches. The administration of a D2 dopamine receptor antagonist triggers c-fos expression and later the increase of aromatase activity in zebra finches, which suggests that vasotocin and dopamine affect mate competition aggression (Kabelik et al., 2010).

Testrosterone treatement of young black-headed gulls (*Larus ridibundus*) increased social behaviour, which was more pronounced in individuals that were previously kept in groups, compared to individually isolated birds. In the gulls kept in groups, the aromatase activity of the POA and the hypothalamus was higher, which confirms the stimulating effect of the increase in androgen-estrogen conversion on social behaviour (Ros et al., 2009).

Estrogen hormones and estrogen receptors. In Japanese quail, both estrogen receptor types (α and β) are present in the preoptic, ventromedial and tuberal areas of the hypothalamus and also in the septum. The neurons in these areas also show a definite (cytoplasmic) aromatase (ARO) activity, however estrogen-binding ability and androgen-estrogen conversion capacity often do not coincide. In the ventromedial and tubular areas of the hypothalamus about 75% of (ARO) immunopositive cells contained estrogen receptors, but among the ARO neurons of the preoptic area and the septum, this number was only 20%. Among the estrogens that regulate neuroendocrine function, the ratio of estrogens formed in the brain by aromatisation and those taken up from the circulation is therefore different in different areas. Nevertheless, it is also possible that estrogens formed in the neurons by aromatisation do not act through classical estrogen nuclear receptors (Balthazart et al., 1991). During the development of the brain, the appearance of estrogen receptors shows sexually dimorphic patterns. In whole brain homogenate from embryonic Japanese quail, the expression of estrogen receptor-α in 9- and 17-day-old female embryo was more intensive than in males. Only estrogen receptor-β was detectable in the POM, the medial area of the bed nucleus of the stria terminalis and the tuberal nucleus of the hypothalamus of 9-day-old female embryo, estrogen receptor-α was not yet present. In 17-day-old embryo, estrogen receptor-β expresses in the POM, the nucleus taenia of the amygdala and the medial part of the bed nucleus of the stria terminalis. Only a small amount of estrogen receptor-α was present in the POM and the nucleus taenia and were absent in the bed nucleus of the stria terminalis (which is sexually dimorphic in adult birds). The relative dominance of estrogen receptor-β during brain development in quail proves

that the functioning of this receptor is crucial during sexual differentiation of the brain (Axelsson et al., 2007).

According to studies on male and female Japanese quail, estrogen receptor-α and β are located in the parts of the brain (POA, accumbent nucleus, bed nucleus of the stria terminalis and other limbic areas) that control sexual behaviour. In these areas, the mRNA of receptor-α and β can express in different ways, suggesting sexual and functional differences (Halldin et al., 2006). The amount of estrogen receptors shows sexual dimorphism: type α is present in higher amounts in the POM of females than in males, but there is more type β in the nucleus taenia of the amygdala of males and in the medio-basal hypothalamus of females. This sexual dimorphism in estrogen receptors can provide physiological background to certain behaviours in quail, but does not explain why testosterone does not trigger male-type copulatory behaviour in females (Voigt et al., 2009).

Studies of seasonal effects in song sparrow show that the hypothalamo-limbic system (POA, ventromedial nucleus of the hypothalamus, bed nucleus of the stria terminalis and periventricular nucleus of the medial striatum) contains both estrogen receptor-α and -β, but the amount of these is equal in reproductive and non-reproductive individuals. Nevertheless, aromatase activity was higher in the ventromedial nucleus of the hypothalamus in sexually active males compared to inactive and moulting birds, and its amount changed seasonally, the same way as the amount of androgen receptors. These results suggest that in male song sparrows the control of changes in estrogen-regulated seasonal behaviour does not originate form estrogens carried to the brain by circulation and then taken up by estrogen receptors, but from estrogens that form in the hypothalamic nuclei as a result of the aromatisation of androgens bound by androgen receptors (Wacker et al., 2010).

Androgen and estrogen receptors of the neurons – in the presence of ligand steroids – play a role in the regulation of neuronal proliferation. The administration of testosterone to a neuron-glia co-culture originating from embryonal chicken hypothalamus was more effective in the case of males than adding estradiol. The stimulatory effect occurred via classic androgen receptors, but aromatisation could also have played a role, as not only the androgen receptor-antagonist flutamide, but also the aromatase-inhibitor letrozol and the estrogen receptor-antagonist tamoxifen effectively decreased the testosterone effect. Adding estrogen to the system had a more effective stimulatory effect and it was inhibited more effectively by tamoxifen (Cao and Zhang, 2007).

The presence of **membrane-bound estrogen receptors** has been confirmed in several areas of the central nervous system. These receptors correspond to the classic type α and β nuclear receptors, which under certain circumstances migrate to the cell membrane and get incorporated into it. Membrane receptors exert their effect through the activation of G-proteins, which leads to the

stimulation of the glutamate receptors (GluR-1a). The next step is the activation of the inositol triphosphate (IP3) receptors, which releases Ca^{2+} from the intracellular calcium depots (such as the endoplasmic reticulum). The increased amount of free, intracellular calcium ensures the biosynthesis of neuro-progesterone, which plays a fundamental role in the steroid synthesis-cascade of the nervous system. The process – based on mammalian data – occurs mostly in the astrocytes. The formed neurosteroid presumably gets into the neurons by trans-synaptic transport (Micevych et al., 2009). Estrogen membrane receptors were also detected in the neuroendocrine system of birds. The binding affinity of membrane receptors located in the neurohyphysis of hens was larger in laying compared to non-laying individuals (Takahashi and Kawashima, 2009). Even though estrogen membrane receptors have not been detected in other areas of the hypothalamo-hypophyseal system of birds yet, their presence is probable, especially in the area of POM.

Progesterone affects the functioning of different areas of the brain through specific receptor isoforms in an age and sex-dependent process. In the hypothalamus, the concentration of progesterone receptor-β is higher in laying hens compared to juvenile or aged individuals. On the other hand, the amount of progesterone receptor-α is high in the cerebellum of aged birds. At the same time, the amount of neither isoforms shows age-dependent changes in the cortex and in the optic tectum. In roosters though, the amount progesterone receptor-β is higher in the hypothalamus of aged individuals. In roosters, no significant changes were detected with age in the area of the cerebellum, cortex and the tectum. In hens, the β isoform is dominant in all areas and at all ages, while in roosters it is only dominant in the hypothalamus of aged individuals (Camacho-Arroyo et al., 2007).

The location and role of **progesterone receptors** and their amount in the cytosol and in the nucleus were studied in relation to their role in the ovulatory cycle. In laying hens, the amount of cytosolic binding cites decreased in both the basal hypothalamus and the adenohypophysis 18 and 8 hours before ovulation, while the amount of nuclear receptors stongly increased 14 and 8 hours before ovulation. According to data, progesterone receptor and progesterone transport from the cytosol into the nucleus is the regulating factor of preovulatory LH release (Kawashima et al., 1980). The location of progesterone receptors was mapped in more detail in chicken brain using immunocytochemical methods. Through this mapping, Sterling et al., (1987) found more marked nuclei in the lamina terminalis, around the preoptic area, and in the dorsal part of the anterior hypothalamus, while fewer receptors were localised around the tuberal and the accumbent nuclei.

In doves, the role of progesterone receptors in the regulation of breeding was shown by Askew et al., (1997). Studying the hypothalamus of males and females, they found cytoplasmic and nuclear progesterone receptors in the

preoptic area, the magnocellular paraventricular preoptic nucleus, the tuberal region and the lateral hypothalamic nucleus. The amount of nuclear progesterone receptors was highest in individuals that were breeding and rearing offspring. As incubation progressed, the amount of progesterone receptors decreased in the tuberal area in males, but no decrease was seen in the area of the POM. This suggests an incubation-stimulating system (possibly VIP neurons) in the progesterone-regulated posterior hypothalamic areas (Lea et al., 2001).

Changes in progesterone receptor distribution as a function of sex, age and breeding cycle were studied in hypothalamus and adenohypophysis of domestic fowl. Estrogens play an important role in the expression and the increase in the amount of progesterone receptors. In juvenile chickens, the adenohypophysis lacks progesterone receptor-containing cells, and there are few such neurons present in the medio-basal hypothalamus. Estrogen treatment increases the number of progesterone receptor-containing cells in the hypothalamus in both juvenile roosters and prepubertal hens, and progesterone receptors also appear in the adenohypophyseal cells. In hens that were beginning to lay, most progesterone receptor-containing cells of the hypophysis were LH-immunopositive (Gasc and Baulieu, 1988). The binding of synthetic progestagen (R5020) to the cytosol and nuclear fraction of hypothalamus from juvenile chickens and laying hens showed that the specific binding increased by a large degree in sexually mature birds. 21-18 and 6-3 hours before laying, progestagene binding decreased in the cytosol and increased in the nuclear fraction, while such change did not occur in juveniles. This data proved the role of the progesterone receptors of the preoptic hypothalamic area in the regulation of preovulatory GnRH release (Kawashima et al., 1994).

In male doves, progesterone plays a role in the development of aggressive courting and breeding behaviour that are related to mate choice, as part of a complex steroidal regulatory system,. During this process, androgen and progesterone receptor-containing neurons of certain hypothalamic areas play a crucial role. These neurons have different functions. Inhibition of aromatase (fadrozole treatment) prevented aggressive courtship behaviour, and decreased the amount of androgen and progesterone receptors in the adenohypophysis, the magnocellular paraventricular preoptic nucleus of the hypothalamus, the POM, the posterior lateral hypothalamic nucleus and the tuberal nucleus. Fadrozole treatment decreased the amount of androgen receptors in the area of the POA in both sexes, and while it had no effect on the number of progesterone receptors in males, their amount decreased in females. Aromatase inhibition decreases the estrogen supply of the hypothalamus, and by this, indirectly the amount of androgen receptors. It also directly increases the amount of progesterone receptors, except for the ones located in the POA. Presumably, fadrozole blocks aggressive courtship behaviour in males because these regulating

– POA-neuron linked – impulses are extinguished by the interruption of the fuctional relationship between the aromatase-dependent androgen receptors and the aromatase-independent progesterone receptors, which are co-located in the neurons. The sight of the partner increases the number of aromatase-independent progesterone receptors in the POA of the males. Estrogen-triggered breeding behaviour is regulated by aromatase-dependent progesterone receptors of other areas, the POM and the basal hypothalamus (Belle et al., 2005).

The modulatory effects of progesterone on behavioural physiology were also studied on female African black coucals (*Centropus grillii*), as the females of this species show aggressive territorial behaviour. Territorial, aggressive females have lower plasma progesterone levels compared to individuals that lack breeding territory. Alternatively, pretreatment with progesterone decreased territorial aggression in non-territorial females, when these individuals were placed in areas suitable for breeding. In this species, progesterone compensates for the territorial aggression-triggering effect of testosterone, and the modulation can occur in the level of the hypothalamic-limbic system (Goymann et al., 2008).

Glycocorticoid receptors, such as a high-affinity, mineralocorticoid-type receptor, and a lower-affinity, glycocorticoid-type receptor, have also been detected in the avian brain. These types bind corticosterone and cortisol with a different affinity, corticosterone binding is typically of lower affinity and has a relatively high B-max value, while cortisol binding occurs with a higher affinity and has a lower B-max value. Brain **cell membrane glycocorticoid receptors** have also been described in zebra finch and house sparrow. The amount of membrane glycocorticoid receptors was lowest during the breeding season, while the amount of cytosol receptors, which have higher and lower binding capacity, was lowest in the winter. These observations prove the seasonal change in (sexual function-regulated) glycocorticoid binding (Breuner and Orchinik, 2001, 2009, Schmidt et al., 2010).

A third option for steroids to influence the neuroendocrine system is through **neurosteroid** biosynthesis, by synthesising steroid hormones de novo in the nervous tissue. This process was discovered about ten years ago, but the physiological role of the steroids synthesised this way is still not well known. The metabolism of neurosteroids was studied in details in the brain of day-old chickens. Following the injection of H3 precursors in the medial mesopallium, pregnenolone, progesterone, dehydroepiandrosteron (DHEA) and dehydroepiandrosteron-sulphate (DHEA-S) formed. The synthesised steroids got excreted from the brain in about five hours. During metabolism, pregnenolone and progesterone got coverted into 20β-dihydroxypregnenolon and 5β-dihydroxyprogesterone, and the latter metabolised into 3α-5β-tetrahydroprogesterone. DHEA and DHEA-S were the most stable metabolites. Because of this, they are assumed to play a role in the early phase of memory formation (Sujkovic et al., 2009). Considering the role of neurosteroids in

memory forming, the medial ventral hyperstriatum and parolfactory lobe can play a crucial role in hatchling chicken, as 5-30 minutes and 60-120 minutes after practical training, a substantial increase was detected in these areas in DHEA and DHEA-S, respectively (Migues et al., 2002).

The enzymes and end products of cholesterol-based steroid biosynthesis were studied in Japanese quail and zebra finch. Along the lateral ventricles and in the vocal control centres of zebra finch, the intermediate and end products, the genes and the enzyme systems of the following conversions were found: cholesterol – pregnenolone (cytochrome P450scc), pregnenolone – progesterone (3β-HSD), pregnenolone – dehydroepiandrosterone (cytochrome P450-17A1, CYP 17), progesterone – androstenedion (CYP 17), androstendione – testosterone (17β-HSD) and testosterone – 17β-estradiol (aromatase: cytochrome P450 19A1, CYP 19). In Japanese quail, primarily in the area of the diencephalon, the enzymes of cholesterol-pregnenolone conversion and the pregnenolone – progesterone, 17α-hydroxyprogesterone, androstenedione and testosteron synthesis were detected, as well as the presence of aromatase, which converts testosterone into 18C estradiol (Matsunaga et al., 2002).

Studying the localisation of certain steroidogenic enzymes, subcellularly, 3β-HSD was mostly localised in the microsomal fraction, and in lower amounts in the mitochrondria and the synaptosomes. 5β-reductase, which mostly acts on androstenedione and testosterone, was only found in the microsomal fraction (Pradhan et al., 2010a).

Only few studies are known that consider the functional aspects of neurosteroids. In Japanese quail, mostly in the in the area of the diencephalon, pregnenolone is also converted into 17α-hydroxypregnenolone, which plays a role in the circadian control of locomotion. Locomotor activity in males shows a circadian pattern: its decrease at night is caused by the increase in melatonin secretion, which decreases the synthesis of 17α-hydroxypregnenolone (Tsutsui et al., 2008, 2009a,b, London et al., 2009). The agression of song sparrows during breeding season is caused by a large amount of testosterone, produced by the testes. However, aggressive behaviour outside the breeding season (in spite of the low testosterone level) can be caused by DHEA, which during this period shows high concentrations in both the plasma and the brain of song sparrows. The amounts of 3β-HSD, which regulates the DHEA – androstenedion conversion in the brain, and the cofactor (NAD+) important in the conversion, substantially increase during this period, indicating that during this time the amount of androgens in the systemic circulation does not play a role in the control of aggression, but neurosteroids do, which presents a more simple solution, using less energy (Pradhan et al., 2010b).

References 5/1., 2.

1. Absil P, Baillien M, Ball GF, Panzica GC, Balthazart J (2001): The control of preoptic aromatase activity by afferent input sin Japanese quail Brain Res.Brain Res.Rev., 37, 38-58.
2. Absil P, Balthazart J (1994): Sex difference in the neurotensin-immunoreactive cell populations of the preoptic area in quail (Coturnix japonica) Cell Tissue Res., 275, 99-116.
3. Absil P, Papello M, Vigletti-Panzica C, Balthazart J, Panzica GC (2002): The medial preoptic nucleus receives vasotocinergic input sin male quail: a tract-tracing and immunocytochemical study J.Chem.Neuroanat., 24, 27-39.
4. Ahmed S, Harvey S (2002): Ghrelin: a hypothalamic GH-releasing factor in domestic fowl (Gallus domesticus) J.Endocrinol., 172, 117-125.
5. Askew JA, Georgiou GC, Sharp PJ, Lea RW (1997): Localization of progesterone receptor in brain and pituitary of the ring dove: influence of breeding cycle and estrogen Horm.Behav., 32, 105-113.
6. Attardo D, Harvey S (1990): Growth hormone-binding sites in chicken hypothalamus J.Mol. Endocrinol., 4, 23-29.
7. Axelsson J, Mattsson A, Brunström B, Halldin K (2007): Expression of estrogen receptor-alpha and-beta mRNA in the brain of Japanese quail embryos Dev.Neurobiol., 67, 1742-1750.
8. Bailhache T, Balthazart J (1993): The catecholaminergic system of the quail brain: immunocytochemical studies of dopamine beta-hydroxylase and thyrosine hydroxylase J.Comp.Neurol., 329, 230-256.
9. Baillien M, Balthazart J (1997): A direct dopaminergic control of aromatase activity in the quail preoptic area J.Steroid Biochem.Mol.Biol., 63, 99-113.
10. Balthazart J, Absil P, Foidart A, Houbart M, Harada N, Ball GF (1996): Distribution of aromatase-immunreactive cells in the forebrain of zebra finches (Taenopygia guttata) imolications for the neural action of steroids and nuclear definition in the avian hypothalamus J.Neurobiol., 31, 129-148.
11. Balthazart J, Baillien M, Charlier TD, Cornil CA, Ball GF (2003): Multiple mechanisms control brain aromatase activity at the genomic and non-genomic level J.Steroid.Biochem.Mol.Biol., 86, 86, 367-379.
12. Balthazart J, Charlier TD, Barker JM, Yamamura T, Ball GF (2010): Sex-steroid induced neuroplasticity and behavioral activation in birds Eur.J.Neurosci., 32, 2116-2132.
13. Balthazart J, Dupierux V, Aste N, Vigletti-Panzica C, Barrese M, Panzica GC (1994): Afferent and efferent connections of the sexually dimorphic medial preoptic nucleus of the male quail revealed by in vitro transport of DiI Cell Tissue Res., 276, 455-475.
14. Balthazart J, Foidart A, Surlemont C, Harada N (1991): Neuroanatomical specificity in the co-localization of aromatase and estrogen receptors J.Neurobiol., 22, 143-157.
15. Belle MD, Lea RW (2001): Androgen receptor immunolocalization in brains of courting and brooding male and female ring doves (Streptopelia risoria) Gen.Comp.Endocrinol., 124, 173-187.
16. Belle MD, Sharp PJ, Lea RW (2005): Aroma-tase inhibition abolishes courtship behaviours in the ring dove (Streptopelia risoria) and reduces androgen and progesterone receptors in the hypothalamus and anterior pituitary gland Mol.Cell Biochem., 276, 193-204.
17. Biczó A, Péczely P (2007): Display activity and seasonality of faecal sexual steroids in male great bustard (Otis tarda) Acta Biol.Hung., 58, 21-33.
18. Blahser, S (1984): Peptidergic pathways in the avian brain J.Exp.Zool., 232, 397-403.
19. Boswell T, Li Q (1998): Cholecystokinin induces Fos expression in the brain of the Japanese quail Horm.Behav., 34, 56-66.
20. Breuner CW, Orchinik M (2001):Seasonal regulation of membrane and intracellular corticosteroid receptors in the house sparrow brain J.Neuroendocrinol., 13, 412-420.
21. Breuner CW, Orchinik M (2009): Pharmacological characterization of intracellular, membrane, and plasma binding sites for corticosterone in house sparrows Gen.Comp.Endocrinol., 163, 214-224.
22. Camacho-Arroyo J, Hernandez-Molina VI, Rivas-Suarez M, Guerra-Araiza C, Gonzalez-Morán MG (2007): Changes in progesterone receptor isoforms content in the brain of immature, mature and aged male and female chickens Gen.Comp.Endocrinol., 150, 381-385.
23. Cao A, Zhang C (2007): Sex-specific effects of androgen and estrogen on proliferation of the embryonic chicken hypothalamic neurons Endocrine., 31, 161-166.

24. Clarkson J, Herbison AE (2006): Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty Mol.Cell Endocrinol., 25, 254-255.
25. Cornil CA, Balthazart J, Motte P, Massotte L, Seutin V (2002): Dopamine activates noradrenergic receptors in the preoptic area J.Neurosci., 22, 9320-9330.
26. Cozzi B, Vigletti-Panzica C, Aste N, Panzica GC (1991): The serotoninergic system in the brain of the Japanese quail. An immunohistochemical study Cell Tissue Res., 263, 271-284.
27. Dridi S, Ververken C, Hillgartner FB, Arckens L, Van der Gucht E, Cnops L, Decuypere E, Buyse J (2006): FAS inhibitor cerulenin reduces food intake and melanocortin receptor gene expression without modulating the other (an) orexigenic neuropeptides in chickens Am.J.Physiol.Regul. Integr.Comp.Physiol., 291, R138-147.
28. Esposito V, Pelagalli GV, De Girolamo P, Gargiulo G (2001): Anatomical distribution of NPY-like immunoreactivity in the domestic chick brain (Gallus domesticus) Anat.Rec., 263, 186-201.
29. Fraley GS, Steiner RA, Lent KL, Brenowitz EA (2010): Seasonal changes in androgen receptor mRNA in the brain of the white-crowned sparrow Gen.Comp.Endocrinol., 166, 66-71.
30. Gasc JM, Baulieu EE (1988): Regulation by estradiol of the progesterone receptor in the hypothalamus and pituitary: an immunohistochemical study in the chicken Endocrinology 122, 1357-1365.
31. Gill SA, Costa LM, Hau M (2008): Males of a single-brooded tropical bird species do not show increases in testosterone during social challenges Horm.Behav., 54. 115-124.
32. Goymann W, Witterzellner A, Schwabl I, Makomba M (2008): Progesterone modulates aggression in sex-role reversed female African black coucals Proc.Biol.Sci., 275, 1053-1060.
33. Haida Y, Ubuka T, Ukena K, Tsutsui K, Oishi T, Tamotsu S (2004): Photoperiodic response of serotonin- and galanin-immunoreactive neurons of the paraventricular organ and infundibular nucleus in Japanese quail, Coturnix coturnix japonica Zoolog.Sci., 21, 575-582.
34. Halldin K, Axelsson J, Holmgren C, Brunström B (2006): Localization of estrogen receptor-alpha and beta mRNA in brain areas controlling sexual behavior in Japanese quail J.Neurobiol., 66, 148-154.
35. Hau M, Stoddard ST, Soma KK (2004): Territorial aggression and hormones during the non-breeding season in a tropical bird Horm.Behav., 45, 40-49.
36. Hau M, Wikelski M, Wingfield JC (2000): Visual and nutritional food cues fine-tune timing of reproduction in a neotropical rainforest bird J.Exp.Zool., 286, 494-504.
37. Kabelik D, Kelly AM, Goodson JL (2010): Dopaminergic regulation of mate competition aggression and aromatase-Fos colocalization in vasotocin neurons Neuropharmacology, 58, 117-125.
38. Karten HJ, Hodos W (1967): A stereotaxis atlas of the brain of the pigeon (Columba livia) The John Hopkins Press, Baltimore, Maryland 21218
39. Kawashima M, Kamiyoshi M, Tanaka K (1980): Relationship between the changes in cytoplasmic progesterone receptor concentration and in nuclear progesterone binding sites in the hen hypothalamus and pituitary during the ovulatory cycle Endocrinol.Jpn., 27, 667-670.
40. Kawashima M, Kamiyoshi M, Tanaka K (1994): Changes in progesterone receptor binding of preoptic hypothalamus during an ovulatory cycle of the hen Poult.Sci., 73, 855-863.
41. Kiss JZ, Péczely P (1987): Distribution of thyrosine-hydroxylase (TH)-immunreactive neurons in the diencephalon of the pigeon (Columba livia domestica) J.Comp. Neurol., 257, 333-346.
42. Klein S, Jurkevich A, Grossmann R (2006): Sexually dimorphic immunoreactivity of galanin and colocalization with arginine vasotocin in the chicken brain (Gallus gallus domesticus) J.Comp. Neurol., 499, 828-839.
43. Knapp R, Silver R (1995): Location of neurons projecting to the hypophysial stalk-median eminence in ring doves (Streptopelia roseogrisea) Cell Tissue Res., 280., 77-86.
44. Korf HW (1984): Neuronal organization of the avian paraventricular nucleus: intrinsic, afferent, and efferent connections 232, 387-395.
45. Kuenzel WJ, van Tienhoven A (1982): Nomenclature and location of avian hypothalamic nuclei and associated circumventricular organs J.Comp.Neurol., 206, 293-313.
46. Lea RW, Clark JA, Tsutsui K (2001): Changes in central steroid receptor expression, steroid synthesis, and dopaminergic activity related to the reproductive cycle of the ring dove Microsc.Res.Tech., 55, 12-26.
47. London SE, Remage-Healey L, Schlinger BA (2009): Neurosteroid production in the songbird brain: a reevaluation of core principles Front Neuroendocrinol., 30, 302-314.

48. Massa R, Bottoni L (1985): The endocrine system and the environment (eds.: BK Follett, S.Ishii, Chandola A) 167, Jap.Sci.Soc.Press, Tokyo, Springer, Berlin
49. Massa R, Sharp PJ (1981): Conversion of testosterone to 5 beta-reduced metabolites in the neuroendocrine tissues of the maturing cockerel J.Endocrinol., 88, 263-269.
50. Matsunaga M, Ukena K, Tsutsui K (2002): Androgen biosynthesis in the quail brain Brain Res., 948, 180-185.
51. Mello CV, Pinaud R, Ribeiro S (1998): Noradrenergic system of the zebra finch brain: immunocytochemical study of dopamine-beta-hydroxylase J.Comp.Neurol., 400, 207-228.
52. Micevych P, Kuo J, Christensen A (2009): Physiology of membrane oestrogen receptor signalling in reproduction J.Neuroendocrinol., 21, 249-256.
53. Migues PV, Johnson AN, Rose SP (2002): Dehydroepiandrosterone and its sulphate enhance memory retention in day-old chicks Neurosci., 109, 243-251.
54. Mikami S (1976): Ultrastructure of organum vasculosum of the lamina terminalis of the Japanese quail, Coturnix coturnix japonica Cell Tissue Res., 172, 227-243.
55. Mikami S, Yamada S (1984): Immunohistochemistry of the hypothalamic neuropeptides and anterior pituitary cells in the Japanese quail J.Exp.Zool., 232, 405-417.
56. Mikami SI, Oksche A, Farner DS, Vitums A (1970): Fine structure of the vessels of the hypophyseal portal system of the white crowned sparrow, Zonotrichia leucophrys gambellii, Zeitschrift Zellforsch., 106, 155-174.
57. Miques PV, Johnston AN, Rose SP (2002): Dehydroepiandrosterone and its sulphate enhance memory retention in day-old chicks Neuroscience 109, 243-251.
58. Montero M, Yon L, Kikuyama S, Dufour S, Vaudry H (2000): Molecular evolution of the growth hormone-releasing hormone/pituitary adenylate cyclase-activating polypeptide gene family. Functional implication in the regulation of growth hormone secretion J.Mol.Endocrinol., 25, 157-168.
59. Moons L, D'Hondt E, Pijcke K, Vandesande F (1995): Noradrenergic system in the chicken brain: immunocytochemical study with antibodies to noradrenaline and dopamine-beta-hydroxylase J.Comp.Neurol., 360, 331-348.
60. Mougeot F, Dawson A, Redpath SM, Leckie F (2005): Testosterone and autumn territorial behavior in male red grouse Lagopus lagopus scoticus Horm.Behav., 47, 576-584.
61. Oksche A, Farner DS (1974): Neurohistological studies of the hypothalamo-hypohyseal system of Zonotrichia leucophrys gambellii, Advances in Anatomy, Embriology and Cell Biology, 48, 1-136.
62. Panzica GC, Spigolon S, Castagna C (1995): Ultrastructural characterization of sexually dimorphic medial preoptic nucleus of male Japanese quail Cell Tissue Res., 279, 517-527.
63. Panzica GC, Vigletti-Panzica C, Balthazart J (1996): The sexually dimorphic preoptic nucleus of quail: a key brain area mediating steroid action on male sexual behaviour Front Neuroendocrinol., 17, 51-125.
64. Péczely P (1969): Effect of different interventions of the amount of ACTH in the median eminence of the domestic pigeon Acta Biol.Acad.Sci.Hung., 20, 399-404.
65. Péczely P (1972): Effect of metopirone, prednisolone and insulin treatment on the domestic pigeon's hypothalamus Acta Morph.Acad.Sci.Hung., 19, 105-122.
66. Péczely P (1987): A Madarak Szaporodásbiológiája, 53-79, 154-155., Mezôgazdasági Kiadó, Budapest
67. Péczely P, Antoni FA (1984): Comparative localisation of neurons containing ovine CRF-like and neurophysin-like immunoreactivity in the diencephalon of the pigeon (Columba livia domestica) J.Comp.Neurol., 228, 69-80.
68. Péczely P, Baylé JD, Boissin J, Assenmacher I (1970): Activité corticotrope et CRF dans l'éminence médiane et activité corticotrope de greffes hypophysaires chez le pigeon C.R.Acad.Sci. (Paris) 270, 3264-3267.
69. Péczely P, Kiss JZ (1988): Immunreactivity to vasoactive intestinal polypeptide (VIP) and thyreotropin releasing hormone (TRH) in hypothalamic neurons of domesticated pigeon (Columba livia). Alterations following lactation and exposure to cold. Cell Tissue Res., 251, 485-494.
70. Péczely P, Kovács K, Barna I (1996): CRF-ACTH system and proopiomelanortin derivates containing neurons in the avian brain Abstr.Avian Brain.Behaviour Tihany, 53-54.

71. Péczely P, Muray T (1967): The effect of KCL and NaCl, hhydration, dehydration on the Subcommissural organ of the domestic pigeon Acta Biol.Acad.Sci.Hung., 18, 115-128.
72. Péczely P, Zboray G (1967): CRF and ACTH activity in the median eminence of the pigeon Acta Physiol.Acad.Sci.Hung., 32, 229-239.
73. Phillips-Singh D, Li Q, Takeuchi S, Ohkubo T, Sharp PJ, Boswell T (2003): Fasting differentially regulates expression of agouti-related peptide, pro-opiomelanocortin, prepro-orexin, and vasoactive intestinal polypeptice mRNAs in the hypothalamus of Japanese quail Cell Tissue Res., 313, 217-225.
74. Pradhan DS, Lau LY, Schmidt KL, Soma KK (2010a): 3-beta HSD in songbird brain: subcellular localization and rapid regulation by estradiol J.Neurochem., 115, 667-675.
75. Pradhan DS, Newman AE, Wacker DW, Wingfield JC, Schlinger BA, Soma KK (2010b): Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season Horm.Behav., 57, 381-389.
76. Ramesh R, Kuenzel WJ, Buntin JD, Proudman JA (2000): Identification of growth- hormone and prolactin-containing neurons within the avian brain Cell Tissue Res., 299, 371-383.
77. Rehák P, Kostová D (1993): Regio preoptica hypothalami in the goose (Anser anser f.domestica) Funct.Dev.Morphol., 3, 157-164.
78. Ros AF, Franco AM, Groothuis TG (2009): Experience modulates both aromatase activity and the sensitivity of agonistic behavior to testosterone in black-headed gulls Physiol.Behav., 97, 30-35.
79. Sartsoongnoen N, Kosonsiriluk S, Prakobsaeng N, Songserm T, Rozenboim I, El Halawani ME, Chaiseha Y (2008): The dopaminergic system in the brain of the native Thai chicken, Gallus domesticus: localization and differential expression across the reproductive cycle Gen.Comp. Endocrinol., 159, 107-115.
80. Schlinger BA, Fivizzani AJ, Callard GV (1989): Aromatase, 5 alpha- and 5 beta-reductase in brain, pituitary and skin of the sex-role reversed Wilson's phalarope J.Endocrinol., 122, 573-581.
81. Schlinger BA, Pradhan DS, Soma KK (2008): 3beta-HSD activates DHEA in the songbird brain Neurochem.Intern., 52, 611-620.
82. Schmidt KL, Malisch JL, Breuner CW, Soma KK (2010): Corticosterone and cortisol binding sites in plasma, immune organs and brain of developing zebra finches: intracellular and membrane-associated receptors Brain Behav.Immun., 24, 908-918.
83. Shen P, Schlinger BA, Campagnoni AT, Arnold AP (1995): An atlas of aromatase mRNA expression in the zebra finch brain J.Comp.Neurol., 360, 172-184.
84. Silverin B, Baillien M, Balthazart J (2004): Territorial aggression, circulating levels of testosterone, and brain aromatase activity in free-living pied flycatchers (2004): Horm.Behav., 45, 225-234.
85. Soma KK (2006): Testosterone and aggression: Berthold, birds and beyond J.Neuroendocrinol., 18, 543-551.
86. Soma KK, Schlinger BA, Wingfield JC, Saldanha CJ (2003): Brain aromatase, 5 alpha reductase, and 5 beta reductase change seasonally in wild male song sparrows: relationship to aggressive and sexual behavior J.Neurobiol., 56, 209-221.
87. Sterling RJ, Gasc JM, Sharp PJ, Renoir JM, Tuohimaa P, Baulieu EE (19879 The distribution of nuclear progesterone receptor in the hypothalamus and forebrain of the domestic hen Cell Tissue Res., 248, 201-205.
88. Strader AD, Schiöth HB, Buntin JD (2003): The role of the melanocortin system and the melanocortin-4 receptor in ring dove (Streptopelia risoria) feeding behavior Brain Res., 960, 112-121.
89. Sujkovic E, Mileusnic R, Fry JP (2009): Metabolism of neuroactive steroids in day-old chick brain J.Neurochem., 109, 348-359.
90. Sun Z, Wang HB, Laverghetta A, Yamamoto K, Reiner A (2005): The distribution and cellular localization of glutamic acid decarboxylase-65 (GAD65) mRNA in the forebrain and midbrain of domestic chick J.Chem.Neuroanat., 29, 265-281.
91. Takahasi T, Kawashima M (2009): Properties of estrogen binding components in the plasma membrane of neurohypophysis in hens and changes in its binding before and after oviposition Poult.Sci., 88, 2206-2211.
92. Tsutsui K, Haraguchi S, Inoue K, Miyabara H, Suzuki S, Ogura Y, Koyama T, Matsunaga M, Vaudry H (2009) Identification, biosynthesis, and function of 7 alpha-hydroxypregnenolone, a new key

neurosteroid controlling locomotor activity, in nonmammalian vertebrates Ann.N.Y.Acad.Sci., 1163, 308-315.
93. Tsutsui K, Inoue K, Miyabara H, Suzuki S, Ogura Y, Haraguchi S (2008): 7 alpha-hydroxypregnenolone mediates melatonin action underlaying diurnal locomotor rhythms J.Neurosci., 28, 2158-2167.
94. Tsutsui K, Inoue K, Miyabara H, Suzuki S, Ogura Y, Tobari Y, Haraguchi S (2009): Discovery of a novel avian neurosteroid, 7 alpha-hydroxypregnenolone, and its role in the regulation of the diurnal rhythm of locomotor activity in Japanese quail Gen.Comp.Endocrinol., 163, 117-122.
95. Veenman CL, Reiner A (1994): The distribution of GABA-containing perikarya, fibers, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets J.Comp.Neurol., 339, 209-250.
96. Vigh B, Manzano e Silva MJ, Franck CL, Vincze C, Czirok SJ, Szabó A, Lukáts A, Szél A (2004): The system of cerebrospinal fluid-containing neurons. Its supposed role in the nonsynaptic signal transmission of the brain Histol.Histopathol., 19, 607-628.
97. Vigh-Teichmann I, Vigh B (1983): The system of cerebrospinal fluid-containing neurons Arch. Histol.Jpn., 46, 427-468.
98. Vigletti-Panzica C, Aste N, Balthazart J, Panzica GC (1994): Vasotocinergic innervation of sexually dimorphic medial preoptic nucleus of the male Japanes quail: influence of testosterone Brain Res., 657, 171-184.
99. Voigt C, Ball GF, Balthazart J (2009): Sex differences in the expression of sex steroid receptor mRNA in the quail brain J.Neuroendocrinol., 12, 1045-1062.
100. Voorhuis TA, de Kloet ER (1992): Immunoreactive vasotocin in the zebra finch brain (Taenopygia guttata): Brain Res.Dev.Brain Res.,69, 1-10.
101. Wacker DW, Wingfield JC, Davis JE, Meddle SL (2010): Seasonal changes in aromatase and androgen receptor, but not estrogen receptor mRNA expression in the brain of the free-living male song sparrow, Melospiza melodia morphna J.Comp.Neurol. 518, 3819-3835.
102. Wacker DW, Wingfield JC, Davis JE, Meddle SL (2010):Seasonal changes in aromatase and androgen receptor, but not estrogen receptor mRNA expression in the brain of free-living male song sparrow, Melospiza melodia morphana J.Comp.Neurol., 518, 3619-3635.
103. Wang Y, Li J, Wang CY, Kwok AH, Leung FC (2007): Identification of the endogenous ligands for chicken growt hormone-releasing hormone (GHRH) receptor: evidence for a separate gene encoding GHRH in submammalian vertebrates Endocrinol., 148, 2405-2416.
104. Wang Y, Li J, Wang CY, Kwok AZ, Zhang X, Leung FC (2010): Characterization of the receptors for chicken GHRH amd GHRH-related peptides: identification of a novel receptor for GHRH and the receptor for GHRH-LP (PRP) Domest.Animal Endocrinol., 38, 13-31.
105. Wingstrand KG (1951): The structure and development of the avian pituitary, Gleerup, Lund, 316.

5.3. Vocal control centres of the avian brain

Behavioural patters of birds show characteristic changes during breeding season. A change in vocalisation occurs, i.e., characteristic calls, whistles and in the case of songbirds, songs carrying complex musical structure develop, which form an important element of reproductive behaviour. These sounds carry signals and mood components, and thereby play a determining role in assuring successful breeding. During breeding season both the sound-forming organ (syrinx) and the brain centres that regulate its function undergo typical functional differentiation, increasing in size and mass. The neuronal system of sound formation shows a determined sexual dimorphism and seasonality. For instance in zebra finch, vocal control centres in the forebrain of males are 5-14 times larger compared to females. The base of this sexual dimorphism is the more intensive androgen production in males.

Females of certain species (wrens, some weavers and bush-shrikes) are known to sing, and these singing females, as well as females that sing a duet with males, even though the size of vocal centres of the forebrain is still larger (and the number of their neurons is higher) in males, the difference in the centres among the sexes is smaller compared to those species, where only males sing. In slate-coloured boubou (*Laniarius funebris*), where the characteristics of male and female song (song repertoire, number and length of song type elements, and the level of frequency modulation) are basically the same, the size of the soma of the neurons is equal in two areas: in the motor nucleus generating vocalisation (nuclei of the tracheo-syringeal part of the hypoglossal nerve) and in the premotor regulatory robust nucleus of the archistriatum (RA). Nevertheless, the size of the ventral hyperstriatum (HVc) is still significantly larger in males compared to females, suggesting the (higher) androgen sensitivity of this area (Gahr et al., 1998).

The functional hyperplasia of the vocal system is caused by increasing androgen (testosterone) secretion at the beginning of the breeding season, and with its decrease in the end of the reproductive period, the mass of the syrinx decreases, and the functional hyperplasia of brain regulatory centres also ceases. In photostimulated white-crowned sparrow, there is a definite correlation between the increase of plasma testosterone levels, the length of the androgen-sensitive cloacal protuberance and the mass increase of the syrinx. Seven days of photostimulation increases the weight of the syrinx from 12.3 mg to 16.2 mg, and substantially increases the amount of androgen receptors in its tissue. At the same time, vocal control centres of the forebrain enlarge, and the number of neurons, their dendritic arborisation and the amount of dendritic spines on them also increase. This results in 70% increase in HVc, 20% in the volume of RA and Area X, while the size of the motor centre and the nucleus of the hypoglossal nerve do not change (Tramontin et al., 2000). The role of androgens

is also proven by the observation that sexual steroid treatment mascularinises forebrain centres responsible for song production in young female birds. The increase of plasma testosterone levels in both sexes leads to the development of reproductive behavioural patterns and at the same time causes differentiation in the vocal centres of the brain. Androgens also play a crucial role in the development of the species-, and rather the tutor-specific song patterns (song learning).

The regulation of vocal control in songbird forebrain consists of a posterior motor and an anterior modulatory system. The modulatory system incorporates learned elements, and its role is essential for the development of vocalisation (species-specific song) in young individuals. Elements of the vocal control system are connected to each other and have functional connections with the ascending auditory tract at several points. Each neuron of the system is song-selective, which means that they react stronger to the song of the same species compared to the song of other species, or different stimuli (noise). (Figure 56, 57.)

Part of the motor, syrinx-regulating **vocal system** is the caudal nucleus of the ventral hyperstriatum (or mesopallium, as it has been recently called). Some of its neurons (high vocal centre: HVc) form descending fibre contact with the robust nucleus of the archistriatum (arcopallium) and the motor neurons located there. The axons of the robust nucleus reach to the myelencephalon, ending in the nucleus of the tracheo-syringeal hypoglossal nerve, as well as the paraambiguus and the retroambiguus nuclei. The tracheo-syringeal nucleus is the vocal control centre in the brainstem, while cells of the latter regulate respiratory frequency. The collaterally-dividing tract therefore coordinates vocalisation and respiratory frequency.

Certain neurons of the ventral hyperstriatum form part of the anterial **vocal modulatory system**, which form axon connections with the Area X of the medial striatum (parolfactory lobe). Area X also receives axons from the medial and lateral magnocellular nuclei (mMAN and lMAN) of the anterior nidopallium (mesostriatum), which are connected to the dorsolateral nucleus of the medial thalamus, the area where a large amount of axons that originate in Area X end. These three areas clearly form a part of a fine-tuning system, through a positive-feedback loop.

Axons running from the lateral magnocellular nucleus of the anterior nidopallium to the robust nucleus of the acropallium connect the anterior and posterior vocal tract. Alternatively, the thalamic modulatory system connects auditory and vocal tracts: it works as a gate, making auditory modulation possible. By evaluating the individual's own along with the voice of unknown birds of the same species, this system forms an important element of song learning. Part of this system is the uvaeform nucleus and the axon bundle that originates here, leading to the nucleus interface of the nidopallium. From this

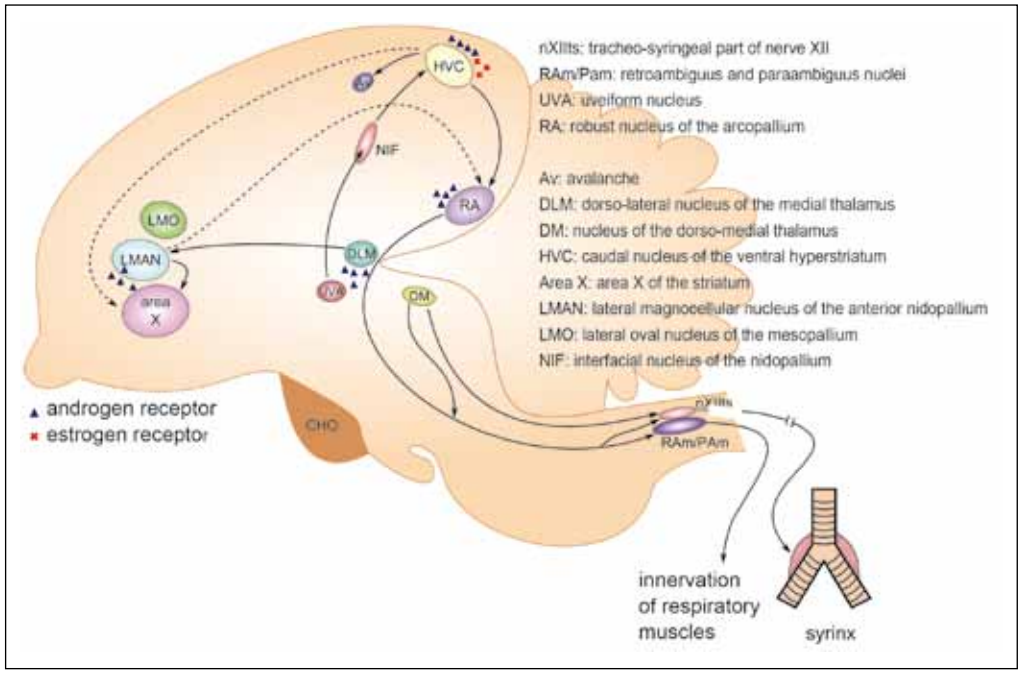

Figure 56. Vocal control centres in the songbird brain (modified after Reiner, et al., 2004)

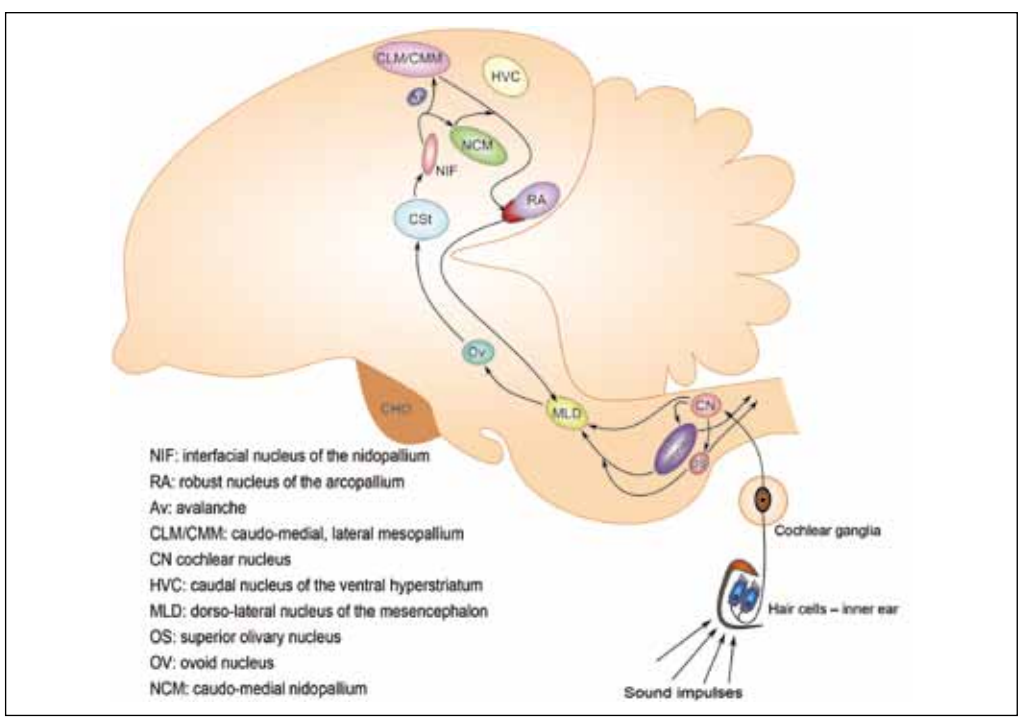

Figure 57. Auditory tracts in songbirds (modified after Reiner et al., 2004)

nucleus axons lead to the ventral hyperstratum of the mesopallium. The uvaeform nucleus receives afferent fibres from the dorsal part of the reticular formation, the lateral lemniscal nuclei and the medial habenular nucleus of the epithalamus (Akutagawa and Konishi, 1994, 2005).

The structure of the **auditory tract** is basically similar among different groups of birds. Therefore in songbirds, similar to others, the hair cells of the inner ear are connected with the centrifugal fibres of the bipolar neurons of the cochlear ganglion, from which ascending (centripetal) axons lead to the cochlear nucleus of the myelencephalon. From here fibres lead to the superior olive, the dorsal, intermedial and lateral parts of the lateral lemniscal nucleus, and to the dorso-lateral part of the mesencephalic nucleus (the area of the inferior colliculus). From the nuclei of the lemniscus and the superior olive also ascending nerves lead into the dorso-lateral nucleus of the mesencephalon. Similarly, descending nerves start from the superior olive and the ventral part of the lateral lemniscal nucleus leading to the ipsilateral cochlear angular nucleus, the laminar nucleus and the lateral lemniscal nucleus. Axons from the succeeding neurons of the tract lead to the central part of the ovoid nucleus of the thalamus. A strong axonal bundle that starts from the cortex of the ovoid nucleus leads to the caudo-lateral and caudo-medial part of the mesopallium and the mediocaudal area of the nidopallidum. From here, associative function fibres lead to the area surrounding the vocal nucleus of the ventral hyperstriatum, and to the area of the robust nucleus of the arcopallium. From the latter arcopallial area axons gather and run to the ovoid nucleus of the thalamus and the dorsolateral nucleus of the mesencephalon. These axons form a functional relationship between the auditory and vocal systems. Impulses arriving from the auditory system influence the occurrence of vocalisation (singing), but do not affect the structure of the vocalisation and do not inhibit the seasonal growth of the vocal control centres (Reiner et al., 2004, 2005, Coleman et al., 2007, Brenowitz et al., 2007, Wild et al., 2010).

Sexual steroids (mostly testosterone) can fuctionally activate the vocal control system directly, though the receptors in the regulatory areas and indirectly, by triggering the synthesis of trophic factors. This manifests through the large seasonal plasticity of the regulatory areas of the cerebrum: in temperate-zone songbirds these areas are much larger in the spring than in the autumn, and the the androgen and estrogen receptor content of the neurons is also higher. Additionally, typically there is increased neurogenesis and transport of new neurons towards the activated areas. These changes are generally much stronger in males than in females, and there is more pronounced sexual dimorphism in the brain of sexually active individuals. In males, there is a strong correlation between the size of the vocal control centres, amount of neurons, dendrite development of the neurons, plasma testosterone level and the song intensity (song stereotypy). At the same time, the size of the vocal control

nuclei and the song repertoire size were not related, proving that the seasonal plasticity of the control system affects song stereotypy and not repertoire size (Smith et al., 1997). The stimulating (masculinising) effect of sexual steroids on song control centres is greater in birds than in any other vertebrate species. The absolute volume and mass of HVc, RA and area X compared to the total brain mass and three thalamic nuclei, which do not participate in song control, is significantly larger in spring than in autumn, and the mass of these nucleus is lower in white-crowned sparrows kept on decreased photoperiod compared to ones on increased photoperiod (Brenowitz et al., 1998, Tramontin et al., 1999). Experimental decrease of testosterone decreased the volume of HVc by 22% in only 12 hours, and in 4 days the amount of neurons decreased by 26%. The decrease of the mass of RA and Area X were significant in 7 days (Thompson et al., 2007). Studies on zebra finches show that the effects of experimentally administered estradiol are nearly equivalent to those of testosterone, while the masculinising effect of androstenedione and progesterone were negligible (Grisham and Arnold, 1995).

Regarding its effect on neuroplasticity, corticosterone is the opposite of sexual steroids, i.e. in song sparrows its physiological dose decreases HVc volume in adult birds. This change can be traced back to the inhibition of new cell production. DHEA protects from the neurogenesis-inhibiting effect of corticosterone, which, at least on this level, proved to be an efficient antiglycocorticoid (Newman et al., 2010).

In several species (zebra finch, canary, song sparrow and starling) a large amount of androgen receptors have been found in the area of HVc, RA, mMAN and lMAN. The amount of these receptors – by binding plasma androgens – can play an important role in song control, especially in song stereotypy. Bilateral intracerebral infusion of androgen-, and estrogen-antagonists inhibited the seasonal increase in song sterotypy (Meitzen and Thompson, 2008). Seasonal changes in the amount of androgen receptor expression (mRNA) were studied in white-crowned sparrow, by comparing them to changes in a limbic area, the lateral bed nucleus of the stria terminalis. The amount of androgen receptors in the HVc and the nuclear area of the stria terminalis was substantially higher during the breeding season, but no seasonal changes were detected in RA and lMAN. This suggests that in the song control nuclei the seasonal plasticity caused by testosterone mostly occurs through the HVc. The androgen-binding ability of HVc is sensitive to changes, and their neurons regulate the functional hyperplasia of other centres by transsynaptical trophic effect (Fraley et al., 2010). The central regulator role of HVc also manifests in the regulation the aromatase activity in RA, and therefore the local estrogen production of the area. The direct effect of increased photoperiod on HVc increases androgen and estrogen receptor expression in the area, the binding of testosterone increased by photogonado-stimulation, and because

of this, its neurons transmit stimulative impulses to the RA, increasing its aromatase activity (Meitzen et al., 2007).

The functional relationship between lMAN and RA nuclei, which contain receptors with different andogen sensitivity, is crucial in song learning. Testosterone treatment increases the length of dendrites, the amount of dendritic spines and the synthesis of N-methyl-aspartate (NMDA) in the lMAN neurons, but not in the RA neurons, so its effect in the latter area is indirect. In the RA neurons, androgen increases the postsynaptic electrical activity trigerred by increased NMDA transmission, and its amount is strongly related to the song learning capacity of young birds. At the same time, NMDA postsynaptic activity experimentally induced in other areas did not affect the effectiveness of song learning, which underlies the functional importance of the lMAND and RA complex. The androgen effect, on the other hand, shortens the period when this steroid can positively influence active song learning, i.e. it also has a negative feedback effect (White et al., 1999).

Neurons located in the medial part of the HVc also contain a large amount of estrogen receptor-α, which can also contribute to the way photo-gonado stimulation can regulate vocal control. These receptors not only bind estrogens present in the systemic regulation, but also play a part in binding hormones produced in the central nervous system (neurosteroids). Through this, HVc can react to both androgen and estrogen effects. The estrogene receptor-β present in other brain areas, such as in the caudo-medial neurostriatum and in the preoptic area presumably binds locally synthesised estrogens, and is not directly involved in the regulation of vocal regulation (Bernard et al., 1999).

The discovery of neurosteroids and the fact that there is a high amount of de novo produced estrogen in the brain of songbirds call attention to the potential role of estrogens in song regulation. Unusually, the aromatase enzyme responsible for androgen-estrogen conversion, unlike in other brain areas, is practically lacking in the soma of the neurons of the vocal control nuclei, but expresses in the fibres and mostly in the synaptic terminals. The aromatase content of synaptosomes was found to be substantially higher in the brain of male birds compared to females, which suggests its role in song control. This assumption is reinforced by the finding that in male song sparrows aromatase activity of the caudal medial nidopallium (CMN), which is part of the auditory system, is significantly higher in individuals singing in the spring compared to individuals in the autumn, during moulting or sexual inactivity in the winter. Similar changes were observed in canary (*Serinus canaria*) and Lapland longspur (*Calcarius lapponicus*).

The analysis of CMN neurons found most of them to be of GABA-erg type, and some of the GABA cells also contained calbindin and estrogen receptors. The sexual dimorphism of CMN can be traced back to this group of neurons. Increased aromatase activity can be the results of increased plasma testosterone

levels, as confirmed by the effect of testosterone injections. The aromatase activity of synaptosomes changes quickly, in courtship-singing male zebra finches placed near females, aromatase activity in the posterior area of the telenceplone increased in 30 minutes, compared to non-singing males. The formed estrogen therefore fills an acute song-regulatory role (Pinaud et al., 2006, Remage-Healey et al., 2010).

In year-round singing and territorial song sparrow population, DHEA can trigger an effect similar to the neuronal estrogen production-increasing effect of testosterone by stimulating aromatase activity. In this species, the plasma DHEA level is high during the sexually inactive period, and at the same time there is high 3β-HSD activity in the NCM area. DHEA increases both 3β-HSD and aromatase activity, and increases estrogen synthesis in the NCM area (Soma and Wingfield, 2001).

The early seasonal development of spring song in Corsican blue tits (*Cyanistes caeruleus*) raises the issue of a more careful interpretation of testosterone-triggered hyperplasia of song control centres in the brain or the consideration of the effect of ecological factors. These birds start singing at the end of January-beginning of February, when their plasma testosterone level is still low and the size of their testes is still small, but the mass of HVc and RA has already increased. Their Mediterranean mixed forest habitat and the ample food supply can explain this phenomenon (Caro et al., 2005).

Sexual steroids (mostly testosterone) can also indirectly stimulate vocal control brain centres. Testosterone is known to activate the preoptic area of the hypothalamus, which is a crucial courtship and mating behaviour-regulating centre in male birds. Neurons that are located here contain a large amount of androgen receptors, and have high testosterone-binding ability. Androgen-activated neurons send impulses to the GnRH secretory system, and their axons also reach several mesencephalic and brainstem areas, which play a modulatory role in vocal control. These areas are the intercollicular dorsomedial nucleus, the gray matter of the periaquaeductal mesencephalon, the ventral tegmental area of Tsai and the catecholamine-containing neurons of the locus coeruleus. In this case, the indirect stimulating effect of testosterone occurs through catecholamine transmission (Riters and Alger, 2004, Ball et al., 2004). These catecholamine-containing neurons react very fast, in zebra finch the simultaneous expression of ZENK (Egr-1) (early gene) and the key enzyme of catecholamine synthesis (thyrosine hydroxilase) occurs in 30 minutes following singing, proving their role in the acute regulation of vocal communication (Lynch et al., 2008).

The steroid receptor coactivator SRC-1 can substantially affect androgen receptor binding and through this, the vocal control regulation of androgens. The synthesis of this protein shows sexual dimorphism, being more pronounced in males. Therefore, it directly contributes to the formation of a

larger HVc in males. On the other hand, the expression of SRC-1 is higher in the ventral tegmental area of Tsai, substantia nigra and locus coereleus, related to POA. All of these areas send catecholamine-containing axons to the vocal control nuclei, and therefore they can indirectly affect singing intensity and song learning (Charlier et al., 2003).

Vasotocin-containing neurons of the brain can also modulate the vocal control effect of (neuro)-steroids. In vertebrates, this peptide is a modulator of social behaviour. In white-crowned sparrow, singing activity can be increased by injecting vasotocin, and further vasotocin receptors were detected in the posterior areas of the telencephalone, especially in the NCM area (Maney et al., 1997, Leung et al., 2009).

Among the mechanisms responsible for the sexual dimorphism of the brain, such as singing characteristic of males, the production of a **neurotrophic factor** (brain-derived neurotrophic factor: BDNF) can have an important role. The production of this factor is substantial in the HVc of male zebra finches in the first 30-35 days after hatching, while its expression is not detected in females. Its increased production can be primarily caused by androgens (testosterone) in the circulation. 35% of the neurotrophic factor-containing neurons of the HVc projects to the RA and the area X, supporting them with BDNF (Dittrich et al., 1999). In white-crowned sparrow, BDNF experimentally administered to the RA causes an enlargement typical of the reproductive season and the neuronal density of the area and the size of the somas increase. Local inhibition of BDNF-binding receptors (TrkB) prevented the enlargement of the RA (Wissman and Brenowitz, 2009).

BDNF production can be initiated by other, testosterone-independent, neuronal activity-increasing effects, such as light or sound, which can cause activity-dependent structural changes in different parts of the brain. To study this phenomenon, male starlings were divided into two groups, one had access to nest holes, and these individuals were singing, while the others had no access and were not singing. Plasma testosterone levels were equal between the two groups, but the volume of HVc was higher in singing birds. In the next step, POM was destroyed in a group of castrated males, which strongly decreased singing activity, and the HVc was smaller in these individuals compared to the ones undergoing sham operation. Testosterone implant, administered to some of the birds that underwent surgery, did not stimulate singing and did not affect HVc volume. Later, in a group of castrated starlings the syrinx was destroyed, and some received testosterone implants. 1. Sham operation + testosterone implant birds had the largest HVc, 2. The volume of HVc was equal between devocalised males with a testosterone implant and 3. Sham operated starlings that did not receive testosterone treatment 4. The smallest HVc belonged to the group of devocalised males that did not receive testosterone. In startlings, the BDNF mRNA content of the area changed in a similar manner

to the volume. This experiment proved that certain volume changes of HVc are activity dependent, stimulated by singing, the effects of testosterone and singing can affect the volume of HVc independently from each other, and the neurotrophic factor expressed by the effect of singing plays an important role in the functioning of the vocal control system (Sartor and Ball, 2005).

The role of **endogen opioids** in the modulation of motivated behaviour, learning and memory development is well known. A considerable amount of met- and leucine-enkephalin has been detected in the vocal control region of the male zebra finch brain. The amount of these increases in intensively in singing males and an opioid receptor antagonist (naloxone) can decrease the frequency of singing. The amount of μ-opioid receptors is higher in the areas of lMAN, Area X, HVc, RA and the dorso-lateral nucleus of the medial thalamic area, which plays a part in audiotory afferentiation, compared to the amount of δ-opioid receptors. The μ-opioid content of vocal control areas is also higher compared to the surrounding structures. Opioid receptors can modify different aspects of vocalisation (song directed towards a particular individual vs. a song containing general mood elements) in different ways, and they can influence the amount of auditory input (Khurshid et al., 2009, 2010).

An important modulator of vocal control is **reelin**, a glycoprotein with a large molecular weight, which is an important factor of neuronal migration within the brain. The expression of reelin can be detected in large areas of the forebrain in starling, such as in the areas surrounding the HVc and the uvaeiform nucleus. Following testosterone treatment, the expression of reelin strongly decreases in the areas of the HVc, the uvaeiform nucleus and the dorsal thalamus, but not in other parts of the brain. The seasonally increasing testosterone level can therefore decrease the incorporation of new neurons into the HVc, and it suggests that reelin plays an important part in the seasonal plasticity of songbird brain (Absil et al., 2003). On the other hand, in canary, 4 weeks of testosterone treatment did not effect reelin-immunopositive neurons in the brain, which suggests its role is not yet fully understood (Balthazart et al., 2008).

Neuroendocrine effects of acoustic signals

Special vocalisation (singing) that appears during the breeding season of birds, forms an important part of courtship behaviour, assisting males and females in finding each other, accelerating maturation of the pair, optimising breeding site and nest construction, making copulation effective and helping in the formation of a stable pair bond. It is also important in offspring rearing. All of this is guaranteed by creating the optimal neuroendocrine state for the breeding cycle, in which ethological aspects also play a role.

Courtship elements contribute to the formation of the endocrine status necessary for the breeding seasone in two ways: 1. The slow mechanism, which means de novo neurogenesis, synaptogenesis and angiogenesis, accompanied by hormone synthesis initiating in the soma of neuroendocrine cells and the functioning of recently formed receptors. This mechanism acts in a period of days and weeks. Such a mechanism is the activation of the hypothalamohypophyseo-pituitary axis in female songbirds, which leads to a strong increase in LH plasma level, and its realisation is affected by male song. 2. The rapid mechanism, which means effects on earlier formed neurons and cell networks, which within minutes or hours results in neurohormone release from the median eminence to the portal circulation of the hypophysis, and leads to a sharp increase in LH plasma level. The rapid effect of behavioural elements and auditory effects are similar to the rapidly realised effects of light change (increased photoperiod) and both lead to cGnRH-I release.

While behavioural changes (including singing) that trigger slow activation affect the neurosecretory elements (cGnRH-I neurons) of the preoptico-septal area, resulting in the expression of "classic genes", the target area of rapid effects, such as the rapid change in the amount of daylight, behavioural elements and auditory effects (song), is not the area that contains the soma of cGnRH-I cells, but the area of axon-terminals and synaptosomes in the mediobasal hypothalamus (MBH), which includes the median eminence and the tubero-infundibular area. The basic role of MBH in c-GnRH-I release is well known in Japanese quail and other bird species. When the photoperiod was increased, in Japanese quails c-fos early gene activation initiated within hours in the medio-basal hypothalamus (but not in the cGnRH-I cells of the preoptic area), accompanied by rapid increase in plasma LH level. Similar results were seen when the song of white-crowned sparrows was played under laboratory conditions to females kept under decreased photoperiod: in the MBH cells early gene expression of egr-1 (ZENK) initiated 42 minutes after the treatment, and plasma LH level also substantially increased. As a result of hearing the song, in females egr-1 expressed in practically all cells of the infundibular nucleus, but it only appeared in a few places among the fibres of the median eminence. It is possible that here immunopositivity appeared in the glia end plates between the cGnRH-I axon endings and the basal lamella, suggesting that these structures undergo a post-simulation, early-gene regulated "withdrawal", which makes intensive neurohormone release into the portal capillaries possible (Maney et al., 2007).

The direct functional relationship between the auditory system and the neuroendocrine system is suggested by the observation that in females two areas of the acoustic tract, in the areas corresponding to the inferior colliculus and the auditory centre (caudal medial neostriatum) of the midbrain also had strong egr-1 expression as a result of hearing male song. Early gene activation

that appeared simulataneously in the auditory system and in the infundibular nucleus of the hypothalamus, and the direct axonal contact detected in the auditory thalamic area of doves to the preoptic and ventromedial areas of the hypothalamus, suggest that auditory impulses can trigger rapid cGnRH-I release in songbirds too. The synchronisation between the auditory and neuroendocrine systems probably does not necessitate the amplifying effect of other behaviours. At the same time, in female birds different hormonal levels, such as the actual plasma estradiol concentration can cause quantitative changes in the LH level that was released as a result of song (Maney et al., 2007).

References 5/3.

1. Absil P, Pinxten R, Balthazart J, Eens M (2003): Effects of testosterone on Reelin expression in the brain of male European starlings Cell Tissue Res., 312, 81-93.
2. Akutagawa E, Konishi M (1994): Two separate areas of the brain differentially guide the development of a song control nucleus in the zebra finch Proc.Natl.Acad.Sci.USA, 91, 12413-12417.
3. Akutagawa E, Konishi M (2005): Connections of thalamic modulatory centers to the vocal control system of the zebra finch Proc.Natl.Acad.Sci.USA, 102, 14086-14091.
4. Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Riters LV, Balthazart J (2004): Seasonal plasticity in the song control system: multiple brain sites of steroid hormone action and the importance of variation in song behavior Ann.N.Y. Acad.Sci., 1016, 586-610.
5. Balthazart J, Voigt C, Boseret G, Ball GF (2008): Expression of reelin, its receptors and its intracellular signaling protein, Disabled 1 in the canary brain: relationships with the song control Neuroscience, 153, 944-962.
6. Bernard DJ, Bentley GE, Balthazart J, Turek FW, Ball GF (1999): Androgen receptor, estrogen receptor alpha, and estrogen receptor beta show distinct patterns of expression in forebrain song control nuclei of european starlings Endocrinology, 140, 4633-4643.
7. Brenowitz EA, Baptista LF, Lent K, Wingfield JC (1998): Seasonal plasticity of the song control system in wild Nuttal's white-crowned sparrows J.Neurobiol, 34, 69-82.
8. Brenowitz EA, Lent K, Rubel EW (2007): Auditory feedback and song production do not regulate seasonal growth of song control circuits in adult white-crowned sparrows J.Neurosci., 27, 6810-6814.
9. Caro SP, Lambrechts MM, Balthazart J (2005): Early seasonal development of brain song control nuclei in male blue tits Neurosci.Letters, 386, 139-144.
10. Charlier TD, Balthazart J, Ball GF (2003): Sex differences in the distribution of the steroid receptor coactivator SRC-1 in the song control nuclei of male and female canaries Brain Res., 959, 263-274.
11. Coleman MJ, Roy A, Wild JM, Mooney R (2007): Thalamic gating of auditory responses in telencephalic song controlnuclei J.Neurosci., 27, 10024-10036.
12. Dittrich F, Feng Y, Metzdorf R, Gahr M (1999): Estrogen-inducible, sex specific expression of brain derived neurotrophic factor mRNA in a forebrain song control nucleus of the juvenile zebra finch Neurobiology, 96, 8241-8246.
13. Fraley GS, Steiner RA, Lent KL, Brenowitz EA (2010): Seasonal changes in androgen receptor mRNA in the brain of the white-crowned sparrow Gen.Comp.Endocrinol., 166, 66-71.
14. Gahr M, Sonnenschein E, Wickler W (1998): Sex difference in the size of the neural song control regions in a duetting songbird with similar song repertoire size of males and females J.Neurosci., 18, 1124-1131.
15. Grisham W, Arnold AP (1995): A direct comparison of the masculinizing effects of testosterone, androstenedione, estrogen, and progesterone on the development of the zebra finch song system J.Neurobiol., 26, 163-170.
16. Khurshid N, Agarwal V, Iyengar S (2009): Expression of mu- and delta-opioid receptors in song control regions of adult male zebra finches (Taenopygia guttata) J.Chem.Neuroanat., 37, 158-169.

17. Khurshid N, Jayaprakash N, Hameed LS, Mohanasundram S, Iyengar S (2010): Opioid modulation of song in male zebra finches (Taenopygia guttata) Behav.Brain.Res., 208, 359-370.
18. Leung CH, Goode CT, Young LJ, Maney DL (2009): Neural distribution of nonapeptid binding sites in two species of songbird J.Comp.Neurol., 513, 197-208.
19. Lynch KS, Diekamp B, Ball GF (2008): Catecholaminergic cell groups and vocal communication in male songbirds Physiol.Behav., 93, 870-876.
20. Maney DL, Goode CT, Lake JI, Lange HS, O'Brien S (2007): Rapid neuroendocrine response to auditory courtship signals Endocrinology, 148, 5614-5623.
21. Maney DL, Goode CT, Wingfield JC (1997): Intraventricular infusion of arginine vasotocin induces singing in a female songbird J.Neuroendocrinol., 9, 487-491.
22. Meitzen J, Moore IT, Lent K, Brenowitz EA, Perkel DJ (2007): Steroid hormones act transsynaptically within the forebrain to regulate neuronal phenotype and song stereotypy J.Neurosci., 27, 12045-12057.
23. Meitzen J,Thompson CK (2008): Season-like growth and regression of the avian song control system: neural and behavioral plasticity in adult male Gambel's white-crowned sparrows Gen. Comp.Endocrinol., 157, 259-265.
24. Newman AE, MacDougall-Shackleton SA, An YS, Kriengwatana B, Soma KK (2010): Corticosterone and dehydroepiandrosterone have opposing effects on adult neuroplasticity in the avian song control system J.Comp.Neurol., 518, 3662-3678.
25. Pinaud R, Fortes AF, Lovell P, Mello CV (2006): Calbindin-positive neurons reveal a sexual dimorphism within the songbird analogue of the mammalian auditory cortex J.Neurol., 66, 182-195.
26. Reiner A, Perkel DJ, Mello CV, Jarvis ED (2004): Songbirds and the revised avian brain nomenclature Ann.NY. Acad.Sci., 1016, 77-108.
27. Reiner A, Yamamoto K, Karten HJ (2005): Organization and evolution of the avian forebrain Anat. Rec. A Discov.Mol.Cell Evol.Biol., 287, 1080-1102.
28. Remage-Healey L, London SE, Schlinger BA (2010): Birdsong and the neural production of steroids J.Chem.Neuroanat., 39, 72-81.
29. Riters LV, Alger SJ (2004): Neuroanatomical evidence for indirect connections between the medial preoptic nucleus and the song control system: possible neural substrates for sexually motivated song Cell Tissue Res., 316, 35-44.
30. Sartor JJ, Ball GF (2005): Social supression of song is associated with a reduction in volume of a song-control nucleus in European starlings (Sturnus vulgaris) Behav.Neurosci., 119, 233-244.
31. Smith GT, Brenowitz EA, Beecher MD, Wingfield JC (1997): Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds J.Neurosci., 17, 6001-6010.
32. Soma KK, Wingfield JC (2001): Dehydroepiandrosterone in songbird plasma: seasonal regulation and relationship to territorial aggression Gen.Comp.Endocrinol., 123, 144-155.
33. Thompson CK, Bentley GE, Brenowitz EA (2007): Rapid seasonal-like regression of the adult avian song control system Proc.Natl.Acad.Sci.USA, 104, 15520-15525.
34. Tramontin AD, Hartman VN, Brenowitz EA (2000): Breeding conditions induce rapid and sequential growth in adult avian song control circuits: a model of seasonal plasticity in the brain J.Neurosci., 20, 854-861.
35. Tramontin AD, Wingfield JC, Brenowitz EA (1999): Contributions of social cues and photoperiod to seasonal plasticity in the avian song control system J.Neurosci., 19, 476-483.
36. White SA, Livingston FS, Mooney R (1999): Androgens modulate NMDA receptor-mediated EPSC sin the zebra finch song system J.Neurophysiol., 82, 2221-2234.
37. Wild JM, Krützfeldt NO, Kubke MF (2010): Connec-tions of the auditory brainstem in a songbird, Taeno-pygia guttata. III. Projections of the superior olive and lateral lemniscal nuclei J.Comp. Neurol., 518, 2149-2167.
38. Wissman AM, Brenowitz EA (2009): The role of neurotrophins in the seasonal-like growth of the avian song control system J.Neurosci., 29, 6461-6471.

5.4. The hypothalamic regulation of pituitary function

5.4.1. Gonadotropin-releasing hormone (GnRH, LH-RH)

The central nervous system regulates gonadal functions behaviour through the effect of decapeptide molecules formed in the hypothalamus on the adenohypophysis. Schally and his colleagues isolated luteinising hormone releasing factor (LH-RH, GnRH) from pig hypothalamus at the beginning of the 1970s, which effectively increases LH and FSH secretion in the pars distalis both in vitro and in vivo. Schally later received the medical Nobel price for this discovery. Several Hungarian researchers worked in Schally's laboratory, who were already studying the synthesis of GnRH analogues, and their biomedical and animal husbandry applications in Hungary, in the end of the 1970s.

They soon managed to synthesise mammalian LH-RH (a neurohormone with the composition of pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) and by applying changes to positions 5 and 7, they created superactive stimulating (agonist) and inhibiting (antagonist) analogues of the decapeptide. Mammalian LH-RH also efficiently increases the gonadotropin (mainly LH) release in other vertebrates, but in these animals soon other GnRH-like decapeptides were found, which were more active in the given species than applied mammalian GnRH. Therefore, considering their optimal effects, these particular GnRH isoforms (of which 15 are currently known) are species specific.

GnRH forms from a much larger pro-hormone, which consists of 69 amino acids along with an additional 23 amino acid-containing signal sequence (prehormonal element). The GnRH decapeptide is found after the signal sequence, i.e. it is the first 10 amino acids of the prohormone. The first 53 amino acids of the pre-prohormone is the gonadotropin-releasing hormone-associated peptide (GAP), which increases prolactin secretion. In spite of its pharmacological stimulating effect, it is not know if it is capable of releasing PRL under physiological conditions.

Two research groups were working practically simultaneously on the isolation of GnRH in birds. Both the Miyamoto-led Japanese team and the South African Millar and King started with over one hundred thousand chicken hypothalamus, and eventually in 1982 obtained 50 μg isolated decapeptide. **Chicken GnRH (cGnRH-I)** was found to differ from mammalian GnRH at the 8^{th} amino acid, which in chicken is glutamic acid, not arginine. Soon, the second decapeptide with GnRH activity was isolated from chicken hypothalamus – the LH releasing effect of cGnRH-II is about eight times stronger compared to cGnRH-I, and their structure differs at 5-His, 7-Trp and 8-Tyr. Both decapeptides have been produced synthetically by both research groups. Phylogenetically, cGnRH-II is a very old molecule, it is present not only in birds, but in the brain of all vertebrates.

According to earlier immunocytochemical studies, in birds most cGnRH-I is produced in the parvocellular neurosecretory cells of the preoptic area (POM), the neurons of which mostly lead to the anterior and posterior median eminence and end in precapillaries there, in the external zone. However, a high percentage of of cGnRH-I immunopositive cells shows a more disperse distribution, located from the ventral preoptic area to the septum and the bed nucleus of the stria terminalis (Millam et al., 1993, van Gils et al., 1993, Teruyama and Beck, 2000, Péczely and Kovács, 2000b,c). Recently, Kuenzel and Golden (2006) separated seven divisions of cGnRH-I neurons in the hypothalamo-septal area of domestic chicken: the paramedial-septal area, the periventricular preoptic nucleus/the hypothalamic periventricular nucleus, the bed nucleus of the pallial commissure, the parvocellular nuclei of the medial and lateral septum, the nucleus of the lateral septum (the cell population located under the inferior horn of the lateral ventricle, which is connected by axons to the choroid plexus), the parvocellular nuclei of the anterior thalamus, and a disperse group of thalamic cGnRH-I cells. A larger amount of cGNRH-I fibres can be found in six areas, the vascular organ of the lamina terminalus (OVLT), preoptic recess (POR), the hypothalamic (infundibular) recess (IR), the periventricular area of the lateral septum, near the choroid plexus, around the subseptal organ, and in the external zone of the median eminence. This hypophyseo-gonadal axis, mostly connected to the liquor, which has a septal or subpalliar neural centre, can also play an important role in the regulation of sexual function. Some cGnRH-I cells are also located in more distant extra-hypothalamic areas, such as the parolfactory lobe, the area of the accumbent nucleus and the lateral thalamus. Their axon endings (ring structures) can be found in the telencephalon, the hippocampus and the nucleus taenia (Sharp and Ciccone, 2004). (Figure 58, 59, 60, 61, 62, 63.)

GnRH neurons originate from the olfactory placode, and can be detected from incubation Day 3 in chicken embryo. They multiply by division and migrate along the axons of the olfactory nerve to the olfactory lobe, where they can be detected from Day 6 of incubation. The neuroblast-like GnRH cells continue to migrate to the preoptic area, and showing continuous division, appear on incubation Day 9 (Mulrenin et al., 1999).

cGnRH-I neurons receive both excitatory and inhibitory afferents. The amounts of these depend on their function: in photorefracter starlings following 11 months of exposure to increased photoperiod, the amount of axosomatic synapses is significantly higher compared to juvenile photosensitive, photogonado-stimulated individuals and during gonadal recession following 1-2 months of photostimulation (Parry and Goldsmith, 1993).

The rich **NPY** innervation is excitatory, which can mean a more general effect in the area of the soma, but precise in the external zone of the median eminence. Most axons of the NPY cells in the tuberal (arcuate) nucleus lead to the median eminence, where they can form synapses with the cGnRH-I axons.

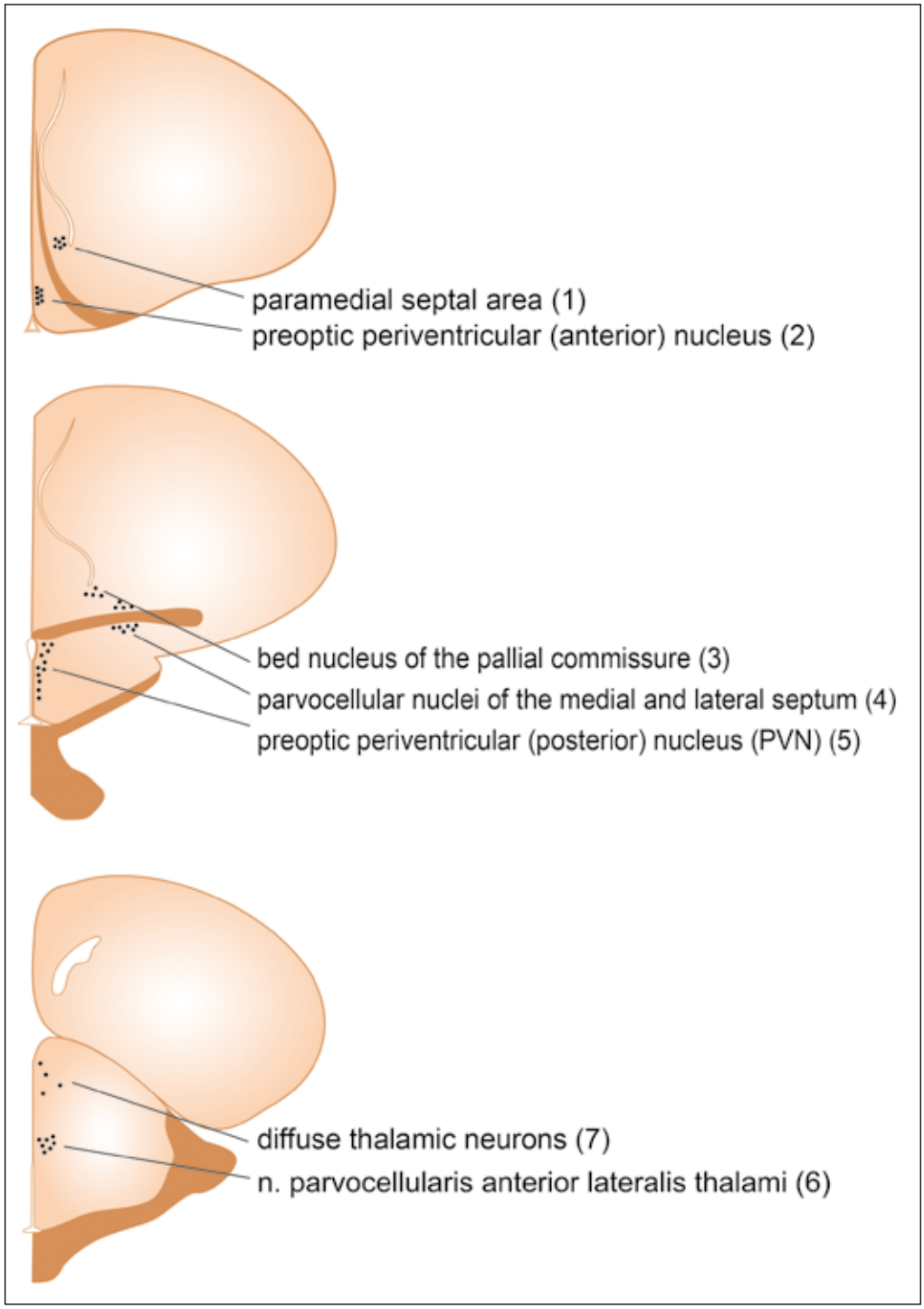

Figure 58. The location of GnRH-I neurons in domestic hen brain (modified after Kuenzel and Golden, 2006)

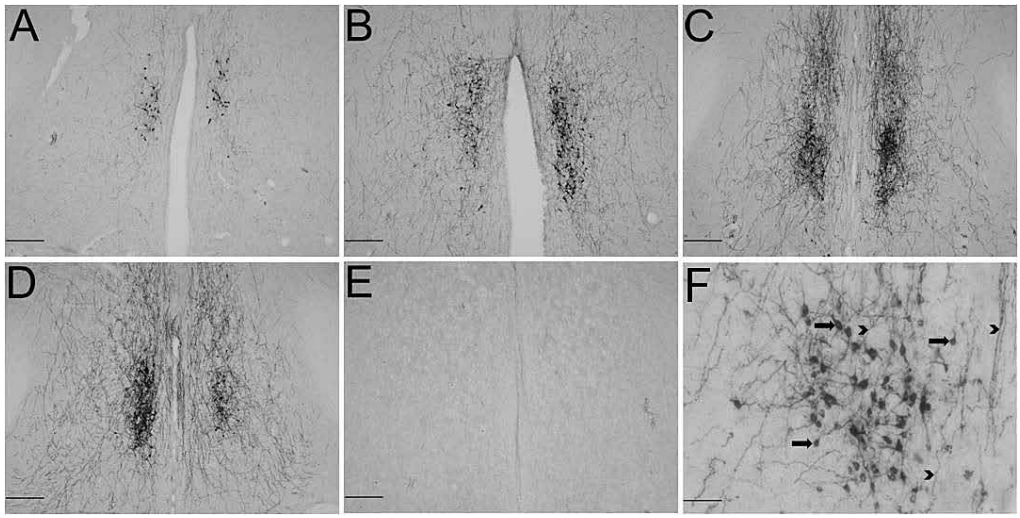

Figure 59. Seasonal changes in the GnRH-I neurons of the medial preoptic nucleus (POM) in male starling. A: beginning of March, B: beginning of April, C: end of April, D: beginning of May, E: end of August, F: close-up of GnRH-I neurons at the beginning of April (photos by Ottó Pintér)

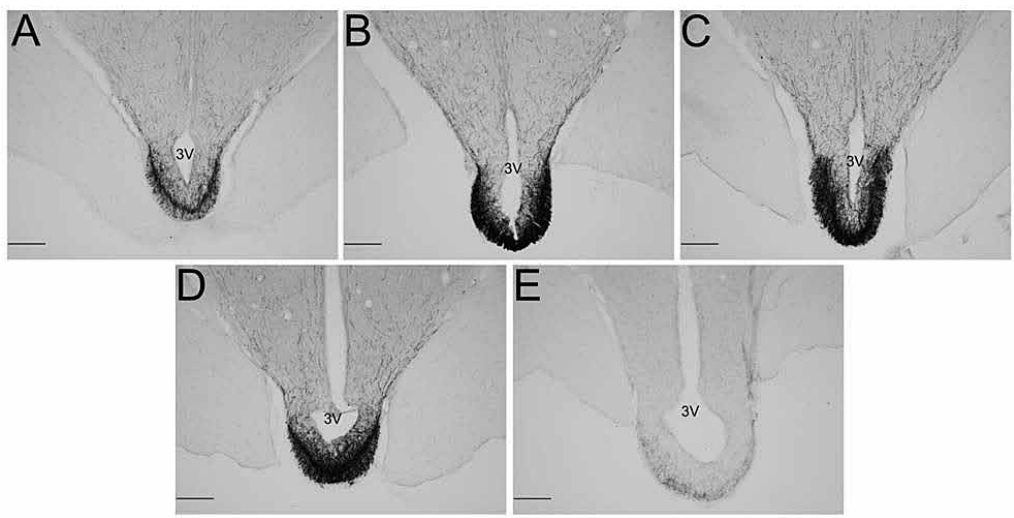

Figure 60. Seasonal changes in the GnRH-I axon endings of the anterior median eminence in male starling. A: beginning of March, B: beginning of April, C: end of April, D: beginning of May, E: end of August (photos by Ottó Pintér)

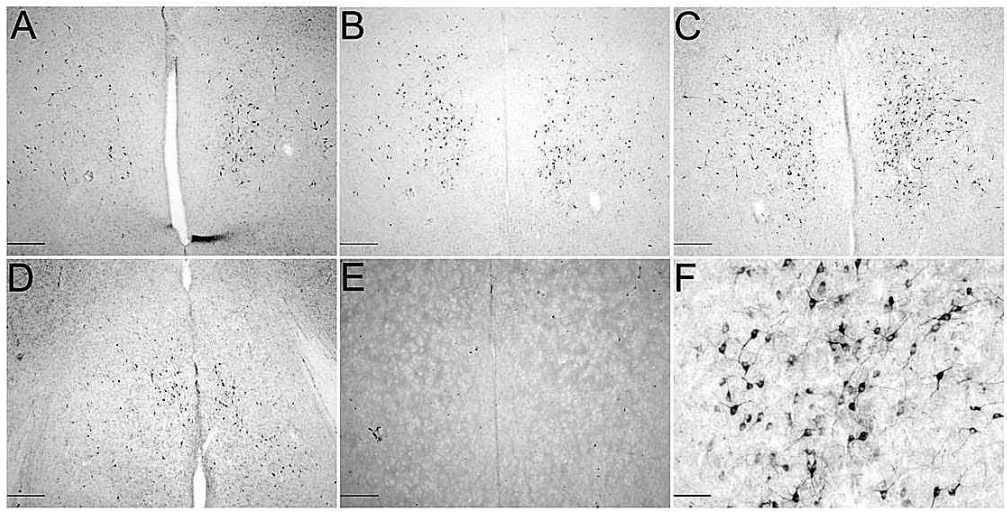

Figure 61. Seasonal changes in aromatase-immunopositive neurons of the medial preoptic nucleus (POM) in male starling. A: beginning of March, B: beginning of April, C: end of April, D: beginning of May, E: end of August, F: close-up of aromatase-immunopositive neurons at the beginning of April (photos by Ottó Pintér)

Figure 62. Anterior median eminence and GnRH-I axons in sexually active female mallard

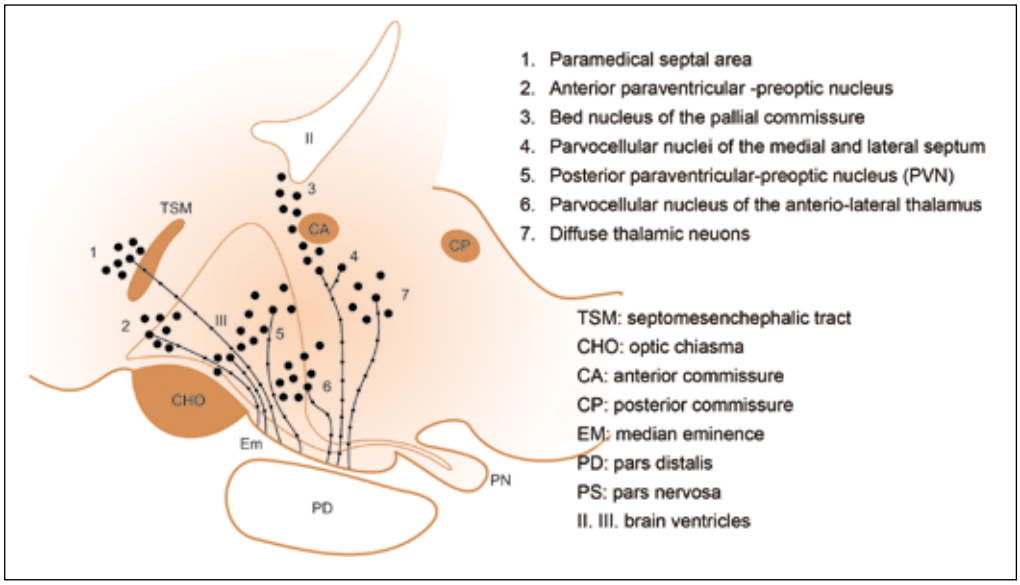

Figure 63. The location of GnRH-I soma and axons in mallard brain, sagittal section

The stimulating effect of NPY can play a role in triggering preovulatory LH release in hens, as the addition of NPY to incubated median eminence tissue in vitro does only increase cGnRH-I release during preovulation, 7 hours before the formation of the LH peak it does not have an effect (Contijoch et al., 1993).

The effect of **glutamate fibres** also seems to be more specific at the level of cGnRH-I somas. Glutamate neurons activate during photogonado-stimulation, and trigger cFOS early gene expression in the preoptic, paraventricular and the tuberal neurons. Simultaneously, significant LH release can be detected in the plasma. Hypothalamic cFOS cells are interneurons, which stimulate cGnRH-I neurons nearby, as cFOS expression cannot be detected in the nucleus itself of the cGnRH-I neuron (Péczely and Kovács, 1998, Meddle et al., 1999). The excitatory effect of glutamate neurons is confirmed by the strong LH release triggered by N-methyl-D-aspartate, a glutamate antagonist (Deviche et al., 2006). Glutamate stimulation is age dependent, but so far its puberty-related appearance has only been studied in mammals. These studies suggest that there is a functional relationship between **GABA** and the glutamate system. According to electrophysiological studies, the activation of GABA receptors precedes the appearance of glutamate signals. In juveniles, the depolarisation of GABA receptors causes GnRH neurons to enter into a hyperpolarised (inhibited) state. This state changes during puberty, when the activation of excitatory glutamate receptors depolarises the membrane of GnRH receptors, causing their activation (Clarkson and Herbison, 2006).

The rich axon mass of the hypothalamus and the relative few somas suggest that in birds γ-aminobutyric acid can also be an important extrahypothalamic

regulating factor of GnRH production. According to studies on mammals, GnRH neurons contain two types of GABA receptors: GABA-A increases (by depolarising) GnRH neuron activity through the influx of Cl^- and the increased functioning of Ca^{2+} channels, while GABA-B hyperpolarises GnRH cell membrane, causing an inhibitory effect by increasing K^+ influx. These GABA-B receptors play a fundamental role in the negative feed-back loop of estrogen (Zhang et al., 2009, Watanabe et al., 2009). In birds, the mechanism of the – probable – facilitatory and inhibitory effect of GABA is not yet known.

Dopamine is also an important excitatory regulating factor of GnRH production. According to early in vitro studies, the noradrenergic, adrenergic and β-adrenergic receptor agonist isoproterenol effectively increased GnRH release in Japanese quail hypothalamus (plus median eminence) superfusion system (Millam et al., 1984). In female turkeys, intracerebroventricular infusion of dopamine results in simultaneous release of LH and PRL, which confirms earlier assumptions about dopamine increasing the production of these two neuropeptides. 30 minutes of electrical stimulation of POM increased plasma LH and PRL levels, and at the same time caused cFOS expression in all cGnRH-I neurons the VIP neurons of the infundibular nucleus of the opposite site. Electrical stimulation also caused cFOS expression in the dopamine-containing neurons of the lateral mammillary nucleus, but dopamine neurons of other areas did not react to the treatment. This study was the first to suggest that dopamine neurons of the mammillary region regulate cGnRH-I production of the POM and VIP production of the infundibulum in an excitatory way, on the level of somas (synthesis) (Al-Zailaie et al., 2006). Immunocytochemical studies found axo-axonic synapses between the axons of the infundibular dopamine neurons projecting into the median eminence and the large amount of cGnRH-I axons found there, and hypothesised their effect on dopamine cGnRH release in the regulation of ovulatory cycle of domestinc chicken. In fasted animals, the lack of ovulation is related to decreased dopamine turnover in the hypothalamus, which decreases the GnRH content and release in the median eminence (Contijoch et al., 1992). During puberty, the immunoactivity of BnST and cGnRH-I cells of PVN, the increased number of thyrosine hydroxylase-containing neurons in the intermedial nucleus, as well as the higher amount of thyrosine hydroxylase axons in the external zone of the median eminence suggest that dopamine plays an important role in the increasing GnRH syntheses and release (Fraley and Kuenzel, 1993). The role of premammillary dopaminergic cells in photogonado-stimulation is supported by the finding that increasing daylight triggered intensive cFOS expression in female turkeys and simultaneously caused an increase in the number of cGnRH-I cells in the POM, as well as larger amount of mRNA in the neurons that produce releasing hormone (El Halawani et al., 2009).

The stimulating effect of **noradrenaline** on GnRH production seems indirect in birds. According to in vitro studies, adding noradrenaline to the incubatory medium inhibits the GnRH-degrading effect of Tyr5-Gly6 endopeptidase and the post-proline slicing effect of certain enzymes, and therefore increases GnRH release from isolated median eminence tissue (Contijoch et al., 1990).

β-endorphin is an inhibitory afferent element, which inhibits LH release on the level of the hypothalamus. Opioid peptide administered to the third brain ventricle inhibits plasma LH level both in the progesterone-stimulated preovulatory phase and the interovulatory low-secretion phase. cGnRH-I neurons receive pro-opiomelanocortin-derivate β-endorphin and enkephaline afferents on both the soma and the axonal levels. Generally, these have inhibitory effects that play a role in steroid feedback mechanisms. In domestic hen, β-endorphin neurons are located in the periarcuate area, and their axons project to the preoptic area (POA) and the external zone of the median eminence. The cGnRH-I content of POA increased four hours before ovulation and remained at this high level until ovulation. On the other hand, β-endorphin content of POA was minimal in during this period, which indicates a temporary ceasing of the β-endorphin inhibitory effect (Contijoch et al., 1993a,b).

Enkephalinergic neurons are located in the PVN area of the hypothalamus, and their immunoreactive axons form synapse-like appositions with cGnRH-I cells and axons around the Broca diagonal tract, in the area of the bed nucleus of the pallial commissure, and in the lateral septal nucleus. These are presumably inhibitory afferents (Millam et al., 2002).

Serotonine (5HT) inhibits LH secretion indirectly, not through the adenohypophysis, as serotonine receptors are lacking there, but by inhibiting GnRH secretion in the hypothalamus (Hall et al., 1986, MacNamee and Sharp, 1989). Serotonine precursor (5-hydroxytryptophan) inhibits preovulatory LH release, stops ovulation and in the frontal hypothalamus its turnover decreases during the preovulatory LH maximum. 5HT turnover and amount decrease in the median eminence four hours before the LH preovulatory peak, and 5HT receptors of the frontal hypothalamus cannot be detected in brooding hens. Therefore, preovulatory LH release can also be related to the temporal ceasing of 5HT inhibition (Sharp et al., 1984, 1989a, 1989b). Immunocytochemical localisational studies also suggest inhibitory effects of 5HT on GnRH. In Japanese quail kept under short daylight cycle, the amount of serotonin-immunoreactive neurons is significantly higher during the dark phase compared to the light phase. Increased photoperiod decreases the amount of 5HT cells, and does not show difference between light and dark periods (Haida et al., 2004).

Nitric oxide (NO), as a fast acting trigger molecule, can play a role in activating the serotonine effect. In Japanese quail, NO seems to be an effective gonadostimulator, and its activator role in the regulation of GnRH secretion has been confirmed in mammals. NO, or the administration of sodium-nitroprusside,

a nitric oxide-donor compound, imitate the effect of increased photoperiod (photo-gonado stimulation), while N-nitro-L-arginine methylester, a nitrite oxide synthetase inhibitor, imitates the effects of short day light conditions (Chaturvedi and Kumar, 2007, Kumar and Chaturvedi, 2008).

Galanin seems to have an inhibitory effect in GnRH afferentation. Galanin-immunopositive cells are located in the area between the paraventricular ependymal organ and the infundibular nucleus. In the external zone of the median eminence their axons get in close contact with the GnRH axons and in the paraventricular area with serotoninergic neurons. The neurons are photosensitive, their amount is substantially higher during short-day light conditions compared to increased photoperiod, but do not show differences between light and dark periods. Galanin neurons can affect GnRH secretion in two ways: directly through the median eminence and indirectly through the paraventricular ependymal organ and the serotonin neurons of the infundibular nucleus (Haida et al., 2004).

The antagonistic relationship between the pituitary gonadotropic system and **prolactin** has been known for a long time. Prolactin, and its hypothalamic regulating factors can inhibit the functioning of the pituitary-gonadal system on several levels. Immunocytochemical studies of the hypothalamus and septal area of Japanese quail kept on decreased and increased photoperiod show a connection between cGnRH-I and VIP neurons. There are typical liquor-contact VIP neurons in the ependymal organ of the lateral septum, which are closely connected to cGnRH-I cells and their axons. This is especially pronounced in the case of the most caudal cGnRH-I cells of the septal area, with which the VIP axon endings form axo-somatic and axo-dentritic contact. The synapses of VIP axons and GnRH neurons are simple in structure, without specialisation. In the external zone of the median eminence, cGnRH-I and VIP axons run parallel, close to each other, with likely axo-axonic synapses between them. Therefore, cGnRH-I neurons presumably receive VIP neuro-modulator impulses at the level of the median eminence, which would provide morphological base to the direct inhibitory effect of VIP to cGnRH-I. On the other hand, VIP neurons of the infundibular area do not show contact with the (few) cGnRH-I cells located here, or the axonic system of cGnRH. This suggests that there are morpho-functional differences between the two areas. These VIP areas also react differently to changes in photoperiod: in male quails the number of VIP immunoreactive cells of the infundibular nucleus increases as a result of long day photoperiod, while the VIP neurons of the lateral septum and the accumbent nucleus remain unaffected by increasing photoperiod (Kiyoshi et al., 1998, Teruyama and Beck, 2001).

Kisspeptin represents an unique regulatory system of GnRH production. KISS1 protein is a metastasis suppressor, and also plays a determining role in the initiation of puberty. It contains 145 amino acids and its sequence differs

from other known proteins. Proteolytic treatment slices it into several derivates, among which metastin (KP-54) and kisspeptin-10 are the best known. Its amide carboxy terminal makes specific receptor binding possible. KP-54 is a modulator of GnRH secretion, which can act directly, through GPR54 receptors bound to the G protein, which express in GnRH cells. Alternatively, KP-54 also stimulates GABA and glutamate receptors, which transmit the stimulating effect of estrogen to GnRH neurons. In male duck, administering kisspeptine into the cerebral ventricule increases plasma LH level, which can be prevented by pretreatment with the GnRH antagonist acyline. Its endogen occurrence is supported by the presence of kisspeptin-immunopositive neurons in the POM area, and kisspeptin axes in several areas of the brain. All kisspeptin-containing cells also have aromatase, which suggests autocrine regulation of kisspeptin expression by estrogens. However, kisspeptin and cGnRH-I are never found colocalised in a neuron, which suggests that in the area of POM, kisspeptin modulates GnRH axo-somatically (Saldanha et al., 2010). (Figure 64, 65, 66.)

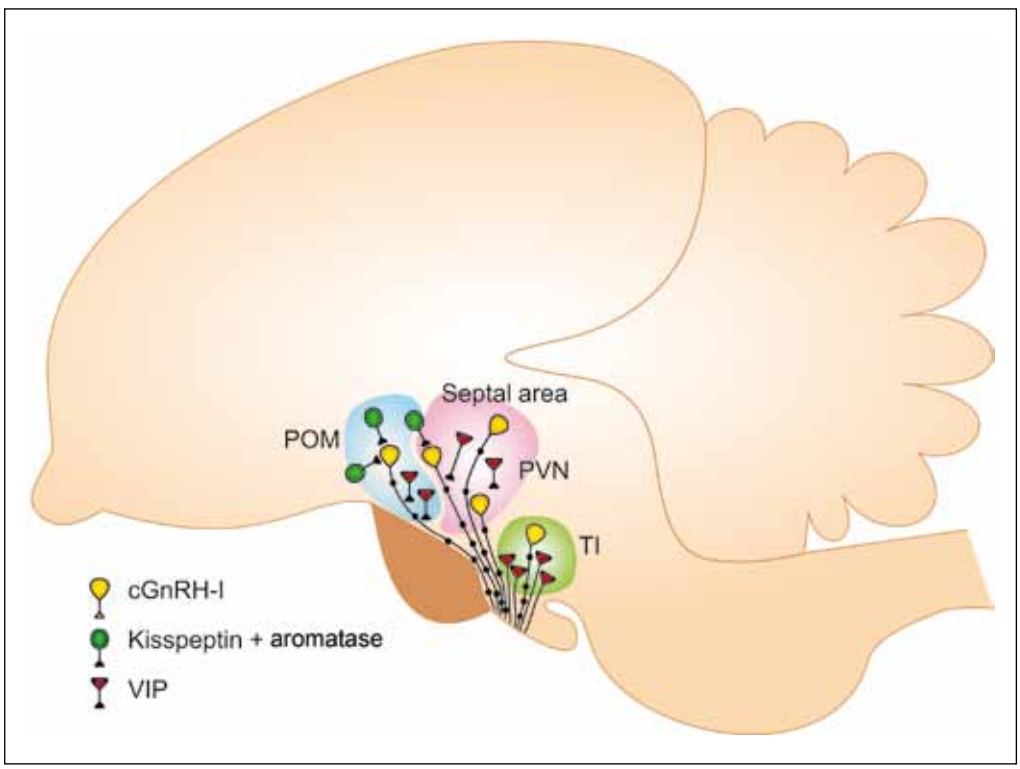

Figure 64. The connections between hypothalamic GnRH-I neurons with VIP and kisspeptin (aromatase) neurons in the avian brain

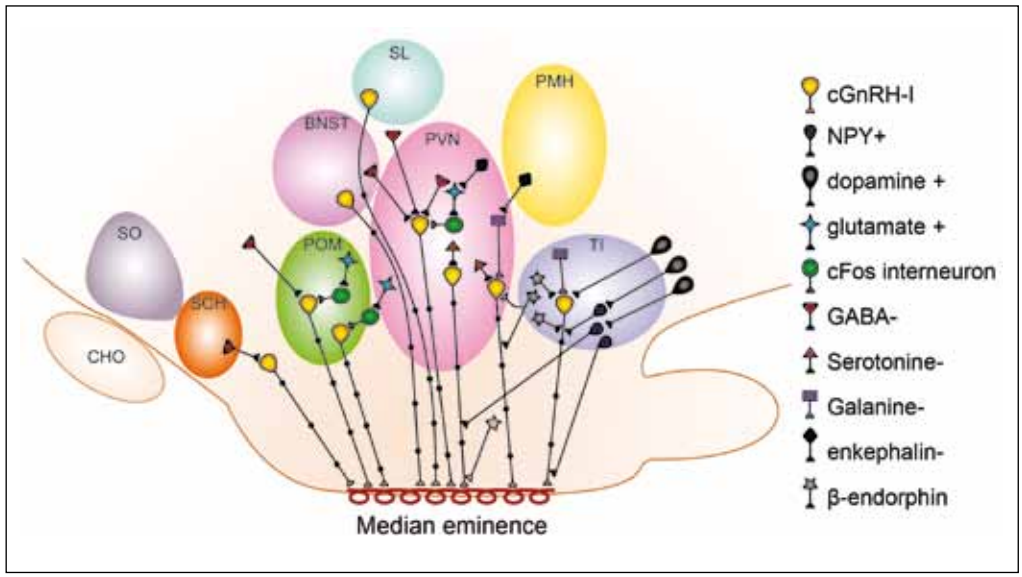

Figure 65. Stimulatory and inhibitory systems of GnRH-I neurons in the avian brain

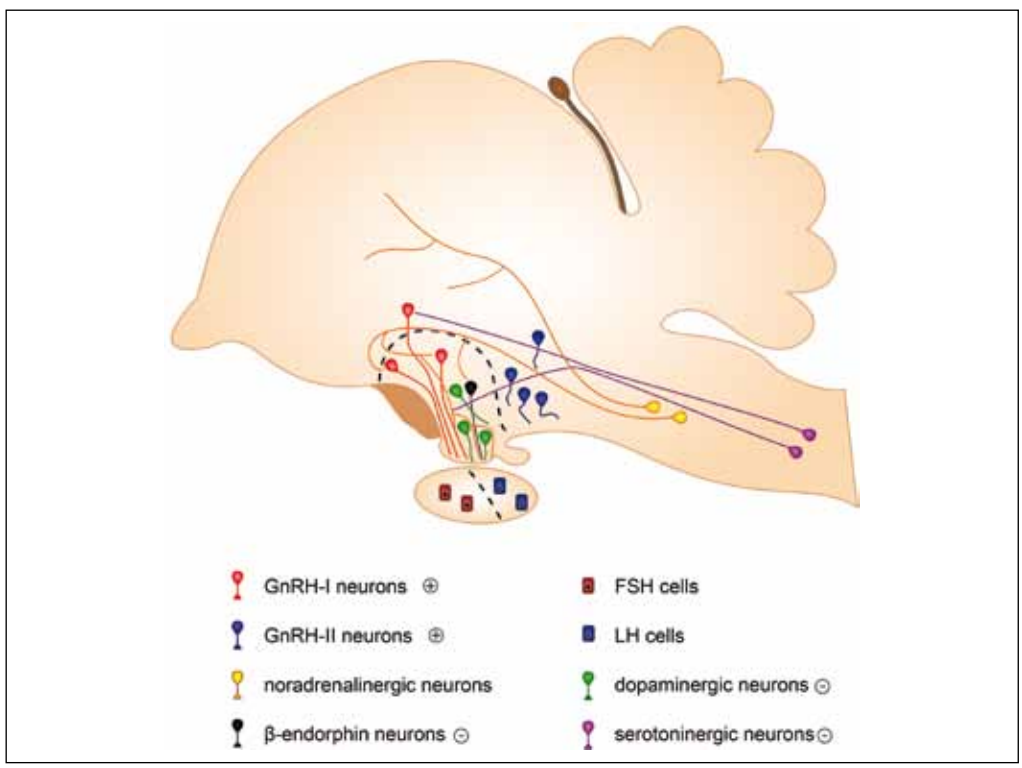

Figure 66. The location of biogenic amine and β-endorphin neurons that regulate pituitary functions in the avian brain

Androgens and estrogens affect cGnRH-I synthesis through negative feedback: castrating roosters increases the amount of GnRH mRNA and peptide in the hypothalamus, while estrogen treatment of castrated individuals supresses the increase of releasing hormone. The negative feedback effect also acts on the level of adenohypophysis: in hypophyseal cell culture the administration of estrogen, testosterone and progesterone inhibits increased LH-release caused by cGnRH-I. There is a peculiar relationship between progesterone and its effect on GnRH: when the effect of progesterone triggers preovulatory LH-release, hypothalamic GnRH release increases and the GnRH-peptide content of the basal hypothalamus decreases, while at the same time the amount of GnRH mRNA does not increase, which means that the fast release from the median eminence is not accompanied by an immediate increase in synthesis in the somas. The LH preovulatory peak therefore does not depend on the increasing GnRH synthesis (Sharp and Ciccone, 2004). The number of cGnRH-I cells is also influenced by social factors, which act through sexual steroids: a higher amount of cGnRH-I immunpositive cells can be detected in the hypothalamus of male starling housed together with females compared to the ones kept separately. In this case, the higher testosterone level of males subject to social impulses can be the direct trigger of the effect (Stevenson and Ball, 2009).

The releasing effect of cGnRH on pituitary cells occurs through specific **cell membran receptors.** The cGnRH receptor is a heptahelical, G protein-linked structure, which contains a long (generally 350 amino acids) extracellular N-terminal ectodomain, a transmembrane helix containing seven hydrophobic elements, and an intracellular C terminal linked to the last intracellular loop (this C terminal is lacking in mammalian GnRH). The transmembrane helices contain hydrophilic extra-, and intracellular loops. Typically, the receptor has extracellular N-linked glycosylation sites and disulphide bridges between the ectodomain and the second extracellular loop and between the first and second extracellular loops. Also, phosphorylated amino acids (generally five) in the intracellular loops are characteristic.

mRNA-s of two cGnRH receptors have been detected in chicken adenohypophysis, and their expression is regulated by estrogen and by GnRH itself. One of them is cGnRH-R-I, which specifically binds cGnRH-I, but shows an even higher binding affinity and inositol phosphate accumulation towards cGnRH-R-II. The other receptor type is cGnRH-R-III, which shows similar binding parameters, but the amount of its mRNA is 1400 times higher in the adenohypophysis. Additionally, its internalisation rate is also higher compared to cGnRH-R-I. While cGnRH-R-I expresses in equal amounts in male and female birds, the occurrence of cGnRH-R-III shows sexual dimorphism, its amount is higher in mature hens compared to roosters, and in juveniles the pattern is the opposite, males have higher concentrations. This R-III type is assumed to be the dominant mediator of the GnRH effect in hypophyseal LH cells (Sun et al.,

2001, Joseph et al., 2009). Besides the adenohypophysis, cGnRH receptors also occur in the GnRH cell region of the hypothalamus (POA and MBH) and in the cerebral cortex. The presence of hypothalamic receptors suggest paracrine regulation by the GnRH cells, while cortex receptors raise the possibility of direct behavioural regulation by GnRH (Sharp and Ciccone, 2004).

The molecular mechanism of **GnRH action** in pituitary cells is only partly known even in mammals. The "classical", gene-expression triggering pathway means that GnRH binding to its specific receptor starts a singal-transduction chain reaction. In the first step, GTP-ase-activating protein (GAP) mRNA production initiates by G-protein signal-2 (RGS2) stimulation, which results in the activation of the G-α-q/G-α-11 membrane-bound protein. This stimulates phospholipase-C-β enzyme, which, causes the phosphorylation of RGS2 through a feedback loop. RGS2 phosphorylation (resetting mechanism) makes it possible for the pulsating effect of another GnRH-GnRH receptor complex to stimulate GAP. Phospholipase-C-β enzyme also increases intracellular Ca^{2+} concentration. This occurs in two steps: first, stimulating endoplasmic reticulum-bound IP3 receptors it releases Ca^{2+} from the intracellular pool, and then by opening non-L-type and L-type Ca^{2+} channels in the LH cells, it causes the influx of extracellular calcium. By stimulating protein kinase-C (PKC), the sudden surge in calcium concentration initiates a cascade mechanism: the mitogen-activated protein kinase (MAPK) phosphorylates cytosol and nuclear proteins through activating extracellular signal-regulated kinase (ERK). As a result of this, gene transcription of gonadotrop subunits and further GnRH receptors begins. The following translational mechanism, through the activation of the Golgi complex, leads to the production and excretion of a large amount of LH/FSH secretory granules (Davidson et al., 1987, Liu et al., 1995, Heather et al., 2006, Naor, 2009). Other studies found that the GnRH-stimulated early growth factor (EGR-1) also has an important role in the expression of gonadotropin subunits. This, bound to the steroidogenetic factor-1 (SF-1) and to the paired-like homeodomain transcription factor-1 (PlTX-1) directly binds to the promoter of the LH-β gene. The three factors together increase LH-β transcription (Fortin et al., 2009).

Similarly based on mammalian studies, the effect of GnRH on pituitary hormones is also known to be affected by other factors. For instance, GnRH stimulates the expression of annexin-A5, a calcium-sensitive phospholipid-binding protein, and this protein contributes to the stimulation of gonadotropin synthesis (Kawaminami et al., 2008). The effect of the insuline-like growth factor-1 (IGF-1) is similar, enhancing the GnRH effect (Yang et al., 2005). The high-voltage-activated L-type Ca^{2+} ion channels are directly stimulated by leptin, increasing the influx of calcium, which also increases the GnRH effect. Nevertheless, leptin alone does not affect pituitary cells, and does not result in LH gene transcription (Avelino-Cruz et al., 2009).

At present, the effects of sexual steroids on GnRH-stimulated gonadotropin secretion are little known even in mammals. GnRH, bound to its receptor, activates the estrogen-response element (ERE) of the LH-β gene, and also stimulates phosphorylation of the two serine molecules in the estrogen-estrogen receptor-α (E-ER) complex. This double effect assists the binding of the E-ER complex to the ERE, and stimulates the formation of the cAMP response element-binding protein-associated factor (PCAF). GnRH also increases early gene expression of c-fos-B, which, along with PCAF, stimulates LH-β gene expression (Chen et al., 2009). In birds, the molecular actions of GnRH are not know, but they are probably similar to those in mammals.

Gonadotropin release regulated by classic genomic processes of course does not explain the rapid trop-hormone release that occurs within hours as the effect of GnRH. In mammals, only GnRH is known to trigger early gene c-Fos expression in pituitary cells, which results in immediate FSH expulsion and FSH-β gene expression. GnRH leads to the activation of calmodulin-linked kinase-II (CamKII), and through this to the phosphorylation of serum response factor (SRF), which in turn results in c-Fos induction. On the other hand, GnRH also leads to the initiation of a rapid mechanism, where ELK-1 is phosphorylated, and then, linked to SRF, enhances its stimulating effect on c-Fos (Ely et al., 2011).

Early gene effects in the GnRH cells are little known in birds. A single day exposure of increased photoperiod has been shown to substantially increase plasma LH levels in Japanese quail and white-crowned sparrow. This single day of photostimulation has significantly increased the synthesis of c-fos and efr-1 proteins in the soma of the GnRH neurons located in the septo-preoptic area of the hypothalamus in white-crowned sparrow. Early gene expression occurred in relatively few GnRH cells, which were located in the MPO. This early gene expression showed sexual dimorphism, being more pronounced in males (Saab et al., 2010).

According to mammalian studies, portal circulation carries GnRH into the adenohypophysis in pulses, and the frequency of this pulse is mostly responsible for the release. Experimental data shows that increasing the frequency of GnRH doses increases the LH releasing effect of the peptide, while decreasing (lowering) the frequency results in the relative increase of FSH release. The discovery that increasing the frequency of GnRH doses past a threshold or a continuous infusion of the peptide initially only increases the synthesis of the common α subunit, and later inhibits the release of both gonadotropin hormones was important for pharmacological reasons.

Considering the molecular mechanism of the GnRH effect, it has been shown in mammals that the amount of synthesised β fragments is closely related to the pulse frequency of GnRH, as well as to the expression of GnRH-stimulated hypophysis adenylate cyclase-activating polypeptide (PACAP) and

its receptor. Lower GnRH pulse frequency resulted in more intensive PACAP gene expression and increased FSH-β synthesis (Purwana et al., 2010). Following the GnRH-GnRH receptor binding, the expression of LH-β, FSH-β and the common α subunit genes is regulated by four, mitogen-activated protein kinases (MAPK): by different combinations of the extracellular signal-regulated kinase (ERK1/2), c-jun-NH_2 terminal kinase (JNK), p38MAPK and ERK5. ERK5 increases the sensitivity of the FSH-β promoter for low-frequency GnRH secretion, thereby increasing FSH synthesis (Lim et al., 2009).

The **episodic (pulsatile) character of the secretion** of both pituitary gonadotropin hormones has also been detected in domestic fowl. By obtaining samples from roosters every 10 minutes using cannulation, the frequency and amplitude of LH pulses were higher than those of FSH. The coocurrence of FSH and LH pulses is <32%, which shows the high independence in the secretion of the two gonadotropin hormones. In roosters with lower testicular mass, lower testosterone and LH levels at the 6.7 ng/ml FSH group no FSH pulses were detectable, while in the group with larger testicular mass at 10.5 ng/ml FSH four FSH pulses were observed during the 12 hour study period. These observations suggest a correlation between pulse and the amount of LH and FSH secreted. In roosters with testes above 10 g, the statistical correlation between plasma LH and testosterone pulses was 83%, while there was no correlation between FSH and testosterone pulses. Therefore, the regulating factor of testosterone secretion is primarily LH (Vizcarra et al., 2004).

In vitro studies on LH and FSH cells isolated from laying hens, while confirming pulsatile regulation, led to contrasting results. High and medium cGnRH-I pulse frequency (15–30 minutes) increased FSH release in a dose-independent way, while low cGnRH-I pulse frequency (60 minutes) increased LH release (Sonez et al., 2010).

GnRH stimulates LH release and also the synthesis of this gonadotropin, as it increases the amount of mRNA of both LH-β and LH-α in hypophyseal cell cultures. However, the amount of the two gonadotropin subunits does not increase in equal proportions, which suggests the the dose and pulse frequency of GnRH affect the regulation of the synthesis of the subunits differently.

The role of cGnRH in the regulation of FSH secretion is not obvious in birds. Earlier studies show that similar to mammals, cGnRH-I is the releasing hormone of both LH and FSH. Hattori et al., (1985, 1986) found that cGnRH-I and cGnRH-II increase Japanese quail adenohypophysis LH and FSH release both in vitro and vivo, by a rate similar to mammalian GnRH. Millar et al., (1986) studied the FSH releasing effect of cGnRH-I and cGnRH-II in chicken adenohypophysis incubated in vitro. Bruggeman et al., (1998) could only detect the FSH releasing effect of cGnRH-I in vivo in ovariectomised chickens, the same treatment was not effective in intact birds. However, another study found that cGnRH-I treatment of photostimulated juvenile chickens did not increase

plasma FSH level (Dunn et al., 2003). According to the results of a more recent in vivo experiment, during intravenous administration of GnRH to 17-week old prepubertal chickens the coadministration of cGnRH-I and cGnRH-II increased plasma LH level more than cGnRH-I alone. In this study, neither cGnRH-I nor cGnRH-II increased plasma FSH concentration. Following intravenous administration of cGnRH-I, cGnRH-II and lGnRH-III to mature roosters, cGnRH-II increased plasma LH level more efficiently than cGnRH-I, but neither of these peptides increased FSH concentration. A very high dose of cGnRH-II resulted in a slight increase in FSH level, but lGnRH-III had no effect at all. These results suggest that in birds FSH secretion is regulated independently from the cGnRHs and lGnRH (Proudman et al., 2006).

The results from a homologous chicken RIA method for FSH, suggest that the FSH-releasing effect of cGnRH-I is insignificant. This method was developed in 1993 and it overwrites the results of the earlier heterologous FSH RIA, which was developed by Follett in 1976 and was used for about 20 years (Krishnan et al., 1993). At the same time, even though cGnRH-I does not act directly, it indirectly increases the amount of subunit β in the pituitary cells, through stimulating the synthesis of FSH-β subunit mRNA. After some time, the increased amount of subunit β forms heterodimers with subunit α, and initiates (increases) FSH synthesis, which leads to the spontaneous release of the produced hormone (Hattori et al., 1986). At the moment, it seems probable that the regulation of FSH production is (also) largely independent of LH in birds, and the GnRH-hypophyseal activin/follistatin system known in mammals can play a role in this process (Sharp and Ciccone, 2004).

In birds, the stimulating effect of GnRH on LH release depends on the age and the physiological state of the individual. In juvenile chickens the stimulating effect is lower than in mature hens, and the LH response also decreases after the egg laying cycle, presumably as a result of a negative feedback caused by the increased steroid hormone concentration in the plasma. During gonadal regression the GnRH sensitivity of LH production is a function of the physiological state. If gonadal regression occurs in photosensitive birds as a result of brooding, the response reaction to GnRH increases, as the steroid negative feedback decreases. However, if the birds are in a photorefractory state, the gonadal regression occurs along with a decrease in LH response.

Increasing day length regulates GnRH neuronal secretion directly and indirectly (Péczely and Kovács, 2000a,b,c). Encephalic photoreceptors and the retino-hypothalamic tract that originates in the retina play an important role in the photogonado-stimulating mechanism. Axons of the opsin-containing encephalic photoreceptor cells in the lateral septum form axo-dentritic synapses on the GnRH neurons of the septum and the POA, and the opsin-positive axons that run radially in the median eminence and the GnRH axons are also in tight contact. These morphological characteristics suggest direct photostimulatory

regulation of GnRH secretion, which occurs both via axo-somatic and axo-axonic contact (Saldanha et al., 2001). In another hypothalamic region, the premammillary area, cells show dopamine-melatonin colocalisation, with the expression of clock genes triggered by photo-impulses. The activation of dopamine-melatonin neurons is signalled by cFoS expression. These premammillary neurons form another encephalic photoreceptor system, which also transmits direct photostimulatory effects towards the cGnRH-I system through dopamine impulses (El Halawani et al., 2009). Presumably, the retino-hypothalamic tract, which ends in the suprachiasmatic nucleus, can also affect the function of cGnRH-I cells indirectly, through the interneurons located here. Therefore, indirect photostimulatory regulation of cGnRH-I secretion becomes possible through the suprachiasmatic nucleus (Péczely, unpublished data).

Increasing GnRH production plays an important role in the initiation of puberty. Compared to free living birds, where sexual maturation is primarily determined by photostimulation, as a result of conditions of husbandry (including artificial light regimes) poultry breeds experience different effects that influence hypothalamic regulation. In domestic roosters living in closed enclosures, at the beginning of puberty the cGnRH-I content of the hypothalamus, its release and the plasma LH level increase does not coincide with an increase in GnRH mRNA, and mRNA level even decreases as a result of testosterone negative feedback. In this case, the non-photoperiodically regulated LH release that initiates puberty is not accompanied by an immediate increase in GnRH synthesis. This is also supported by the observation that the number of pro-cGnRH-I immunpositive neurons does not increase, which suggests that GnRH peptide release and its degradation that later becomes dominant play a key role in the regulation of puberty (Sharp and Ciccone, 2005).

In photosensitive birds puberty is initiated as a result of long day light conditions (photostimulation). In Japanese quails changing the light conditions from 8L to 20L caused increased plasma LH level within 20-26 hours (suggesting increased GnRH release) and increased cGnRH-I mRNA (increased GnRH synthesis). Photostimulation continuously increases both release and synthesis, causing a constantly high plasma LH level and hypothalamic cGnRH-I mRNA concentration (Perrera and Follett, 1992, Dunn and Sharp 1999).

Photoperiodic stimulation increases the amount of immunopositive cGnRH-I in the neurons of the hypothalamo-septal area, and also increases the amount of cGnRH-I cells, which suggests a simultaneous increase in mRNA synthesis. Studies on male starlings found that as a result of photostimulation, the amount of cGnRH-I mRNA increased mostly in the medial and lateral nuclei of the central hypothalamus, as well as in the neurons of the medio-caudal hypothalamus, suggesting that certain cGnRH-I neurons have different photosensitivity (Stevenson et al., 2009). Similar results were found in female turkey, where following photostimulation cGnRH-I mRNA expression was most

pronounced in the pallial commissure nucleus around the OVLT, and was lowest in the lateral septal nucleus, the cortico-habenular – cortico-septal area and in the POM. Studies on photostimulated female turkey showed that in laying hens the cGnRH-I mRNA content was higher than in non-photostimulated brooding birds and photorefracter individuals showed the lowest levels (Kang et al., 2006). Short (one day) photostimulation results in early gene expression in some cGnRH-I neurons and causes the appearance of cFOS and EGR-1 protein in the POM area by the following morning. Therefore, acting through early gene expression, photostimulation can trigger an effect within hours. The appearance of early genes was more intensive in male white-crowned sparrows than in females (Saab et al., 2010).

Photorefractoriness that closes the breeding cycle of photosensitive birds occurs along with a substantial decrease in hypothalamic cGnRH content and plasma LH level, which lead to gonadal atrophy. At the beginning of the photorefractory stage the negative feedback effect of steroids, the consistently high prolactin level and the continuous degradation of the GnRH peptide cause a drastic drop on in releasing factor outflow. The decrease of the mRNA content of the neurons does not start yet, suggesting that the inhibition of cGnRH-I gene expression occurs later after the inhibition of the release. Recent studies on white-crowned sparrow and mallard confirm this statement, showing that the first step in the onset of photorefractoriness is the decrease of cGnRH-I release (Péczely and Kovács, 2000a, Sharp et al., 1998, Dawson et al., 2002, Meddle et al., 2006).

cGnRH-II neurons are located in the caudal basal hypothalamus, the parvocellular elements of the lateral hypothalamus and in the magnocellular area of the oculomotor complex of the mesencephalon. The location of the cGnRH-II fibres is not obvious, some studies have described only a very small amount in the external zone of the median eminence (Millam et al, 1998), while according to others there is a substantial amount of immunopositive cGnRH-II axons here (Clerens et al., 2003). On the other hand, cGnRH-II fibres are found across the entire hypothalamus, hippocampus and septum, the habenular area, the hyperstriatum, the pyriform cortex and the nucleus taenia. In Japanese quail, cGnRH-II axons are found in the area of the POA and the lateral septum, and in quail as well as in some songbirds they are also located in the median eminence (Teruyama and Beck, 2001, Sharp and Ciccone, 2005, Stevenson et al., 2007). The uncertain immunolocalisation of cGnRH-II possibly orginates from two different cGnRH-II antisera, which were developed in two different laboratories by Millam et al., and Vandesande et al., respectively.

Studies also disagree with regard to the function of cGnRH-II. Active immunisation of laying hens for cGnRH-I caused ovarian regression, but this effect did not occur after active immunisation for cGnRH-II. During puberty, the amount of cGnRH-I increases in the hypothalamus, while cGnRH-II remains

stable. Castration increases the amount of hypothalamic cGnRH-I in roosters and it decreases following estrogen treatment, however these changes do not occur in the case of cGnRH-II. In hens, the preovulatory LH peak co-occurs with the decrease of the amount of hypothalamic cGnRH-I, while the concentration of cGnRH-II does not decrease (Wilson et al., 1990, Sharp and Ciccone, 2004). Nevertheless, some findings contradict the generally accepted view that cGnRH-II lacks gonadotropin releasing activity. Rozenboim et al., (1993) found the hypothalamic cGnRH-I and cGnRH-II content higher in laying turkey compared to non-laying photosensitive or brooding photorefracter females. On the other hand, in laying hens it is not the amount of cGnRH-I, but the hypothalamic concentration of cGnRH-II that decreases at the time of peak preovulatory LH level (Wilson et al., 1990).

Several studies concluded that in birds (maybe in addition to other minor roles) cGnRH-II functions as a neuromodulator/neurotransmitter. The intracerebroventricular injection of cGnRH-II increased courtship behaviour in male white-crowned sparrow, while cGnRH-I did not elicit this effect. The neuromodulatory function of cGnRH-II is supported by the observation that it increases willingness to copulate in females (Millam et al., 1993, 1998, Clerens et al., 2003, Stevenson and MacDougall-Shackleton, 2005, Maney et al., 1997, Bentley et al., 2006).

A **third GnRH** was detected in songbird brain, identical to the LGnRH-III of jawless fishes. Its structure differs from cGnRH-I at 3-Tyr, 5-Leu, 6-Glu, 7-Tyr and 8-Lys. It is assumed that in mammals (in rat and cattle, where this peptide occurs in the preoptic-paraventricular area), LGnRH would play the role of a physiological FSH releasing factor (McCann et al., 2001, 2002, Yu et al., 2002). In birds, LGnRH has a strong LH-releasing effect, but its effect on FSH is not known. In songbirds, this neuropeptide is found in certain song-regulating hypothalamic nuclei. Presumably, it is connected to song learning and complex vocalisations, therefore its role is more neuromodulatory as opposed to being a releasing hormone (Bentley et al., 2004, Stevenson et al., 2007).

The glia cell system of the median eminence, in particular the astrocytes and the tanycytes, which are specialised elements of the ependyma located at the base of the infundibular recess, play an important role in transporting GnRH into the portal veins. Their connection to GnRH axons is functionally important. Two mechanisms are known, one triggers the synthesis of growth factors through receptors that activate tyrosine kinase, while the other ensures the plastic appearance of the glia-neuron adhesion. GnRH axons reach the external zone directed by the fibroblast growth factor that acts near the basal membrane. The release of the neurohormone from the axon endings is regulated by an insuline-like growth factor and several epidermal growth factors. Numerous adhesion molecules are located in the external zone of the median eminence, for example the neuronal cell-adhesion molecule, synaptic cell

adhesion molecule and neuronal contactin, which interacts with the tyrosine phosphatase transmembrane receptor-like molecule of the glia cells (Ojeda et al., 2008). In the GnRH cells, especially in the median eminence and the axons leading to the OVLT, contactin and its cis parter, Caspr1 express. In the hypophyseal astrocytes tyrosine phosphatase-β receptor is formed, and its carbonic anhydrase extracellular subdomain can bind neuronal contactin. These receptor relationships are important for GnRH release, as their amount can influence GnRH efflux into the portal veins (Parent et al., 2007).

Glial "feet" that end on the basal lamina also play a role in GnRH release mechanism and their development affects the intensity of the efflux. The structure of the median eminence changes seasonally, as a function of photostimulation. The amount of photoperiodically stimulated triiodothyronine increases the amount of thyroid hormones in the median eminence by upregulation, and the intensive thyroid hormone effect increases the size of the axon terminals that reach the basal lamina, which makes a more intensive GnRH release possible. In the case of short day light conditions, the axon terminals are smaller and they are separated from the basal lamina by glial endfeet, which decreases GnRH efflux (Yamamura et al., 2004).

5.4.2. The gonadotropin inhibitory hormone (GnIH)

GnIH was discovered in 2000, when Tsutsui et al. isolated a peptide from Japanese quail brain containing 12 amino acids and forming an amide on the C terminal (Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH2) that decreased gonadotropin release in quail adenohypophyseal cell culture in a dose-dependent fashion (Tsutsui és mtsai., 2000). The five amino acid-containing C terminal of this dodecapeptide was equal to a pentapeptide isolated from chicken hypothalamus, which has already been assumed to be broken off from a larger molecule (Dockray et al., 1983, Dockray and Dimaline, 1985). The C terminal was also similar to the cardio-excitatory Phe-Met-Arg-Phe-NH2 (FMRF-amide), which was described in 1977 from the ganglion of Venus' girdle (*Cestum veneris*). This molecule was later found in the nervous system of several invertebrates and vertebrates. Among the particular brain areas, the highest concentration of GnIH is in the hypothalamus, and immunocytochemical localisation shows that in Japanese quail most GnIH somas are in the PVN, and some positive cells are located in the septal area. The largest amount of GnIH axes are in the external zone of the median eminence, but they are also found in the hypothalamic POA, septum, mesencephalon, ventral area of the paleostriatum, optic tectum, motor nucleus of the dorsal vagus, and in the area of the myelencephalon (Ukena et al., 2003). Later, similar GnIH localisations have been described from the brain of several songbird species (Tsutsui et al., 2009).

The mRNA of the GnIH receptor (GnIH-R) expresses in relatively larger amounts in the diencephalon compared to the adenohypophysis and the ovary. In chickens, the amount of adenohypophysis receptors is larger in juvenile birds compared to mature individuals, which is the effect of sexual steroids: estradiol or estrodiol+progesterone treatment decreases the amount of GnIH-R mRNA in the pars distalis. GnIH-R mRNA can be detected in both the cephalic and the caudal adenohypophyseal lobe, and it shows a particular colocalisation in the LH-β and FSH-β containing cells (Maddieni et al., 2008b).

The physiological effect of GnIH was first studied in vitro, in Japanese quail adenohypophyseal cell culture, determining its effect on LH, FSH and prolactin. GnIH inhibits LH release in a dose-dependent way, and inhibits FSH release in nearly the same way. In chickens, GnIH only decreases LH release in the hypophysis of immature individuals, not affecting mature hens. GnIH does not affect prolactin production in isolated hypophyseal cells. GnIH does not only inhibit trop hormone release, but also gonadotropin biosynthesis, inhibiting the synthesis of the common α subunit and the FSH β subunit, but not affecting the synthesis of the LH β subunit. As the half time is longer in the case of the LH β subunit, the technique of measurement could have influenced the results (Tsutsui et al., 2000, Maddineni et al., 2008b).

GnIH is also efficient in vivo, i.e. when white-crowned sparrows captured during breeding season were given GnIH intravenously, their plasma LH level significantly decreased within 2 minutes, and it was not detectable 10 minutes later. This observation suggests rapid GnRH-inhibitory action of GnIH, presumably at the level of the median eminence (Tsutsui et al., 2005). According to mammalian data, GnIH does not only decrease GnRH release by direct inhibition, but can also block the stimulating effect of kisspeptin and glutamatergic neurons (Wu et al., 2009a,b).

GnIH binds to a specific, heptahelical, G protein-linked transmembrane receptor, which is synthesised in adenohypophyseal gonadotropic cells, hypothalamic GnRH neurons, theca and granulosa cells of the ovary and Sertoli cells of the testis. The gonadotropin release-inhibitory effect of GnIH can act on both LH and FSH, as GnIH-R-mRNA and the receptor protein both express in the hypophyseal LH-β- and FSH-β-containing cells. The direct GnRH-inhibitory effect of GnIH is confirmed by the close contact between GnIH axons and the cGnRH-I and cGnRH-II neurons, and the presence of GnIH receptors in the membrane of cGnRH-I and cGnRH-II neurons (Ubuka et al., 2008, Tsutsui et al., 2005). The functional connection between GnIH and GnRH is characterised by the fact that in chicken adenohypophysis the level of GnIH-R mRNA changes in the opposite direction than GnRH-R-III in sexually inactive and active birds. The chicken GnIH-R signal, which inhibits adenylcyclase cAMP production, binds to the G-α subunit. This inhibitory effect efficiently decreases the dose-dependently inducted activation of the cAMP-responsive element (CRE) by

GnRH and the intracellular level of cAMP in the adenohypohysis. The GnIH/GnRH ratio has a determining role in this process, which, by changing the sensitivity of the hypophysis, can switch between inhibitory and stimulatory effects (Shimizu and Bédécarrats, 2010). The presence of GnIH receptors in the follicular cells of the ovary and the Sertoli cells of the testis makes the GnIH effect more extensive. These receptors and the binding of GnIH also act on the „G-α"-adenylcyclase – cAMP axis, and modulate the effect of FSH and LH on gonadal maturation in autocrine or paracrine way (Bédécarrats et al., 2009).

In the ovary, GnIH receptors are located in both the theca and the granulosa cells, and their amount is larger in prehierarchical than in preovulatory follicles. GnIH treatment decreses the viability of granulosa cells, and this effect does not occur in the presence of FSH. The formation of GnIH receptors is steroid dependent, their expression is inhibited by both estrogens and progesterone (Maddineni et al., 2008a).

GnIH neurons, which presumably also originate from the olfactory placode, migrate to the hypothalamus along the olfactory nerve around hatching.

Melatonin probably has a role in the regulation of the functioning of GnIH. The effects of melatonin on gonadal function and gonadotropin secretion have been long known. In Japanese quail, epiphysectomy and enucleation combined with it decreases GnIH mRNA expression and GnIH peptide content in the PVN and the median eminence. Melatonin treatment of the individuals that undergone surgery increased GnIH mRNA expression and neurohormone production in a dose-dependent manner. GnIH secretion changed according to light regimes: it increased during short day light conditions, when the melatonin production also increased and decreased during long day photoperiod, when melatonin production decreased. Under in vitro conditions GnIH release showed a circadian rhythm, its intensity increased during the dark period and decreased during light period, running along the changes in melatonin secretion. Hypophyseal LH release changed the opposite way compared to GnIH secretion. Melatonin probably affects GnIH directly, which is supported by the expression of Mel(1c) receptors in the GnIH neurons **of** PVN (Ubuka et al., 2005, Chowdhury et al., 2010).

The extent of areas with GnIH fibres and terminals in the cerebrum suggests that this dodecapeptide plays a role in the development of different behaviours. GnIH can act through direct inhibition of cGnRH-I and cGnRH-II neurons: there is close contact between cGnRH-I and cGnRH-II axons in the median eminence, and in the mesencephalon of white-crowned sparrow there are GnIH terminals on the cGnRH-II neurons and in their close proximity. In female white-crowned sparrows pretreated with estrogen, cGnRHII increased copulatory willingless in response to the male's song. However, intracerebroventricular application of GnIH did not only decrease LH plasma level, but also immediately inhibited willingness to copulate in cGnRHII-stimulated female white-crowned sparrows (Bentley et al., 2006).

5.5. Hypothalamic regulation of prolactin production

In the 1950s and 60s, early bioassays using pigeon crop sac assay showed that the regulation of prolactin synthesis is different in mammals and birds. In mammals, except for the period of lactation, prolactin production is under tonic inhibition by the hypothalamus, while in birds it seemed to be under a central, stimulatory regulation. However, recent studies show that there are stimulatory and inhibitory factors present in the regulation of prolactin secretion in both mammals and birds, and the only difference is in physiological dominance. A general stimulatory factor of hypothalamic regulation is the **vaso(active)intestinal polipeptide (VIP)**, which belongs to the group of gastrointestinal bioactive peptides (secretin, glucagon and pituitary adenylate cyclase-activating polypeptide (PACAP)). VIP behaves as a prolactin-releasing factor, while **dopamine (DA)** is a generally inhibitory regulating factor (with PIF-like action).

VIP is a 28 amino acid-containing polypeptide, which shows close structural relationship with secretin, glucagon, growth hormone releasing factor and PACAP. VIP was first isolated from chicken intestine (in 1974), and its structure proved to be very similar to a polypeptide isolated from pig intenstine, differring only in four amino acids. The polypeptide is a typical regulating factor of the gastro-intestinal, circulatory, immune and reproductive systems, and considering its origin and receptors also of the central nervous system, including the regulation of behaviour**.**

In the central nervous system VIP primarily occurs in the hypothalamus. Light microscopic immunocytochemical studies on pigeon, quail, bantam chicken and turkey found that the largest amount of VIP somas is located in the medial and lateral area of the ventromedial nucleus of the medial hypothalamus and in the infundibular nucleus. A smaller amount of neurons is found along the base of the third ventricle, in the area of the preoptic, supraoptic and paraventricular nuclei (Mikami and Yamada, 1984, Péczely and Kiss, 1988, Mauro et al., 1989, Esposito et al., 1993). According to other studies, they are also present in the area of the accumbent nucleus and the lateral area of the hypothalamus, particularly around the lateral septal organ at the ventricle, where some of the VIP cells are typical liquor contact neurons (Kuenzel and Blasher, 1994, Teruyama and Beck, 2001).

The axons of the periventricular neurons lead to the median eminence, and end in pericapillaries in the external zone. These axons play a determining role in the hypophyseal regulating role of VIP, fulfilling a prolactin releasing function. From the median eminence the portal circulatory system transports VIP into the adenohypophysis, and increases secretion of prolactin cells (mainly in the cephalic lobe).

The prolactin-releasing function of VIP was mostly shown in laying and brooding bantam hens and turkeys. The immune-reactivity of hypothalamic VIP, hypothalamic VIC mRNA content and VIP concentration in the portal blood show stong correlation with the circulating prolactin level. VIP increases prolactin secretion by the adenohypophysis, the stability of prolactin mRNA and prolactin gene expression in domestic hen and female turkey under in vivo and in vitro conditions (Sharp et al., 1989, El Halawani et al., 1990, Kansaku et al., 1998, You et al., 2001). Alternatively, passive and active immunisation of bantam hens and turkeys with VIP stopped hypothalamic VIP release and significantly decreased adenohypophyseal prolactin mRNA and plasma prolactin levels (Sharp et al., 1989, El Halawani et al., 1996).

Besides its hypophysiotropic effect, VIP also plays a neuromodulatory role, suggested by the widespread occurrence of its immunopositive axon system in the central nervous system. There are extrahypothalamic VIP axes in the medial habenula, the dorsal and posterior archistriatum, the pyriform cortex, the Tsai of the ventral area and in the parolfactory lobe. These fibres suggest a neuromodulatory role and that VIP influences behaviour through the limbic system (Kuenzel and Blasher, 1994). VIP neurons and axons located in the lateral septum and the bed nucleus of the stria terminalis can play a role in the development of agression that occurs in song sparrows independently of the reproductive period. When aromatase activity was inhibited and/or plasma DHEA concentration experimentally increased the aggression of the birds increased, when the VIP immunereactivity of the studied areas substantially changed. Increasing androgen dominance, at least partly, increases aggression by activating VIP neurons (Wacker et al., 2008).

The functional relationship between VIP and GnRH is confirmed by the morphological connections between their neurons. In the lateral septum and the preoptic area VIP axons are in tight apposition, and form synapses with the soma and dendrites of the cGnRH-I neurons, which are visible electronmicroscopically. VIP axons have been assumed for some time to transmit impulses from the photosensitive neurons of the septal area towards the cGnRH-I system, and in this case fulfil a photostimulatory-neuromodulatory role (Kiyoshi et al., 1998). This assumption has been proven right recently, when VIP neurons of the septal area in domestic hen were found to function as encephalic photoreceptors. Additionally, photostimulation triggers VIP synthesis in these cells, which increases GnRH gene expression through VIP receptors located in the cGnRH-I neurons of the nucleus of the pallial commissure (Li and Kuenzel, 2008).

There are certain similarities between the production of GnRH and VIP: photostimulation increases both, but considering the peak value, there is a phase difference. In turkey, photostimulation increases hypothalamic VIP content, reaching its maximum in brooding and photorefracter birds. Photostimulation also increases cGnRH-I and II, but they reach their maxima earlier,

during egglaying, and during brooding and photorefractoriness it decreases to a low, non-photostimulated level. Decreasing amount of daylight decreases the levels of both cGnRH-I and II in the hypothalamus (Rozenboim et al., 1993). Similar results were seen in photostimulated and photorefracter dark-eyed juncos. Long day light conditions increased the amount of GnRH and VIP immunoreactive neurons, but VIP neurons reached the peak value later, with the development of the photorefracter stage (Saldanha et al., 1994). In turkey, the coactivation of hypothalamic cGnRH-I and VIP neurons following the electrical stimulation of the medial preoptic area suggests that this effect occurs through the dopamine-containing neurons of the lateral mammillary nucleus (Al-Zailaie et al., 2006).

VIP acts through its specific receptors (VIP-R) in different tissues. Its structure has mainly been studied in chicken and turkey adenohypophysis, finding that it mostly expresses in the cephalic lobe, and binds to the membrane of prolactin cells. Another important location of the receptor is the hypothalamus, where its mRNA has been detected in the infundibular nucleus, lateral septum, nucleus of the pallial commissure, POM, POA and PVN. The amount of VIP-R only shows substantial changes with regard to the reproductive cycle in the infundibular nucleus. In birds, the VIP receptor can also fulfil an important neuro-behavioural role in areas of the cerebral cortex: the receptor was detected in the membrane fraction of cortical cells from goose and turkey (Zawilska et al., 2004a,b). The VIP receptor consists of 457 amino acids in turkey, while in chicken it is a 446 amino acid containing heptahelical peptide, which acts through the G-protein system, stimulating adenylate cyclase and producing cAMP. In its ectodomain (131 amino acids) there are six cystein residues, and it carries characteristic conservative motives (transmembrane domain 248 amino acids) in the intracellular loop between the 5^{th} and the 6^{th} transmembrane regions. Its entodomain contains 64 amino acids. Chicken and turkey VIP-R are about 55% similar to the mammalian VIP-1R (Kansaku et al., 2001, You et al., 2001, Chaisea et al., 2004).

An important regulating factor of prolactin secretion is **dopamine (DA)**. Earlier studies suggested that similarly to mammals, where the dopamine-containing neurons of the arcuate nucleus inhibit prolactin secretion, dopamine also inhibits prolactin secretion in the pars distalis in birds. Later, in vitro studies showed that when dopamine and pimozide, its receptor antagonist, were added to the incubation medium, they did not affect prolactin secretion of the pars distalis. Nevertheless, TRH-stimulated prolactin release was inhibited by dopamine in a dose-dependent way, and this effect was suspended by pimozide. Prolactin secretion by the pars distalis incubated together with hypothalamic tissue was increased by the addition of pimozide, which suggests that the tonic inhibitory effect of dopamine also plays a role in establishing the base level of prolactin secretion (Hall and Chadwick, 1984). The discovery of

VIP and its stimulatory, releasing effect suggested that in birds, as opposed to mammals, hypophyseal prolactin secretion is regulated by a stimulatory mechanism.

According to our current knowledge, the regulation of prolactin secretion is under the control of a stimulating (VIP) and a more complex (DA) system. On one hand, DA has an inhibitory effect on the adenohypophysis: the D2-type dopamine receptor agonist (propylnorapomorphine-HCl) inhibits prolactin gene transcription and release in isolated pars distalis cell culture, and this inhibition was substantially decreased by the addition of a D2 dopamine receptor antagonist (eticlopride-HCl) to the system. The inhibitory effect of the dopamine receptor agonist did not only occur on the level of basic secretion, but it also substantially decreased the stimulating effect of VIP on prolactin secretion (Al Kahtane et al., 2003).

In the adenohypophysis only inhibitory (hypoprolactinemic) D2 receptors are found, D1-type receptors are lacking (Youngren et al., 1998). On the other hand, in the hypothalamus both receptor types are present, and their expression can be detected in VIP cells, mostly in the lateral hypothalamus and in the infundibular nucleus. The amount of D1 receptors in the hypothalamus is 6.8 times higher compared to the amount of D2 receptors. During the breeding season, the amount of D1 receptors considerably increases in certain areas of the hypothalamus. In brooding hens, further substantial increase only occurred in the area of the infundibular nucleus, the high levels in the areas of the POA and the ventromedial nucleus typical of laying hens did not change. In photorefracter, non-laying hens the amount of D1 receptors substantially decreased in all studied hypothalamic areas. The amount of D2 receptors in the hypothalamus does not change during the breeding season, except for the infundibular nucleus, where its amount increased substantially in photorefracter hens. These results clearly show that the amount of hypothalamic D1 dopamin receptors that are part of the prolactin-stimulating system increases in hyperprolactinemic, brooding hens, while the amount of the hypophyseal D2 receptors that are part of the prolactin secretion-inhibitory system increases in hypoprolactemic photorefracter hens, i.e. dopamine has a double effect (Chaisea et al., 2003).

The prolactin-release stimulating effect of dopamine occurs through the facilitation of VIP neurons. In the dopaminergic system of the brainstem and in the hypothalamus the immunopositivity of the tyrosine-hydroxilase (TH) containing, dopamine-producing cells of the intramedial nucleus shows strong correlation with changes in plasma prolactin levels in Thai chickens under extensive husbandry. The TH immunopositivity of the intramedial nucleus continuously increased during egg laying, and reached its maximum during brooding (Sartsoongnoen et al., 2008).

Serotonine (5HT) also affects prolactin secretion at the level of the hypothalamus, however, this is not a direct effect, but probably occurs through the

dopaminergic system. In turkey, 5HT administered to the third ventricle or the electrical stimulation of the POM and the ventromedial nucleus both incleased plasma prolactin level. When a D1 dopamin receptor antagonist was administered into the infundibular area, this increase in prolactin concentration did not occur. The administration of 5HT-2A receptor agonist into the ventricle triggered an increase in prolactin secretion, which was effectively inhibited by a pretreatment with a receptor antagonist. The effect of 5HT receptor agonist was also effectively inhibited by the pretreatment with a dopamine D1 receptor antagonist, which suggests that adrenergic activation can (also) occur through 5HT-2A receptor stimulation of the infundibular VIP neurons. 5HT-1A receptors make the inhibitory effect of serotonin on prolactin secretion possible, in a way that the 5HT-1A effect inhibits the stimulation of 5HT-2A on the adrenergic system (Chaisea et al., 2010).

The role of **histamine** also seems probable in the regulation of prolactin secretion. Histamine acts through H2 G-protein-linked receptors, increasing cAMP release in isolated hypophyseal cell culture. Other histamine receptors (H1 and H3) do not play a role in this process. Presumably there is synergism between the histamine-H2 receptor and the activation of the VIP neurons, which together cause specific cAMP release in hypophyseal prolactin cells (Zawilska et al., 2005).

The regulatory/modulatory effect of **pars nervosa hormones** on adenohypophyseal functions is accepted in mammals. Prolactin cells contain oxytocin receptors, in which oxytocin causes increased prolactin secretion, and oxytocin antagonists decrease prolactin secretion (Kennett and McKee, 2012). In birds the similar effects of arginine-vasotocin and mesotocin-oxytocin are little known. In chicken adenohypophysis, using an antiserum against vasotocin receptor (VT2R), membrane-linked receptor immuneactivity could be detected in technically all ACTH and MSH cells and in about 10% of the prolactin cells. However, vasotocin receptors never appeared in the membrane of gonadotropic, thyreotropic and somatotropic cells. This study supports the regulatory/modulatory role of vasotocin in some prolactin cells (Jurkevich et al., 2008). In the supraoptic and paraventricular hypothalamic nuclei mesotocin immunopositive neurons have been identified. Their amount was higher in brooding turkeys compared to laying individuals. The appearance of brooding behaviour was accompanied by the increase in c-fos activity in the mesotocin neurons of the two nuclei, and with the increase of plasma prolactin level. Therefore, mesotocin has a determined prolactin releasing activity in turkey (Thayananuphat et al., 2011).

Since the 1970s, the presence of a compound with prolactin-releasing activity has been known in the hypothalamus. However, it was not the later identified VIP. The molecule called **prolactin-releasing peptide (PrPR)** turned out to be a new releasing hormone, which possessed a specific heptahelical receptor,

and resulted in prolactin release in hypophyseal cell culture (Hinuma et al., 1998). Prolactin-releasing peptide has also been isolated from chicken brain in 2010, which was earlier isolated not only from mammalian brain, but also from amphibians and bony fish. The compound consisting of 20 and 31 amino acids is similar to the peptide that occurs in several isoforms (containing 20, 31, 32 and 37 amino acids) and is also similar to earlier identified forms. cPrRP 20 was 100% similar to the compound in fish, containing the same amino acids, 95% similar to the amphibian and 70% similar to the mammalian form. cPrRP31 was 90% similar to the *Xenopus* and 52-55% similar to the mammalian homologue. Central or peripheral administration of cPrRP20 does not have endocrine and behaviour-forming effect in chicken. Peripheral administration of cPrRP31 increased plasma prolactin level, and decreased it when administered int the cerebral ventricle. Central administration increased feeding behaviour in the treated birds, and the growth hormone content of the plasma decreased in case of both applications (Tachibana et al., 2010).

The **peptide histidine-isoleucine (PHI)**, another peptide with a potential prolactin-releasing effect, unlike in mammals, turned out to be not having a prolactin-release increasing effect in birds (Kulick et al., 2005).

5.6. The structure of the limbic system and its relationship with the neuroendocrine regulation

The avian limbic system is a neuronal network similar to that in mammals, but given brain structural differences between the two groups, they show a different anatomical localisation. The most important components of the limbic system are the bed nucleus of the stria terminalis (BnST), the lateral and medial septum, the accumbens nucleus, the hippocampus, the parahippocampal area, the pyriform cortex, the praepyriform cortex, the dorso-lateral part of the corticoid area, the nucleus taenia of the amygdala, the posterial pallial part of the amygdala, the caudo-ventral nidopallium, the dorso-medial and lateral nuclei of the anterio-median thalamus, and the lateral part of the mammillar nucleus. (Figure 67.)

It is connected to the hypothalamus by several reciprocal axon connections, and its functions, based on relatively less data than in mammals, seems to be similar to the mammalian limbic system. The limbic neuronal circuit, based on the hypothalamic-centered neurohumoral regulatory system, develops and integrates several behavioural patterns and projects them towards higher telencephalic units. Based on sensory information, it modulates attention, activity, hormonally determined behaviours, such as feeding, reproduction and parental behaviour, as well as social interactions, the appearance of locomotor activity, takes part in the development of spatial orientation, memory forming,

learning processes and actualising circadian rhythms. Substantially less is known about its pallial associative connections.

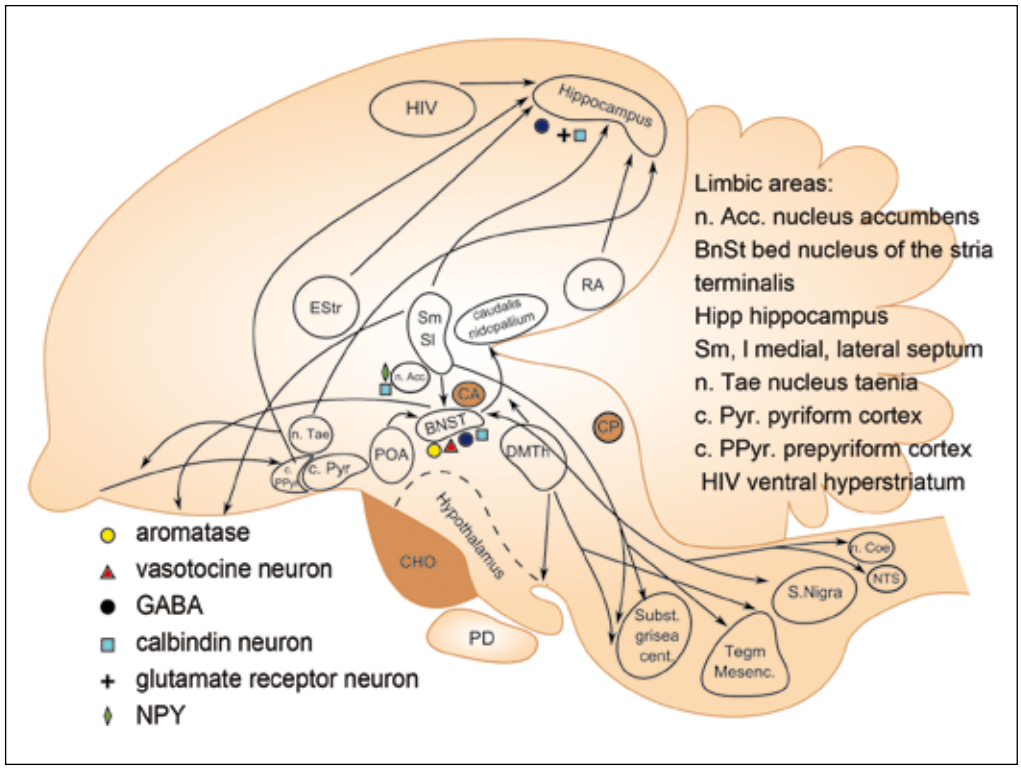

Figure 67. The structure of the limbic system in bird brain and the known neuron types

Among these areas, the **bed nucleus of the stria terminalis (BnST)**, similar to the POM, possesses testosterone-dependent aromatase activity and neurons that can express vasotocin in a sexually dimorphic way. Several of its cells contain neurotensin (Panzica et al., 2001). Its lateral area can be divided into three parts based on cytoarchitectonic and immuncytochemical characteristics. Its central region consists of larger cells, the majority of which are GABA positive. This area is an important regulatory centre of copulatory behaviour, and along with the central part of the BnST shows intentinse cFOS expression in quail that mate regularly (Taziaux et al., 2008). Its two peripheral regions have lower neuron density, and these neurons show weaker GABA expression. In one of the peripheral regions, a large amount of NO synthetise and calretinin-containing axons run, and some calretinin-positive neurons are also detectable. In the other peripheral region calbindin somas can be detected among the calbindin fibres (Real et al., 2008). The bed nucleus has rich reciprocal fibre connections with the preoptic area, which suggests that they form a functional unit. Its efferent fibres run to the caudal nidopallium, the olfactory tubercule and

the accumbent nucleus, and it is in a reciprocal connection with the substantia nigra, the dorsal subcaeruleus nucleus, the coeruleus nucleus and the nucleus of the solitary tract. It receives a stong axon bundle from the hippocampus (Atoji et al., 2006). Studies using LIM-homeobox chicken genes show that in birds the bed nucleus of the stria terminalis (both its medial and lateral parts) is of pallido-pedunculo-preoptic origin, its ventral part is homologous to the median part of the bed nucleus of the stria terminalis in mammalian amygdala (Abellán and Medina, 2008).

The **hippocampal area** is an ancient part of the brain (archipallium), it is a complex structure, which consists of at least seven regions: dorso-lateral, dorso-medial, triangular, V-shaped area, magnocellular, parvocellular and cell-poor region. The V-shaped layer shows structural-functional similarity to the mammalian dentate gyrus, and its medial division is similar to the Ammon-horn and the subiculum.

Dorso-laterally it is attached to the **parahippocampal area**, and more laterally to the **dorso-lateral corticoid area**, which forms a thin sheet covering the dorsal part of the ventricle. From these areas, and also from the caudo-ventral nidopallium, which can be considered a limbic area, several fibres run medially into the hippocampus, which mostly transfer visual impulses. Unlike in mammals, the hippocampus probably does not receive direct fibres from the pallial associative areas. The **caudo-ventral nidopallium**, which has an integrative function, also unlike the mammalian prefrontal cortex, does not receive direct hippocampal or parahippocampal efferents.

Its neurons can be divided into projection and local. Projection neurons are pyramidal, pyramid-like and multipolar cells, which are characterised by a large amount of denritic spines. Pyramidal and pyramid-like cells are only found in the central region. Among the local neurons, there are several inhibitory GABA and excitatory NMDA receptor-containing glutamate cells, which play a role in memory formation (Tömböl et al., 2000, Gibbs and Bowser, 2009). In songbirds, its dorsolateral, dorsomedial and ventral nuclei show calbindin immunopositivity (Székely, 1999). There are reciprocal fibre connections between its divisions, and its contralateral fibres run in the pallial commissure. The V-shaped area contains intrinsic neurons.

It receives sensory afferents from the optic and olfactory centres and from the septum, while its efferents project to several telencephalic areas, the septum and (mostly the lateral nucleus of) the hypothalamus. The retino-thalamic neurons are characteristic, branching out in the parvocellular superficial nucleus of the thalamus and projecting to the hyperpallium (optic Wulst) and the hippocampus (Trottier et al., 1995).

The hippocampus has an integrative function within the limbic system, as well as in the whole neuroendocrine unit, including the whole brain. Its function plays an important role regulating behavioural processes, such as

homing behaviour, visual discrimination, finding (stored) food (spatial memory), imprinting, and sexual behaviour. Task completion learning, such as finding food is accompanied by the appearance of ZENK and FOS early genes in the whole area of the hippocampus. Recalling previously obtained knowledge also triggered early gene activation similar to the actual learning (training) process. Its electrophysiological characteristics are unlike those in mammals: it does not form rhythmic theta waves when the individual is awake and in a REM sleep stage. This is supported by the observation that the number of medial septal afferents, which play an important role in theta wave generation, is very small in birds (Karten and Hodos, 1967, Atoji and Wild, 2006, Bischof et al., 2006, Mayer et al., 2010, Rattenborg and Martinez-Gonzalez, 2011).

The **septum** consists of **medial and lateral** nuclei, and it receives ipsilateral afferent fibres from the hippocampus, the dorsolateral corticoid area, the pyriform cortex, the pallial amygdala and the ventral pallidium. Contralateral afferents arrive from the lateral septum, the pallial amygdala and the lateral hypothalamus. Efferent fibres project from the ipsi- and contralateral divisions to the nucleus of the diagonal bundle, ventral paleostriatum, parolfactory lobe, accumbent nucleus, olfactory tubercule, archistriatum, pyriform cortex and the anterior neostriatum. Fibres also run to the preoptic region of the hypothalamus, into the ventromedial, paraventricular and periventricular nuclei, the lateral hypothalamic area and the mammillary region. Septal efferents also reach the dorsal thalamus, the habernular region and the central substantia grisea of the mesencephalon, as well as the ventral tegment. Its function manifests in the modulation of behavioural forms: attention and learing, memory forming, hormonal regulation of behaviour, social interactions, modulation of locomotor function and aligning the body to the circadian rhytm (Montagnese et al., 2004).

The **nucleus taenia of the amygdala** is a relatively small nucleus of the posterior and central archistriatum. It sends efferent fibres to the hypothalamus, the parahippocampal area, the hyperstriatum, the intermediate archistriatum and the parolfactory lobe. It receives afferent fibres from the olfactory bulb, the ovoidal and subrotundal nuclei and the hippocampus. Considering its function, it plays a role in the regulation of social behaviour, influencing active phases and adaptive relevances (Cheng et al., 1999).

The **dorso-medial thalamic nucleus** is an important relay nucleus, sending several fibres to the ventral paleostriatum, septal area, Wulst area of the hyperstriatum, neostriatum, archistriatum, dorsolateral corticoid area, dorsal thalamic nuclei and the hypothalamus. Its anterior area projects more to the hypothalamus, while its posterior area to the dorsal thalamus. It has efferent connections with several mesencephalic areas, vestublar and brainstem-brain nerve nuclei. Its role is important in tuning together attention, fear, pain, memory connections and hormonally regulated behavioural patterns (Montagnese et al., 2003).

The **posterior pallial amygdala**, together with the outer group of cells of the bed nucleus of the stria terminalis form a functional unit in the limbic system, suggested by their bilateral fibre connection system in the stria terminalis. The posterior pallial amygdala forms a reciprocal connection with several areas of the nidopallium and the pyriform cortex, as well as with the rostral part of the cerebral hemispheres (fronto-medial and fronto-lateral nidopallium and denso-cellular hyperpallium). The pallial amygdala connects to the dorsomedial thalamic nuclei and the lateral hypothalamic area through the occipitomesencephalic tract. The area receives a large amount of afferent fibres from the hippocampus. It is assumed to be an important part of the limbic-visceral system (Atoji et al., 2006).

The **accumbent nucleus** is a group of neurons wedged into the septal area at the ventral side of the ventricule. It contains two cell populations, one central and another shell-like covering it. The nucleus is located laterodorsally from the bed nucleus of the stria terminalis, and the shell covers the nucleus ventrally and ventrolaterally. Compared to the nucleus, the shell part has more intensive calbindin and neuropeptide-Y immunopositivity. The shell receives several fibres from the nucleus of the solitary tract, and this structure is similar to the one described in mammals (Bálint and Csillag, 2007).

The **pyriform cortex** is the posterior part of the ventrolateral surface of the cerebrum, the area behind the **prepyriform cortex** and the **parolfactory lobe-olfactory bulb compex** in front of it. It connects dorsolaterally to the dorsolateral corticoid area of the hippocampal area. It is part of the ancient olfactory cortex. It receives a strong fibre bundle from the olfactory bulb, to where its efferent fibres lead. Besides, it has reciprocal fibre connections with the parahippocampal area and the hippocampus, and also with the anterior telencephalic areas (pyriform cortex and dorsal hyperstriatum). Its function was primarily studied in homing pigeons, where it plays an important role in spatial orientation, the development of the so-called navigational map (Reiner and Karten, 1985, Bingman et al., 1994)

5.7. The hypothalamo-hypophyseal portal circulation

The hypophysis receives arterial blood through the **infundibular artery**, which splits off from the internal carotid artery near the posterior edge of the optic chiasma. This short blood vessel divides into anterior, central and posterior branches. The anterior vessels lead to the lateral part of the chiasma and the optic tract, the central group runs to the pars tuberalis, which covers the median eminence as a coat, and the vessels of the posterior branch lead to the pars nervosa (Wingstrand, 1951). The blood vessels of the pars nervosa link to the posterior primary capillary plexus though some side branches, but there is

no direct vasicular connection between the pars nervosa and the pars distalis. The short portal vessels described in mammals do not develop in birds, as the dural ossification that separates the pars nervosa and the pars distalis creates an anatomical barrier (Vitums et al., 1964).

The branching blood vessels of the central group form a **primary capillary plexus** (Mantel plexus), which covers the basal surface of the median eminence, creating tight contact. In some species (e.g., white-crowned sparrow, Alexandrine paraket *Psittacula eupatria*, little owl *Athene noctua*, kingfisher *Halcyon sp.*, bulbul *Pycnonotus* sp., Eurasian golden oriole *Oriolus oriolous*, tree pie *Dendrocitta sp.*, bunting Emberiza sp. and house sparrow) this capillary plexus forms two bundles, an anterior (laying on the anterior median eminence) and a posterior (laying on the posterior median eminence. In other species (e.g., domestic chicken, pigeon, dove, ibis, and European roller *Coracias garrulus*) the capillary plexus is uniform.

Blood vessels that collect from the anterior capillary plexus run to the cephalic lobe of the pars distalis adenohypophysis, while vessels from the posterior capillary plexus run to the posterior lobe. There is no anastomosis between the two afferent blood vessel bundles, even in those types, where the two capillary plexuses anastomise with each other multiple times (Vitums et al., 1964, Dominic and Singh, 1969, Singh and Dominic, 1975) .

In birds, the primary capillary plexus does not enter into the tissue of the median eminence, the screwdriver vessels typical of mammals, which deeply penetrate the nervous tissue from the surface blood vessel network are lacking (Assenmacher, 1952, Singh and Dominic, 1970, Neumeler and Lametschwandtner, 1994). In birds, the vessels of the primary plexus lay on the pliated basal surface (superficial stratum) of the external zone of the median eminence, embedded in its basal lamina. Hypothalamic axons that lay radially on the basal surface make neuro-heamal contact here, through the basal lamina. In the pericapillary space between the axon endings and the portal capillaries are the glial processes and their widened end feet, the thickness (amount) of which changes seasonally and depending on their function (Matsui, 1969, Péczely and Calas, 1970).

The blood vessels of the primary portal plexus are f**enestrate**d **capillaries,** where thin endothelial cells form windows and make it possible for large protein molecules that leave the pericapillary axon endings to enter the lumen of the capillary. In other areas endothelial cells form protusions into the lumen, which can have a role in the regulation of circulation. The pericytes are arranged in a characteristic spiral pattern, capable of contraction, which can also fine tune the amount of blood flow. Portal vessels, the parenchyma bundles of the pars tuberalis arranged around them in a cylinder-like fashion and the axon and glial elements that end in pericapillaries are all linked into a morpho-functional unit by a system of reticular fibres. Besides optimising neuro-vascular material

flow and paracrine function, this system can also play an important role regulating blood flow through the portal vessels (Mikami et al., 1970).

The portal vessels lead into the lacunae located between the parenchymal columns in the adenohypophysis, providing blood flow to rinse around the secretory cells. Venous blood gets into the cavernous sinus formed by the connective tissue capsule around the pars distalis by two short, non-anastomising blood vessels. The veins from the pars nervosa also lead into the cavernous sinus. Through several short veins blood from the cavernous sinus eventually flows into the jugular vein.

Similar to mammals, in the avian hypothalamus there is also a **deep-running capillary system** in the subependymal stratum of the median eminence under the infundibular recess. There are axon- and hypendyma fibre endings around the walls of the rich blood vessel system. Precapillary arterioles can anastomise with the basally located Mantel plexus and also with the infundibular arteries that are located higher. This system shows certain morphofunctional similarity to the Mantel plexus, making neuro-haemal connection possible for mostly the infundibular neuro-endocrine cells and it forms vascular connection between higher hypothalamic areas and the portal circulation of the hypophysis (Duvernoy et al., 1969, Péczely and Calas, 1970).

References 5/4., 5., 6., 7.

1. Abellán A, Medina L (2008): Expression of cLhx6 and cLhx7/8 suggest a pallido-pedunculo-preoptic origin for the lateral and medial parts of the avian bed nucleus of the stria terminalis Brain Res. Bull., 75, 299-304.
2. Al Kahtane A, Chaiseha Y, El Halwani M (2003): Dopaminergic regulation of avian prolactin gene transcription J.Mol.Endocrinol., 31, 185-196.
3. Al-Zailaie KA, Kang SW, Youngren OM, Thayananuphat A, Bakken T, Chaiseha Y, Millam JR, Proudman JA, El Halawani ME (2006): Identification of dopamine, gonadotrophin-releasing hormone-I, and vasoactive intestinal peptide neurones activated by electrical stimulation to the medial preoptic area of the turkey hypothalamus: a potential reproductive neuroendocrine circuit J.Neuroendocrinol., 18, 514-525.
4. Assenmacher I (1952 – 1953): Anatomy of the arteries of the head and neck in the bird Arch.Anat. Histol.Embryol., 35, 181-202.
5. Atoji Y, Saito S, Wild JM (2006): Fiber connections of the compact division of the posterior pallial amygdala and lateral part of th ebed nucleus of the stria terminalis in the pigeon (Columba livia) J.Comp.Neurol., 499, 161-182.
6. Atoji Y, Wild JM (2006): Anatomy of the avian hippocampal formation Rev.Neurosci., 17, 3-15.
7. Avelino-Cruz JE, Cebada J, Mellon PL, Felix R, Monjaraz E (2009): Leptin increases L-type Ca^{2+} chanel expression and GnRH-stimulated LH release in Lbeta T2 gonadotropes Mol.Cell Endocrinol., 298, 57-65.
8. Bálint E, Csillag A (2007): Nucleus accumbens subregions: hodological and immunohistochemical study in the domestic chick (Gallus domesticus) Cell Tissue Res., 327, 221-230.
9. Bédécarrats GY, McFarlane H, Maddineni SR, Ramachandran R (2009): Gonadotropin-inhibitory hormone receptor signaling its impact on reproduction in chickens Gen.Comp.Endocrinol., 163, 7-11.
10. Bentley GE, Jensen JP, Kaur GJ, Wacker DW, Tsutsui K, Wingfield JC (2006): Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH) Horm.Behav., 49, 550-555.

11. Bentley GE, Moore IT, Sower SA, Wingfield JC (2004): Evidence for a novel gonadotropin-releasing hormone in hypothalamic and forebrain areas in songbirds Brain Behav.Evol., 63, 34-46.
12. Bingman VP, Casini G, Nocjar C, Jones TJ (1994): Connections of the pyriform cortex in homing pigeons (Columba livia) studied with fast blue and WGA-HRP Brain Behav.Evol., 43, 206-218.
13. Bischof HJ, Lieshoff C, Watanabe S (2006): Spatial memory and hippocampal function in a non-foodstoring songbird, the zebra finch (Taenopygia guttata) Rev.Neurosci., 17, 43-52.
14. Bruggeman V, D'Hondt E, Berghman L, Onagbesan O, Vanmontfort D, Vandesande F, Decuypere E (1998): The effect of food intake from 2 to 24 weeks of age on LHRH-I content in the median eminence and gonadotrophin levels in pituitary and plasma in female broiler breeder chickens Gen.Comp.Endocrinol., 112, 200-209.
15. Chaisea Y, Kang SW, Leclerc B, Kosonsiriluk S, Sartsoongnoen N, El Halawani ME (2010): Serotonin receptor subtypes influence prolactin secretion in the turkey Gen.Comp.Endocrinol., 165, 170-175.
16. Chaisea Y, Youngren OM, El Halawani ME (2004): Expression of vasoactive intestinal peptide receptor messenger RNA in the hypothalamus and pituitary throughout the turkey reproductive cycle Biol.Reprod., 70, 593-599.
17. Chaiseha Y, Youngren O, Al-Zailaie K, El Halawani M (2003): Expression of D1 and D2 dopamine receptors in the hypothalamus and pituitary during the turkey reproductive cycle: colocalization with vasoactive intestinal peptide Neuroendocrinology, 77, 105-118.
18. Chaturvedi CM, Kumar P (2007): Nitic oxide modulates gonadal and adrenal function in Japanese quail Coturnix coturnix japonica Gen.Comp.Endocrinol., 151, 285-299.
19. Chen J, An BS, Cheng L, Hammond GL, Leung PC (2009): Gonadotropin-releasing hormone-mediated phosphorilation of estrogen receptor-alpha contributes to fosB expression in mouse gonadotrophs Endocrinology, 150, 4583-4593.
20. Cheng M, Chaiken M, Zuo M, Miller H (1999): Nucleus taenia of the amygdala of birds: anatomical and functional studies in ring doves (Streptopelia risoria) and European starlings (Sturnus vulgaris) Brain.Behav.Evol., 53, 243-270.
21. Chowdhury VS, Yamamoto K, Ubuka T, Bentley GE, Hattori a, Tsutsui K (2010): Melatonin stimulates the release of gonadotropin-inhibitory hormone by the avian hypothalamus Endocrinology, 151, 271-280.
22. Clarkson J, Herbison AE (2006): Development of GABA and glutamate signaling at the GnRH neuron in relation to puberty Mol.Cell Endocrinol., 25, 254-255.
23. Clerens S, D'Hondt E, Berghman LR, Vandesande F, Arckens L (2003): Identification of c GnRH-II in the median eminence of Japanese quail (Coturnix coturnix japonica): Gen.Comp.Endocrinol., 131, 48-56.
24. Contijoch AM, Gonzalez C, Singh HN, Malamed S, Advis JP (1992): Dopaminergic regulation of luteinizing hormone-releasing hormone release at the median eminence level: immunocytochemical and physiological evidence in hens Neuroendocrinology, 55, 290-300.
25. Contijoch AM, Johnson AL, Advis JP (1990): Norepinephrine-stimulated in vitro release of luteinizing hormone-releasing hormone (LHRH) from median eminence tissue is facilitated by inhibition of LHRH-degrading activity in hens Biol.Reprod., 42, 222-230.
26. Contijoch AM, Malamed S, McDonald JK, Advis JP (1993): Neuropeptide Y regulation of LHRH release in the median eminence: immunocytochemical and physiological evidence in hens Neuroendocrinology, 57, 135-145.
27. Contijoch AM, Malamed S, McDonald JK, Advis JP (1993b): Neuropeptide Y regulation of LHRH release in the median eminence: immunocytochemical and physiological evidence in hens Neuroendocrinology, 57, 135-145.
28. Contijoch AM, Malamed S, Sarkar DK, Advis JP (1993a): Beta-endorphin regulation of LHRH release at the median eminence level: immunocytochemical and physiological evidence in hens Neuroendocrinology, 57, 365-373.
29. Davidson JS, King JA, Millar RP (1987): Luteinizing hormone release from chicken pituitary cells: synergism between calcium and protein kinase C and its inhibition by calmodulin antagonists Endocrinology, 120, 692-699.
30. Dawson A, Talbot RT, Dunn IC, Sharp PJ (2002): Changes in basal hypothalamic chicken gonadotropin-releasing hormone-I and vasoactive intestinal polypeptide associated with a

photo-induced cycle in gonadal maturation and prolactin secretion in intact and thyroidectomized starlings (Sturnus vulgaris) J.Neuroendocrinol., 14, 533-539.
31. Deviche P, Sabo J, Sharp PJ (2008): Glutaminergic stimulation of luteinising hormone secretion in relatively refractory male songbirds J.Neuroendocrino9l., 20, 1191-1202.
32. Dockgray GJ, Dimaline R (1985): FMRF amide- and gastrin /CCK-like peptides in birds Peptides 6, Suppl.3, 333-337.
33. Dockray GJ, Reeve JR Jr, Shively J, Gayton RJ, Barnard CS (1983): A novel active pentapeptide from chicken brain identified by antibodies to FMRFamide Nature, 305, 328-330.
34. Dominic CJ, Singh RM (1969): Anterior and posterior groups of portal vessels in the avian pituitary Gen.Comp.Endocrinol., 13, 22-26.
35. Dunn IC, Lewis PD, Wilson PW, Sharp PJ (2003): Acceleration of maturation of FSH and LH responses to photostimulation in prepubertal domestic hens by oestrogen Reproduction, 126, 217-225.
36. Dunn IC, Sharp PJ (1999): Photo-induction of hypothalamic gonadotrophin releasing hormone-I mRNA in the domestic chicken: a role for oestrogen? J.Neuroendocrinol., 11, 371-375.
37. Duvernoy H, Gainet F, Koritké JG (1969): Vascularization of the avian hypophysis J.Neurovisc. Relat., 31, 109-127.
38. El Halawani ME, Kang SW, Leclerc B, Kosonsinluk S, Chaiseha Y (2009): Dopamine-melatonin neuron sin the avian hypothalamus and their role as photoperiodic clocks Gen.Comp.Endocrinol., 163, 123-127.
39. El Halawani ME, Pitts GR, Sun S, Silsby JL, Sivanandan V (1996): Active immunization against vasoactive intestinal peptide prevents photo-induced prolactin secretion in turkeys Gen.Comp. Endocrinol., 104, 76-83.
40. El Halawani ME, Silsby JL, Mauro LJ (1990): Vasoactive intestinal peptide is a hypothalamic prolactin-releasing neuropeptide in the turkey (Meleagris gallopavo) Gen.Comp.Endocrinol., 78, 66-73.
41. Ely HA, Mellon PL, Coss D (2011): GnRH induces the c-Fos gene via phosphorilation of SRF by the calcium/calmodulin kinase II pathway Mol.Endocrinol., 25, 669-680.
42. Esposito V, De Girolamo O, Gargiulo G (1993): Immunoreactivity to vasoactive intestinal polypeptide (VIP) in the hypothalamus of the domestic fowl, Gallus domesticus Neuropeptides, 25, 83-90.
43. Ferris HA, Shupnik MA (2006): Mechanisms for pulsatile regulation of the gonadotropin subunit genes by GNRH1 Biol.Reproduct., 74, 993-998.
44. Fortin J, Lamba P, Wang Y, Bernard DJ (2009): Conser/vation of mechanisms mediating gonadotrophin-releasing hormone 1 stimulation of human luteinizing hormone beta subunit transcription Mol.Hum.Reprod., 15, 77-87.
45. Fraley GS, Kuenzel WJ (1993): Immunocytochemical and histochemical analyses of gonadotrophin releasing hormone, tyrosine hydroxylase, and cytochrome oxidase reactivity within the hypothalamus of chicks showing early sexual maturation Histochemistry, 99, 221-229.
46. Gibbs ME, Bowser DN (2009): Astrocytes and interneurons in memory processing in the chick hippocampus: roles for G-coupled protein receptors, GABA (B) and mGluR1 Neurochem.Res., 34, 1712-1720.
47. Haida Y, Ubuka T, Ukena K, Tsutsui K, Oishi T, Tamotsu S (2004): Photoperiodic response of serotonin- and galanin-immunoreactive neurons of the paraventricular organ and infundibular nucleus in Japanese quail, Coturnix coturnix japonica Zoolog.Sci., 21, 575-582.
48. Hall TR, Chadwick A (1984): Effects of synthetic mammalian thyrotrophin releasintg hormone, somatostatin and dopamine on the secretion of prolactin and growt hormone from amphibian and reptilian pituitary glands incubated in vitro J.Endocrinol., 102, 175-180.
49. Hall TR, Cheung A, Harvey S (1986): Serotoninergic inhibition of LH secretion in the domestic fowl J.Endocrinol., 110, 239-244.
50. Hattori A, Ishii S, Wada M (1986): Different mechanisms controlling FSH and LH release in Japanese quail (Coturnix coturnix japonica) evidence for an inherently spontaneous release and production of FSH J.Endocrinol., 108, 239-245.
51. Hattori A, Ishii S, Wada M, Miyamoto K, Hasegawa Y, Igarashi M, Sakakibara S (1985): Effects of chicken (Gln8)-and mammalian (Arg8)-luteinizing hormone-releasing hormones on the release

of gonadotrophins in vitro and in vivo from the adenohypophysis of Japanese quail Gen.Comp. Endocrinol., 59, 155-161.
52. Hinuma S, Habata Y, Fujii R, Kawamata Y, Hosoya M, Fukusumi S, Kitada C, MasuoY, Asano T, Matsumoto h, Sekiguchi M, Kurokawa T, Nishimura O, Onda H, Fujino M (1998): A prolactin-releasing peptide in the brain Nature, 393, 272-276.
53. Joseph NT, Morgan K, Sellar R, McBridge D, Millar RP, Dunn IC (2009): The chicken type III GnRH receptor homologue is predominantly expressed in the pituitary, and exhibits similar ligand selectivity to the type I receptor J.Endocrinol., 202, 179-190.
54. Jurkevich A, Berghman LR, Cornett LE, Kuenzel WJ (2008): Immunohistochemical characterization of chicken pituitary cells containing the vasotocin VT2 receptor Cell Tissue Res., 333, 253-262.
55. Kang SW, Thayananuphat A, Rozenboim I, Millam JR, Proudman JA, El Halawani ME (2006): Expression of hypothalamic GnRH-I mRNA in the female turkey at different reproductive states and following photostimulation Gen.Comp.Endocrinol., 146, 91-99.
56. Kansaku N, Shimada K, Ohkubo T, Saito N, Suzuki T, Matsuda Y, Zadworny D (2001): Molecular cloning of chicken vasoactive intestinal polypeptide receptor complementary DNA, tissue distribution and chromosomal localization Biol.Reprod., 64, 1575-1581.
57. Kansaku N, Shimada K, Saito N, Hidaka H (1998): Effects of protein kinase A inhibitor (H-89) on VIP- and GRF-induced release and mRNA expression of prolactin and growth hormone in the chicken pituitary gland Comp.Biochem.Physiol. C Pharmacol.Toxicol Endocrinol., 119, 89-95.
58. Karten HJ, Hodos W (1967): A stereotaxic atlas of the brain of the pigeon (Columba livia) Baltimore, The John Hopkins Press
59. Kawaminami M, Uematsu N, Funahashi K, Kokubun R (2008): Gonadotropin releasing hormone (GnRH) enhances annexin A5 mRNA expression through mitogen activated protein kinase (MAPK) in L-beta-T2 pituitary gonadotrope cells Endocrine Journal, 55, 1005-1014.
60. Kennett JE, McKee DT (2012): Oxytocin: an emetging regulator of prolactin secretion int he female rat J. Neuroendocr., 24, 403-412.
61. Kiyoshi K, Kondoh M, Hirunagi K, Korf H (1998): Confocal laser scanning and electron-microscopic analyses of the relationship between VIP-like and GnRH-like-immunoreactive neuron sin the lateral septal-preoptic area of the pigeon Cell Tissue Res., 293, 39-46.
62. Krishnan KA, Proudman JA, Bolt DJ, Bahr JM (1993): Development of an homologous radioimmunoassay for chicken follicle-stimulating hormone and measurement of plasma FSH during the ovulatory cycle Comp.Biochem.Physiol.Comp.Physiol., 105, 729-734.
63. Kuenzel WJ, Blasher S (1994): Vasoactive intestinal polypeptide (VIP)-containing neurons: distribution throughout the brain of the chick (Gallus domesticus) with focus upon the lateral septaal organ Cell Tissue Res., 275, 91-107.
64. Kulick RS, Chaiseha Y, Kang SW, Rozenboim I, El Halawani ME (2005): the relative importance of vasoactive intestinal peptide and peptide histidine isoleucine as physiological regulators of prolactin int he domestic turkey Gen.Comp.Endocrinol., 142, 267-273.
65. Kuenzel WJ, Golden CD (2006): Distribution and change in number of gonadotropin-releasing hormone-1 neurons following activation of the photoneuroendocrine system in the chick, Gallus gallus Cell Tissue Res.,325, 501-512.
66. Kumar P, Chaturvedi CM (2008): Correlation of nitric oxide (NO) activity and gonadal function in Japanese quail, Coturnix coturnix japonica following temporal phase relation of serotoninergic and dopaminergic oscillations Anim.Reprod.Sci., 106, 48-64.
67. Li H, Kuenzel WJ (2008): A possible neural cascade involving the photoneuroendocrine system (PNES) responsible for regulating gonadal development in an avian species, Gallus gallus Brain Res.Bull., 76, 586-596.
68. Lim S, Pnueli L, Tan JH, Naor Z, Rajagopal G, Melamed Ph (2009): Negative feedback governs gonadotropefrequency-decoding of gonadotropin releasing hormone pulse-frequency PlosONE 4, e7244.
69. Liu RC, Lea RW, Sharp PJ (1995): Sexually differentiated role of calcium ion sin chicken GnRH-I-stimulated release of LH from anterior pituitary glands from adult domestic chickens Gen.Comp. Endocrinol., 100, 267-272.

70. MacNamee MC, Sharp PJ (1989): The functional activity of hypothalamic 5-hydroxytryptamine neurones in broody bantam hens J.Endocrinol., 120, 125-134.
71. Maddineni S, Ocon-Grove OM, Krzysik-Walker SM, Hendricks GL 3rd, Proudman JA, Ramachandran R (2008): Gonadotrophin-inhibitory hormone receptor expression in the chicken pituitary gland:potential influence of sexual maturation and ovarian steroids J.Neuroendocrinol., 20, 1078-1088.
72. Maddineni SR, Ocon-Grove OM, Krzysik-Walker SM, Hendricks GL 3rd, Ramachandran R (2008): Gonadotropin-inhibitory hormone (GnIH) receptor gene is expressed in the chicken ovary: potential role of GnIH in follicular maturation Reproduction, 135, 267-274.
73. Maney DL, Richardson RD, Wingfield JC (1997): Central administration of chicken gonadotropin-releasing hormone-II enhances courtship behaviour in a female sparrow Horm.Behav., 32, 11-18.
74. Matsui T (1969): Fine structural difference between the anterior and posterior divisions in the pigeon median eminence. Seminar on Hypothalamic and Endocrine Functions in Birds, Tokyo 19-20.
75. Mauro LJ, Elde RP, Youngren OM, Phillips RE, El Halawani ME (1989): Alterations in hypothalamic vasoactive intestinal peptide-like immunoreactivity are associated with reproduction and prolactin release in the female turkey Endocrinology, 125, 1795-1804.
76. Mayer U, Watanabe S, Bischof HJ (2010): Hippocampal activation of immediate early genes Zenk and c-Fos in zebra finches (Taenopygia guttata) during learning and recall of a spatial memory task Neurobiol.Learn.Mem., 93, 322-329.
77. McCann SM, Karanth S, Mastronardi CA, Dees WL, Childs G, Miller B, Sower S, Yu WH (2001): Control of gonadotropin secretion by follicle-stimulating hormone-releasing factor, luteinizing hormone-releasing hormone, and leptin Arch.Med.Res., 32, 476-485.
78. McCann SM, Karanth S, Mastronardi CA, Dees WL, Childs G, Miller B, Sower S, Yu WH (2002): Hypothalamic control of gonadotropin secretion Prog.Brain Res., 141, 151-164.
79. Meddle SL, Bush S, Sharp PJ, Millar RP, Wingfield JC (2006): Hypothalamic pro-GnRH-GAP, GnRH-I and GnRH-II during the onset of photorefractoriness in the white-crowned sparrow (Zonotrichia leucophrys gambellii) J.Neuroendocrinol., 18, 217-226.
80. Meddle SL, Maney DL, Wingfield JC (1999): Effects of N-methyl-D-aspartate on luteinizing hormone release and Fos-like immunoreactivity in the male White-crowned sparrow (Zonotrichia leucophrys gambellii) Endocrinology, 140, 5922-5928.
81. Mikami S, Yamada S (1984): Immunohistochemistry of the hypothalamic neuropeptides and anterior pituitary cells in the Japanese quail J.Exp.Zool., 232, 405-417.
82. Mikami SI, Oksche A, Farner DS, Vitums A (1970): Fine structure of the vessels of the hypophysial portal system of the white crowned sparrow, Zonotrichia leucophrys gambelii Z.Zellforsch. Mikrosk.Anat., 106, 155-174.
83. Millam JR, Burke WH, El Halawani ME (1984): Release of gonadotropin releasing hormone from the japanese quail hypothalamus in vitro Gen.Comp.Endocrinol., 53, 293-301.
84. Millam JR, Faris PL, Youngren OM, El Halawani ME, Hartman BK (1993): Immunohistochemical localization of chicken gonadotropin-releasing hormones I and II (cGnRH I and II) in turkey hen brain J.Comp.Neurol., 333, 68-82.
85. Millam JR, Ottinger MA, Craig-Veit CB, Fan Y, Chaisea Y, El Halawani M (1998): Multiple forms of GnRH are released from perifused medial basal hypothalamic/preoptic area (MBH/POA) explants in birds Gen.Comp.Endocrinol., 111, 95-101.
86. Millam JR, Wang R, Craig-Veit CB,Siopes TD (2002): Apposition of enkephalinergic axons with cGnRH I-containing perikarya in turkey hen brain Gen.Comp.Endocrinol., 127, 128-135.
87. Millar RP, Milton RC, Follett BK, King JA (1986): Receptor binding and gonadotropin-releasing activity of a novel chicken gonadotrophin-releasing hormone (/His5, Trp7, Tyr8/GnRH) and a D-Arg6 analog Endocrinology, 119, 224-231.
88. Montagnese CM, Mezey SE, Csillag A (2003): Efferent connections of the dorsomedial thalamic nuclei of the domestic chick (Gallus domesticus) J.Comp.Neurol., 459, 301-326.
89. Montagnese CM, Székely AD, Ádám, A, Csillag A (2004): Efferent connections of septal nuclei of the domestic chick (Gallus domesticus) an anterograde pathway tracing study with a bearing on functional circuits J.Comp.Neurol., 469, 437-456.

90. Mulrenin EM, Witkin JW, Silverman AJ (1999): Embryonic development of the gonadotropin-releasing hormone (GnRH) system in the chick: a spatio-temporal analysis of GnRH neuronal generation, site of origin, and migration endocrinology, 140, 422-433.
91. Naor Z (2009): Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor Front.Neuroendocrinol., 30, 10-29.
92. Neumeier C, Lametschwandtner A (1994): The vascularization of the pituitary gland of the chicken (Gallus domesticus). A scanning electron microscope study of vascular corrosion casts Arch.Histol. Cytol., 57, 213-233.
93. Ojeda SE, Lomniczi A, Sandau US (2008): Glial-gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion J.Neuroendocrinol., 20, 732-742.
94. Panzica G, Vigletti-Panzica C, Balthazart J (2001): Sexual dimorphism in the neuronal circuits of the quail preoptic and limbic regions Microsc.Res.Tech., 54, 364-374.
95. Parent AS, Mungenast AE, Lomniczi A, Sandau US, Peles E, Bosch MA, Ronnekleiv OK, Ojeda SR (2007): A contactin-receptor-like protein tyrosine phosphatase beta complex mediates adhesive communication between astroglial cells and gonadotropin-releasing hormone neurones J.Neuroendocrinol., 19, 847-859.
96. Parry DM, Goldsmith AR (1993): Ultrastructural evidence for changes in synaptic input to the hypothalamic luteinizing hormone-releasing hormone neurons sin photosensitive and photorefractory starlings J.Neuroendocrinol., 5, 387-395.
97. Péczely P, Calas A (1970): Ultrastructure de l'éminence médiane du pigeon dans diverses conditions expérimentales Z.Zellforsch.Mikrosk.Anat., 111, 316-345.
98. Péczely P, Kiss JZ (1988): Immunoreactivity to vasoactive intestinal polypeptide (VIP) and thyreotropin-releasing hormone (TRH) in hypothalamic neurons of the domesticated pigeon (Columba livia). Alterations following lactation and exposure to cold Cell Tissue Res., 251, 485-494.
99. Péczely P, Kovács KJ (2000a): Photostimulation affects gonadotropin releasing hormone immunoreactivity and activates a distinct neuron population in the hypothalamus of the mallard Neurosci.Letters, 290, 205-208.
100. Péczely P, Kovács KJ (2000b): Effect of season and photostimulation on the neural activation and GnRH immunoreactivity in the brain of mallard Abstr.VII.Intern.Symp.Avian Endocrinol., Varanasi 2.18 P.
101. Péczely P, Kovács KJ (2000c): Regulatory role of photosensitive and glutaminergic hypothalamic neuron sin the GnRH secretion of mallards Abstr.11th Intern.Congr.Endocrinol., Sydney, 110.
102. Perera AD, Follett BK (1992): Photoperiodic induction in vitro: the dynamics of gonadotropin-releasing hormone releasing hormone release from hypothalamic explants of the Japanese quail Endocrinology, 131, 2898-2908.
103. Proudman JA, Scanes CG, Johannsen SA, Berghman LR, Camp MJ (2006): Comparison of the ability of the three endogenous GnRHs to stimulate relase of follicle-stimulating hormone and luteinizing hormone in chickens Domest.Animal Endocrinol., 31, 141-153.
104. Purwana IN, Kanasaki H, Oride A, Mijiddorj T, Shintani N, Hashimoto H, Baba A, Miyazaki, K (2010): GnRH induced PACAP and PAC1 receptor expression in pituitary gonadotrophs: a possible role int he regulation of gonadotropin subunit gene expression Peptides 31, 1748- 1755.
105. Rattenborg NC, Martinez-Gonzalez D (2011): A bird-brain view of episodic memory Behav.Brain Res., 222, 236-245.
106. Real MA, Pineda D, Dávila JC, Guirado S (2008): Development and adult organization of the lateral part of th ebed nucleus of the satria terminalis in the chicken Brain Res.Bull., 75, 410-413.
107. Reiner A, Karten HJ (1985): Comparison of olfactory bulb projections in pigeons and turtles Brain Behav.Evol., 27, 11-27.
108. Rozenboim I, Silsby JL, Tabibzadeh C, Pitts GR, Youngren OM, El Halawani ME (1993): Hypothalamic and posterior pitutary content of vasoactive intestinal peptide and gonadotropin-releasing hormones I and II in the turkey hen Biol.Reprod., 49, 622-626.
109. Saab SS, Lange HS, Maney DL (2010): Gonadotrophin-releasing hormone neuron sin a photoperiodic songbird express fos and egr-1 protein after a single long day J.Neuroendocrinol., 22, 196-207.

110. Saldanha CJ, Deviche PJ, Silver R (1994): Increased VIP and decreased GnRH expression in photorefractory dark-eyed juncos (Junco hyemalis) Gen.Comp.Endocrinol., 93, 128-136.
111. Saldanha CJ, Silverman AJ, Silver R (2001): Direct innervation of GnRH neurons by encephalic photoreceptors in birds J.Biol.Rhytms., 16, 39-49.
112. Saldanha CJ, Walters BJ, Fraley GS (2010): Neurons that co-localize aromatase- and kisspeptin-like immunoreactivity may regulate the HPG axis of the mallard drake (Anas platyrhynchos) Gen. Comp.Endocrinol., 166, 606-613.
113. Sartsoongnoen N, Kosonsiriluk S, Prakobsaeng N, Songserm T, Rozenboim I, El Halawani ME, Chaiseha Y (2008): The dopaminergic system in the brain of the native Thai chicken, Gallus domesticus: localization and differential expression across the reproductive cycle Gen.Comp. Endocrinol., 159, 107-115.
114. Sharp PJ, Ciccone N (2005): The gonadotrophin releasing hormone neurone: key to avian reproductive function In: Functional Avian Endocrinology (eds.: A. Dawson, PJ. Sharp) Narrosa Publishing House, New Delhi, Chennai, Mumbai, Kolkata, 59-72.
115. Sharp PJ, Dawson A, Lea RW (1998): Control of luteinizing hormone and prolactin secretion in birds Comp.Biochem.Physiol. C Pharmacol.Toxicol.Endocrinol., 119, 275-282.
116. Sharp PJ, MacName MC, Talbot RT, Sterling RJ, Hall TR (1984): Aspects of the neuroendocrine control of ovulation and broodiness in the domestic hen J.Exp.Zool., 232, 475-483.
117. Sharp PJ, Sterling RJ, Talbot RT, Huskisson NS (1989a): The role of hypothalamic vasoactive intestinal polypeptide in the maintenance of prolactin secretion in incubating bantam hens: observations using passive immunization, radioimmunoassay and immunohistochemistry J.Endocrinol., 122, 5-13.
118. Sharp PJ, Talbot RT, MacName MC (1989b): Evidence for the involvement of dopamine and 5-hydroxytryptamine in the regulation of the preovulatory release of luteinizing hormone in the domestic hen Gen.Comp.Endocrinol., 76, 205-213.
119. Shimizu M, Bédécarrats GY (2010): Activation of the chicken gonadotropin- inhibitory hormone receptor reduces gonadotropin releasing hormone receptor signaling Gen.Comp.Endocrinol., 167, 331-337.
120. Singh KB, Dominic CJ (1975): Anterior and posterior groups of portal vessels in the avian pituitary: incidence in forty nine species Arch.Anat.Microsc.Morphol.Exp., 64, 359-374.
121. Singh RM, Dominic CJ (1970): Disposition of the portal vessels of the avian pituitary in relation to the median eminence and the pars distalis Experientia, 26, 962-964.
122. Sonez MC, Sonez CA, Mugnaini MT, Haedo M, Romera SA, Lombardo DM, Delhon GA (2009): Effects of differential pulse frequencies of chicken gonadotrophin-releasing hormone-I (cGnRH-I) on laying hen gonadotrope responses in vitro Biotech.Histochem., 85, 355-363.
123. Stevenson TJ, Arckens L, MacDougall-Shackleton SA (2007): Distribution of gonadotropin releasing-hormone-II in the house sparrow brain (Passer domesticus) Gen.Comp.Endocrinol., 150, 96-105.
124. Stevenson TJ, Ball GF (2009): Anatomical localization of the effects of reproductive state, castration, and social milieu on cells immunoreactive for gonadotropin-releasing hormone-I in male European starlings (Sturnus vulgaris).
125. Stevenson TJ, Bernard DJ, Ball GF (2009): Photoperiodic condition is associated with region-specific expression of GnRH1 mRNA in the preoptic area of thr male starling (Sturnus vulgaris) Biol.Reprod., 81, 674-680.
126. Stevenson TJ, MacDougall-Shackleton SA (2005): Season- and age-related variation in neural cGnRH-I and cGnRH-II immunoreactivity in house sparrows (Passer domesticus) Gen.Comp. Endocrinol., 143, 33-39.
127. Sun YM, Dunn IC, Baines E, Talbot RT, Illing N, Millar RP, Sharp PJ (2001b): Distribution and regulation by oestrogen of fully processed and variant transcripts of gonadotropin releasing hormone I and gonadotropin releasing hormone receptor mRNAs in the male chicken J.Neuroendocrinol., 13, 37-49.
128. Sun YM, Flanagan CA, Illing N, Ott TR, Sellar R, Fromme BJ, Hapgood J, Sharp PJ, Sealfon SC, Millar RP (2001a): A chicken gonadotropin-releasing hormone receptor that confers agonist activity to mammalian antagonists. Identification of D-Lys(6) in the ligand and extracellular loop two of the receptor as determinants J.Biol.Chem., 276, 7754-7761.

129. Székely A (1999): The avian hippocampal formation: subdivisions and connectivity Behav.Brain Res., 98, 219-225.
130. Tachibana T, Moriyama S, Takahashi A, Tsukada A, Oda A, Takeuchi S, Sakamoto T (2011): Isolation and characterization of prolactin-releasing peptide in chicks and its effect on prolactin release and feeding behaviour J.Neuroendocrinol., 23, 74-81.
131. Taziaux M, Kahn A, Moore J 3rd, Balthazart J, Holloway KS (2008): Enhanced neural activation in brain regions mediating sexual responses following exposure to a conditioned stimulus that predicts copulation Neuroscience, 151, 644-658.
132. Teruyama R, Beck MM (2000): Changes in immunoreactivity to anti-cGnRH-I and -II are associated with photostimulated sexual status in male quail Cell tissue Res., 3000, 413-426.
133. Teruyama R, Beck MM (2001): Double immunocytochemistry of vasoactive intestinal peptide and cGnRH-I in male quail: photoperiodic effects Cell Tissue Res., 303, 403-414.
134. Tömböl T, Davies DC, Németh A, Sebestény T, Alpár A (2000): A comparative Golgi study of chicken (Gallus domesticus) and homing pigeon (Columba livia) hippocampus Anat.Embryol. (Berl), 201, 85-101.
135. Thayananuphat A, Youngren OM, Kang SW, Bakken T, Kosonsiriluk S, Chaiseha Y, El Halawani ME (2011): Dopamine and mesotocin neurotransmission during the transition from incubation to brooding int he turkey Horm.Behav., 60, 327-335.
136. Trottier C, Repérant J, Miceli D (1995): Anatomical evidence of a retino-thalamo-hippocampal pathway in the pigeon (Columba livia) J.Hirnforsch., 36, 489-500.
137. Tsutsui K, Bentley G, Ciccone N (2005): Structure, action and functional significance of GnIH In: Functional Avian Endocrinology (eds.: Alistar Dawson, Peter J Sharp), 73-82., Narosa Publishing House New Delhi, Chennai, Mumbai, Kolkata
138. Tsutsui K, Sagioh E, Yin H, Ubuka T, chowdhury VS, Osugi T, Ukena K, Sharp PJ, Wingfield JC, Bentley GE (2009): A new key neurohormone controlling reproduction, gonadotrophin-inhibitory hormone in birds: discovery, progress and prospects J.Neuroendocrinol., 21, 271-275.
139. Tsutsui K, Saigoh E, Ukena K, Teranishi H, Fujishawa Y, Kikuchi M, Ishii S, Sharp PJ (2000): A novel avian hypothalamic peptide inhibiting gonadotropin release Biochem. Biophys. Res. Communications, 661-667.
140. Ubuka T, Bentley GE, Ukena K, Wingfield JC, Tsutsui K (2005): Melatonin induces the expression of gonadotropin-inhibitory hormone in the avian brain Proc.Natl.acad.Sci. USA, 1023052-3057.
141. Ubuka T, Kim S, Huang YC, Reid J, Jiang J, Osugi T, Chowdhury VS, Tsutsui K, Bentley GE (2008): Gonadotropin-inhibitory hormone neurons interact directly with gonadotropin-releasing hormone-I and -II neuron sin European starling brain Endocrinology, 149, 268-278.
142. Ukena K, Ubuka T, Tsutsui K (2003): Distribution of a novel avian gonadotropin-inhibitory hormone in the quail brain Cell Tissue Res., 312, 73-79.
143. van Gils J, Absil P, Grauwels L, Moons L, Vandesande F, Balthazart J (1993): Distribution of luteinizing hormone-releasing hormones I and II (LHRH-I and -II) in the quail and chicken brain as demonstrated with antibodies directed against synthetic peptides J.Comp.Neurol., 334, 304-323.
144. Vitums A, Mikami SI, Oksche A, Farner DS (1964): Vascularization of the hypothalamo-hypophyseal complex in the white-ceowned sparrow, Zonotrichia leucophrys gambelii Z.Zellforsch.Mikrosk. Anat., 64, 541-569.
145. Vizcarra JA, Kreider DL, Kirby JD (2004): Episodic gonadotropin secretion in the mature fowl: serial blood sampling from unrestrained male broiler breeders (Gallus domesticus) Biol.Reprod., 70, 1798-1805.
146. Wacker DW, Schlinger BA, Wingfield JC (2008): Combined effects of DHEA and fadrozole on aggression and neural VIP immunoreactivity in the non-breeding male song sparrow Horm. Behav., 53, 287-294.
147. Watanabe M, Sakuma Y, Kato M (2009): GABAA receptors mediate excitation in adult rat GnRH neurons Biol.Reprod., 81, 327-332.
148. Wilson SC, Chairil RA, Cunningham FJ, Gladwell RT (1990): Changes in the hypothalamic contents of LHRH-I and –II and in pitutary responsiveness to synthetic chicken LHRH-I and –II during the progesterone-induced surge of LH in the laying hen J.Endocrinol., 127, 487-496.

149. Wingstrand KG (1951): The structure and development of the avian pituitary Gleerup, Lund 316.
150. Wu M, Dumalska I, Morozova E, van den Pol AN, Aireja M (2009a): Melanin- concentrating hormone directly inhibits GnRH neurons and blocks kisspeptin activation, linking energy balance to reproduction Proc.Natl.Acad.Sci. USA, 106, 17217-17222.
151. Wu M, Dumalska I, Morozova E, van den Pol AN, Aireja M (2009b): Gonadotropin inhibitory hormone inhibits basal forebrain vGluT2-gonadotropin-releasing hormone neurons via direct postsynaptic mechanism J.Physiol., 587, 1401-1411.
152. Yamamura T, Hirunagi K, Ebihara S, Yoshimura T (2004): Seasonal morphological changes in the neuro-glial interaction between gonadotropin-releasing hormone nerve terminals and glial endfeet in Japanese quail Endocrinology, 145, 4264-4267.
153. Yang D, Caraty A, Dupont J (2005): Molecular mechanisms involvedd in LH release by the ovine pituitary cells Domest.Anim.Endocrinol., 29, 488-507.
154. Youngren OM, Chaiseha Y, El Halawani ME (1998): Regulation of prolactin secretion by dopamine and vasoactive intestinal peptide at the level of the pituitary in the turkey Neuroendocr., 68, 319-325.
155. You S, Hsu CC, Kim H, Kho YJ, El Halawani ME, Farris J, Foster DN (2001): Molecular cloning and expression analysis of the turkey vasoactive intestinal peptide receptor Gen.Comp.Endocrinol., 124, 53-65.
156. Yu WH, Karanth S, Mastronardi CA, Sealfon S, Dean C, Dees WL, McCann SM (2002): Lamprey GnRH-III acts on its putative receptor via nitric oxide to release follicle-stimulating hormone specifically Exp.Biol.Med. (Maywood) , 227, 786-793.
157. Zawilska JB, Niewiadomski P, Nowak JZ (2004a): Receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide in turkey cerebral cortex: characterization by /125/-VIP binding and effects on cyclic AMP synthesis Gen.Comp.Endocrinol., 137, 187-195.
158. Zawilska JB, Gendek-Kubiak H, Woldan-Tambor A, Wiktorowska-Owczarek A, Nowak JZ (2005): Histamine-induced cyclic AMP formation in the chick hypothalamus: interaction with vasoactive intestinal peptide Pharmacol.Rep., 57, 188-194.
159. Zawilska JB, Niewiadomski P, Nowak JZ (2004b): Receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide in the goose cerebral cortex Pol.J.Pharmacol., 56, 203-211.
160. Zhang C, Bosch MA, Rennekleiv OK, Kelly MJ (2009): Gamma-aminobutyric acid B receptor mediated inhibition of gonadotropin-releasing hormone neurons is supressed by kisspeptin-G protein-coupled receptor 54 signaling Endocrinology, 150, 2388-2394.
161. Zhou M, Lei M, Rao Y, Nie Q, Zeng H, Xia M, Liang F, Zhang D, Zhang X (2008a): Polymorphism of vasoactive intestinal peptide receptor-1 gene and their gene-tic effects on broodiness in chickens Poult. Sci., 87, 893-903.

5.8. The gonadotropic function of adenohypophysis

The avian adenohypophysis consists of the sheet-like pars tuberalis that leans on the median eminence, and the pars distalis, which fills the bony sella turcica. In birds, the pars intermedia is not separated, its characteristic hormone, the melanocyte-stimulating hormone (MSH) is produced by specialised pars distalis cells or ACTH cells. The pars distalis consists of an anterior or cephalic and a posterior or caudal lobe, which contain different cells. Comparing differential staining methods using light microscopy, electon microscopic studies and immunocytochemical results shows that adrenocorticotropic hormone (ACTH), prolactin (PRL), thyrotropin stimulating hormone (TSH) and melanocyte-stimulating hormone (MSH) producing cells are located in the cephalic lobe. Follicle-stimulating hormone (FSH) producing cells are also located in the cephalic lobe. Separated cells of the caudal lobe produce growth hormone (STH) and luteinising hormone (LH). In some species, the production of glycoprotein hormones is not separated between the two lobes, FSH, LH and TSH cells can be found in the cephalic, as well as the caudal lobes (Mikami and Yamada, 1984, Mikami, 1986, Proudman et al., 1999).

The embryonal and postembryonal development of pituitary hormone synthesis and the ontogenesis of FSH and LH cells are relatively little known.

Gonadotropic cells appear in chicken adenohypophysis on Day 8 of incubation, when FSH and LH-immunopositive cells can be detected in the caudal lobe of the adenohypophysis by using antibodies developed for FSH-β and LH-β subunits. Using RT-PCR analysis, LH-mRNA can first be detected on Day 4 on incubation and FSH mRNA on Day 7. Up to Day 10 of incubation, the two gonadotropic cells are only found in loose formation in the caudal lobe, however, from Day 12 round and cuboid gonadotropes also appear in the cephalic lobe. LH and FSH cells are found in higher numbers in the caudal lobe during the entire embryonic development (Maseki et al., 2004).

Studies conducted on chicken show that plasma FSH level starts low (7.4 ng/ml), and increases slowly and gradually until the end of the premature stage. During maturation it continues to increase sharply, reaching 225ng/ml (Scanes et al., 1977a,b). In roosters, LH concentration is 4.5 ng/ml in the first weeks after hatching, it slightly decreases in weeks 7-9, reaches 7.5 ng/ml during the second-third month, and continues to increase during maturation to reach 9.7 ng/ml (Williams and DeReviers, 1981). Similar changes in LH level were described in young great tits (*Parus major*), where GnRH sensitivity already appread in one day-old birds, suggesting the early appearance of GnRH receptors in LH cells (Silverin and Sharp, 1996).

Using light microscopic staining techniques, the two gonadotropic cells stain PAS positive and are basophilic. In domestic duck and pigeon, electron microscopic studies show FSH (β) cells that contain smaller, strongly electron

dense granula in the cephalic lobe, while larger LH (γ) cells that contain less dense, larger sized and strongly heteromorphous granula are located in the caudal lobe (Tixier-Vidal and Follett, 1973, Péczely and Szokoly, 1969, Péczely and Szokoly, unpublished data).

Avian gonadotropic hormones were first purified and isolated from chicken adenohypophysis in 1969 by Stockel-Hartree and Cunningham. Bioassays showed that FSH increased spermatogenesis in the testis of roosters both on its own and in synergy with testosterone and LH increased testicular testosterone production. Testis prepared from young ducks was found to be especially sensitive to LH (Bona-Gallo et al., 1983, Chase, 1982). Using purified avian gonadotropin, sensitive RIA methods were developed to identify the following molecules in plasma, cell culture and tissue incubate: chicken LH (Follett et al., 1972), chicken FSH (Scanes et al., 1977b), turkey LH (Burke et al., 1979), ostrich (*Struthio camelus*) LH and FSH (Bona-Gallo et al., 1983). The produced antibodies (antisera) made it possible to develop heterologue RIA methods that also work well in other bird species.

Studying the structure of avian gonadotropins showed that they are glycoprotein hormones of 25-30 kDa molecular weight that contain about 6-14% carbohydrates. Both hormones have a heterodimeric structure, consisting of an α and a β subunit, and the α subunit (similar to all known glycoprotein hormones) is the same in FSH and LH. The ***α* subunit** is responsible for receptor binding and singnal transduction, while the β subunit provides the specific activity of the hormone and species specificity. The two subunits are synthesised independently and are expressed by different genes. In the cytoplasm, they bind to each other posttranslationally by non-covalent bonds to form a biologically active heterodimer. Isolating, sequencing and cloning the complementer (c)-DNA of the subunits made it possible to identify their entire amino acid sequence.

Subunit α was studied in turkey, ostrich, domestic duck and Muscovy duck (Foster and Foster, 1991, Koide et al., 1996, Ya-Lun Hsieh, 2001). It contains 96 amino acid (residues), and shows 100% similarity within birds and 70-80% similarity with known vertebrate α subunit amino acid sequences. A 24 amino acid-containing signal polypeptide sequence binds to this. There are 10 cystein residues in subunit α, which form 5 disulphid bridges. The N-linked glycosylation sites on two, aspartic acid-residues at the 57th and 82nd positions are also characteristic to the molecular structure. In a hypophyseal cell culture mRNA synthesis of the α subunit is increased by GnRH and TRH, and inhibited by estradiol, testosterone and thyroid hormones.

In chicken, the **β subunit** consists of 111 amino acids, with a 20 amino-acid long signal protein bound to it. The molecule contains 12 cystein residues, which form six disulphide bridges and two aspartic acid-linked glycosylational sites and four proline residues that regulate species specificity and receptor binding

of the molecule. Chicken FSH β subunit is 98% similar to the one in Japanese quail, 93% to ostrich, and 66-70% to FSH β from different mammals. It only shows 37% similarity to chicken LH β and 40% to chicken TSH β (Shen and Yu, 2002, Kikuchi et al., 1998).

The LH β subunit was studied in ostrich, turkey, chicken and Japanese quail. In turkey, the molecule contains 39 signal amino acids and 120 apoprotein-forming amino acids. In Japanese quail, LH β consists of 47 and 119 amino acid residues and in ostrich 128. Adding GnRH to isolated adenohypophyseal cells increased LH β expression, while prolactin decreased LH β synthesis (Koide et al., 1996, You et al., 1995, Ando and Ishii, 1994, Noce et al., 1989).

In female birds, FSH is the factor primarily responsible for the development of ovarian follicles, its binding can be detected in granulosa, theca and stromal cells. Its effect is especially pronounced in prehierarchical (white and small yellow) follicles and gradually decreases in large yellow follicles from F6 to F1. In atretising follicles FSH binding decreases substantially. Besides stimulating mitotic division and inhibiting apotosis in granulosa cells, in vitro FSH also slightly increases progesterone, androgen and estrogen production in prehierarchical follicles in theca tissue. In males, it increases mitosis in the Sertoli cells, as well as their testosterone production and also initiates and increases spermatogenesis.

The specific binding of **FSH** to cells is realised by **membrane receptors** that form in the membrane of those cells. Chicken FSH receptor (cFSH-R) has a heptahelical structure and it contains 693 amino acids, the first 17 of which forming the signal sequence. Its molecular weight is 78,767 Dalton. The FSH receptor binds to the G protein, and conducts signal transduction through the adenylcyclase-protein kinase A and the phospholipase-C phosphatidylinositol systems.

The (extracellular) ectodomain with multiple accordion-like plaits ends in an N-domain, and contains 349 amino acids. It has three N-linked glycosylation sites and 11 cysteine residues. The transmembrain domain that forms the seven pliae consists of 264 amino acids, and its intracellular loops contain several tyrosine, serine amd threonine residues, which provide potential sites for phosphorylation. Two elements (Thr 555 and Ser 596) of the transmembrane domain are sites for protein kinase-C phosphorylation, there are nine cysteine residues and one N-linked glycosylation site is this section. The intracellular domain ending in a C-terminal is substantially shorter, consisting of 63 amino acids, with two phosphorylational sites at Thr 632 and Thr 658 (You et al., 1996). Japanese quail FSH receptor amino acid sequence shows 47.4% similarity to its LH receptor sequence (Akazome et al., 1996).

In females, LH increases progesterone synthesis of the granulosa cells in the largest yellow follicles and it is inefffective in white and small yellow follicles. Before ovulation, the sudden LH surge caused by the progesterone trigger causes rupure of the F1 follicular wall and ovulation. In males, increasing

LH plasma level causes increased testosterone production in the Leydig cells of the testis.

Avian **LH** binds to heptahelical **membrane receptors**, which have similar structure to mammalian LH and avian FSH. The structure of avian LH receptor was studied in Japanese quail testicular cell culture and in the granulosa cells of the ovarian follicles by cDNA sequencing and cloning. The receptor peptide isolated from granulosa cell culture begins with a hydrophilic ectodomain (extracellular part) of 19 signal amino acids, followed by 366 amino acid residues, forming an accordion-like, multiple-times bent element. The transmembrane domain of seven loops consists of 267 amino acid residues. The intracellular section consists of 76 amino acid residues, which end in the C-terminal. The amino acid sequence of chicken LH receptor is 67-69% similar to the amino acid sequence of known mammalian (human) LH receptors and 51% similar to the sequence of the chicken FSH receptor (Mizutani et al., 1998). Receptors of the avian LH, with a structure very similar (98%) to that of chicken, have also been detected in Japanese quail and turkey (You et al., 2000). The cDNA of the complete receptor expresses in granulosa cell culture obtained from yellow follicles of chicken, and the addition of cLH causes this system to produce cAMP in a dose-dependent way. cLH-R mRNA expression can not be detected in granulosa cells from little white follicles (3-8 mm) from chicken ovary, it only initiates in follicles of 9-12 mm size, and continues to significantly increase in granulosa cell cultures of F3-F2-F1 follicles (Akazome et al., 1994, Johnson et al., 1996, Mizutani et al., 1998).

5.9. Adenohypophyseal prolactin production and the physiological role of prolactin in birds

Classic light microscopic staining methods have shown that the erythrosinophil-acidophil (η) cells located in the cephalic lobe of the pars distalis produce prolactin (Tixier-Vidal and Follett, 1973, Mikami et al., 1975). Later immunocytochemical studies confirmed these results (Mikami and Yamada, 1984). According to electromicroscopic studies, prolactin cells are quite large, oval structures, often aligned in lines, with well-developed granular endoplasmic reticulum, strong Golgi complex and a large amount of mitochondria. The granula are 4-600 nm in diameter and are polymorphic (Mikami and Yamada, 1984).

A **prolactin gene** containing five exons and four introns has been identified in goose adenohypophysis. It was 98.4% similar to duck, 92.2% similar to turkey, 92% similar to chicken and 91.9% similar to Japanese quail cDNA. Among its transcriptional binding sites, Pit-1 (–130/–122) and VIP (-64/-53) seem to be functionally important. However, estrogen binding sites are not found in the

regulatory region of the prolactin gene, which suggests that it does not have a direct effect. Expressed pre-prolactin contains 229 amino acids. The peptide hormone consists of a 30 amino acid signal sequence and a 199 amino acid-long active molecular stem, which shows over 90% similarity and equal secondary and tertiary structure with duck, quail, chicken and turkey prolactin. It is also 54–78% homologous to mammalian prolactins. In goose and chicken five isoforms have been identified. Its molecular weight is approximately 26 kDa. The amino acid sequence of prolactin is 26% similar to chicken growth hormone (Hanks et al., 1989, Watahiki et al., 1989, Wong et al., 1991, Kurima et al., 1995, Van As et al., 2006, Liu et al., 2008).

Prolactin binds specifically in several tissues. The cDNA of its **membrane receptor** has been isolated from pigeon crop sac, as well as turkey and duck kidney tissue, and during its cloning an 830-832 amino acid-long peptide was isolated, with a molecular weight of 91.5 kDa. Prolactin receptor (Pr-R) is a peptide of the prolactin/growth hormone/cytokine receptor group. Its extracellular (ecto)domain consists of two homologous, repetitive units, its part farther away from the membrane contains 204 amino acids, while the part closer to the membrane contains 212. There are cysteine residues in both parts. The transmembrane domain of a single chain contains 24 amino acid residues, and the proline-rich intracellular domain contains 368. Pigeon crop sac prolactin receptor also binds rat prolactin with a high affinity (Chen and Horseman, 1994, Zhou et al., 1996, Wang et al., 2009).

According to comparative measurements in turkey, among the 26 tissues examined, prolactin receptor concentration was high in the adenohypophysis, hypothalamus, crop lining, duodenum and abdomen (Zhou et al., 1996). Besides the prolactin receptor content of the crop lining of doves, it was detected in significant amount in the ventromedial nucleus of the hypothalamus, which is the regulating centre of hyperphagia that occurs during chick rearing. Ovine prolactin injected into the ventromedial nucleus increases feeding behaviour in doves and anti-prolactin receptor antibody administered to the same location strongly decreased the effect of prolactin (Li et al., 1995).

Hypophyseal prolactin gene expression and plasma prolactin concentration change seasonally as well as during certain phases of the reproductive cycle. They can also differ in males and females. Several factors can influence prolatin secretion and changes in its plasma level. Such factors can be the direct or indirect effects of gonadotropins and sexual steroids and also the prolactin secretory-regulatory effects of long photoperiod, which can be related to the effects of photostimulation on other endocrine functions. Developing photorefractoriness can be in a cause-effect relationship with prolactin secretion. Prolactin is also a determining factor in the regulation of brooding, and it is an important component of the regulation of the complex processes of moulting and migratory behaviour.

Photostimulation increases plasma prolactin concentration in birds, irrelevant of their maturity. In Bantam hens and roosters, photostimulation increases plasma prolactin levels well before the birds reach maturity (16-18 weeks old), significant increase was detected at the ages of 4, 8, 12 and 16 weeks (Sreekumar and Sharp, 1998). After photostimulation, plasma prolactin level in photosensitive male and female song sparrows increases nearly parallel to photogonadostimulation, i.e. the increase of the size of the gonads (Wingfield and Goldsmith, 1990). After photostimulation plasma prolactin levels also start to increase immediately in male ducks, but its intensity is lower compared to the level of LH (Sharp et al., 1986). During photogonadostimulation, the amount of prolactin mRNA and prolactin in the hypophysis, as well as plasma prolactin levels more than double and during brooding following egg laying, all three parameters increase to 8-10 times their baseline level (Shimada et al., 1991). Photostimulation does not change the circadian rhythm of prolactin plasma levels in female turkeys, but the amplitude of the daily curve becomes higher at both the maximum (morning) and minimum (evening) levels (Proudman, 1998). In male turkeys, the effect of photostimulation is slower, triggering a smaller increase in prolactin plasma levels, and the drop in hormonal levels at the beginning of the photorefracter period typical to females does not occur in males. In males, prolactin levels start to decrease later, after the postnuptial moult has initiated (Proudman and Siopes, 2005). In female and male goose, prolactin plasma level increases during the period of photogonadostimulation, is high (showing maximum value) at the beginning of photorefractoriness, and decreases by a large amount in a later phase of photorefractoriness. During the postrefracter period prolactin plasma concentration stays at this low level (Péczely et al., 1993). In starling, on the other hand, gonadal regression associated with photorefractoriness coincides with a definite increase in plasma prolactin level (Dawson, 2006).

Changes associated with **photorefractoriness** are variant in different bird species. The development of photorefractoriness (ceasing of egg laying and ovarian regression) is associated with decreasing prolactin plasma levels in laying end/or brooding turkey. However, prolactin levels hardly change in those individuals that do not become photorefracter (Proudman and Siopes, 2002, 2006). The functional relationship between prolactin and thyroid hormones can also play a role in the development of photorefractoriness, but these effects are species specific. According to earlier studies, thyroxine treatment of starling resulted in the ceasing of breeding and induced photorefractoriness. Plasma prolactin level increased in T4-treated starlings (Goldsmith, Nicholls, 1984a). Thyroidectomy decreased plasma prolactin level and these individuals later did not become photrefracter (Goldsmith and Nicholls, 1984b). Thyroxine treatment of thyroidecomised starlings increased prolactin level, and these birds became photorefracter following photostimulation (Goldsmith et al.,

1985). During photostimulation the increasing prolactin level of female turkeys gives a peak value during the peak of egg production or right after it, and drastically declines when photorefractoriness is developing. During photostimulation plasma prolactin and T4 concentration are lower in those individuals that later become photorefracter (Proudman and Siopes, 2002). On the other hand, in female and male geese, plasma prolactin, T3 and T4 levels all increase after the peak of the breeding period, at the beginning of photorefractoriness. However, in a later stage of photorefractoriness prolactin levels decrease considerably, while T3 and T4 plasma concentrations show moderately high values. Thyroidectomy does not affect plasma prolactin levels neither in egg laying, nor in photorefractory geese (Péczely et al., 1993).

In species, where photogonado-stimulation does not trigger the initiation of the breeding cycle, plasma prolactin increase can also be observed along with gonadal development. Hyperprolactinemia can be related to a change in steroid levels. Zebra finch is an Australian opportunistic breeder. In this species, breeding is not triggered by the change in light regimes, but by the abundance of food after ample rainfall. Prolactin plasma level is lowest in non-paired individuals, it increases when birds form pairs and reaches its maximum in breeding pairs. The plasma level in this species does not show difference between males and females. Injecting VIP to non-breeding individuals causes maximum prolactin level increase, and has no effect in brooding birds. This response is different from photosensitive birds, where only individuals in the reproductive phase respond to VIP treatement. Therefore, in the opportunistic zebra finch the physiological prerequisite for prolactin secretion (PrR expression) is continuously present, and breeding can initiate very rapidly (Christensen and Vleck, 2008).

The changes in prolactin plasma level in laying hens during the egg laying cycle show inverse correlation with egg production. There is also negative correlation between prolactin plasma concentration, plasma estradiol and progesterone levels and egg production in untreated and bromo-ergocripitin treated hens, where prolactin secretion is inhibited (Reddy et al., 2002). Bromoergocripitin treated hens develop low prolactin level, but substantially higher plasma LH concentration, more intensive LH pulsation, and higher estradiol and progesterone levels compared to control individuals, and their egg production is also higher (Reddy et al., 2007).

During the reproductive cycle, prolactin can have a direct inhibitory effect on ovarian follicle proliferation and steroid production. Repeated injection of mammalian prolactin can cause atrophy in the large yellow follicles in chicken and turkey (Opel and Proudman, 1980). In vitro observations found that prolactin directly inhibited estradiol release in the white follicles, while in yellow preovulatory follicles it can have inhibitory or stimulatory effect, depending on the actual developmental stage of the follicle (Hrabia et al., 2004).

During maturation and in a sexually active stage, ovarian steroids can affect plasma prolactin levels. During sexual maturation in female turkey plasma prolactin level increases, but this increase does not occur in ovariectomised individuals. In ovariectomised geese, prolactin level increases slower during the growth of the individual and shows lower values than the high level characteristic of egglaying, mature individuals, and the peak typical of the beginning of the photorefractory stage is also significantly lower. The prolactin-secretion increasing effect of estrogen and progesterone is demonstrated by the experiment when estrogen and progesterone injected into ovariectomised turkey increased their plasma prolactin level (El Halawani et al., 1983, Péczely et al., 1993).

On the other hand, testosterone does not affect the increase in prolactin secretion that occurs during maturation in males: in castrated geese it did not affect the high plasma prolactin level (Péczely et al., 1993).

Prolactin and LH secretion, following their parallel increase typical at the beginning of maturation, change course during times of sexual activity and the subsequent periods of brooding and chick rearing. Their relation is mostly known in females. At the beginning of egg laying, the increase in LH during maturation is replaced by highly fluctuating plasma levels caused by the preovulatory peaks. Prolactin level continues to increase after sexual maturation, showing a lag compared to LH, obtaining its maximum value at the beginning of brooding and during the photorefracter phase. At this time, plasma LH is already strongly decreasing. High plasma prolactin concentration, along with progesterone that initially stays high, can be the primary determining (maybe even initiating) factor of brooding, to ensure hatching of the laid eggs. In a later stage of brooding, hatching or chick-rearing, high plasma prolactin concentration substantially decreases, meanwhile plasma LH level starts to increase again. Observing breeding canaries after disturbance and when they renested showed that brooding birds had high prolactin and low LH levels. Within 24 hours after the loss of the nest their prolactin level substantially decreased and at the same time the LH level sharply increased and when they renested, prolactin increased and LH concentration decreased again (Goldsmith et al., 1984a, 1984b, Péczely et al., 1993, Richard-Yris et al., 1998, Bédécarrats et al., 1999).

In doves that breed two-three times in a row, LH (and sexual steroids) and prolactin plasma levels change largely independently from each other. In females, LH level is high during courtship, and it gradually decreases following egg laying and at the beginning of incubation. Plasma prolactin level is low during courtship and remains low at the beginning of brooding, but increases sharply from mid-incubation, when the crop lining starts to thicken. In the first few days when the chicks hatch plasma prolactin level is high (the period of feeding them with crop milk), then it decreases and at the same time the crop lining becomes thinner. After the young become independent, plasma LH level starts to increase again, and a new courtship phase begins.

In males, the production of the two hypophyseal hormones also changes independently from one another, there is neither synergy nor explicit anagonism between their plasma levels. Prolactin plasma level is higher in male doves than in females (Goldsmith et al., 1981). Further studies suggested that the increase of prolactin level in doves during incubation is more related to the thickening of the crop lining (crop milk production) than to the development of brooding behaviour (Silver, 1984). Alternatively, other studies suggested that prolactin initiates chick feeding by regurgitation, as opposed to triggering crop milk secretion associated with the thickening of crop lining. In doves that have not incubated yet, repeated subcutaneous and intracerebroventricular injection of ovine prolactin initiated hyperphagia and chick-feeding behaviour (regurgitation), while there was no change in the thickness of crop lining (Buntin et al., 1991).

The role of prolactin is crucial in the initiation of **crop milk secretion** and the regulation of its production in pigeons, doves, flamingos and the emperor penguin (*Aptenodytes forsteri*). Crop milk is a defining nutrient during the first few days of life of the developing chicks, and depriving hatchlings of it leads to their death. The formation of crop milk is best known in pigeons, where its production starts about two days before the chicks hatch. In *Columbiformes,* the crop develops in the lower section of the esophagus, where it lacks propria glands. Here, at the medial sides of the esophagus two sac-like structures protrude. In the inactive state the wall of these structures is thin, but later they develop into large, thick-walled sacs that bulge out sideways. Active crop sacs are characterised by intensive proliferation of the epithelial layer, causing its thickening, while the fibrous connective tissue also thickens and becomes rich in blood vessels. Tissue proliferation does not occur in the medial part of the esophagus, but it develops gradually in a medio-lateral direction. The thickening of the mucosa is not uniform around the crop sac, it shows a reticulated surface and crypt-like pouches form on the luminar surface. The inward bulging crypts are separated by thin, prominent connective tissue hedges. The crypts that open into the luminar surface contain whitish-gray, rice grain-like concrements, formed by a mass of epithelial cells showing fatty degeneration. Regurgitation during feeding pushes these concrements into the lumen of the crop and the esophagus, where they mix with the product of the propria glands from higher sections (saliva). The resulting pulp-like material, the crop milk, is regurgitated by the parent into the mouth of the chick. The detached epithelial cells are continuously replaced by intensive division in the basal layer. As prolactin causes crop sacs to enlarge in a dose-dependent way, sensitive bioassays were developed in the 1950s and 60s. In this assay, an unknown sample is subcutaneously injected above one of the crop sacs in an inactive pigeon and a standard containing a known amount of prolactin is injected above the other one. From the mass and amount of tissue proliferation of the prepared crop

sac, the prolactin content of the sample can be deduced (Péczely, unpublished data). Later this method was replaced by a more sensitive radioreceptor assay based on the same principle (Forsyth et al., 1978).

The composition of the dry weight of pigeon crop milk is about 60% protein, 35% lipid, 1-3% carbohydrate, calcium, potassium, sodium and phosphates, as well as a significant amount of IgA, and a specific, EGF-like growth factor. Its lipid content is made up by triglycerides, and its carbohydrates are lipid-, and protein-bound sialic acid, fucose, glucosamine, galactose, mannose and glucose (Gillespie et al., 2011, Shetty et al., 1992, 1994).

The secretion of crop milk is triggered and maintained by the increasing plasma prolactin level during incubation. In the first days (week) of chick feeding, the mass of the crop sac increases about five fold, and this effect can also be achieved by systemic dosing with 1 mg/day prolactin for four days (Garrison and Scow, 1975). Many details of this process are well known. The prolactin-binding ability of the proliferated crop mucosa from pigeon parents feeding chicks is 4-5 times higher compared to non-feeding or juvenile birds, which indicates the increase in the amount of prolactin receptors expressing as a result of prolactin exposure (Kledzik et al., 1975). The binding of prolactin to epithelial cell membrane receptors leads to increased amount of signal transducer and activator of transcription (STAT) protein, activated by tyrosine phosphorylation (Horseman and Buntin, 1995). Prolactin causes the expression of a large number of genes in the mitotically dividing epithelial cells, the upregulation of 542 genes and downregulation of 639 genes have been observed. Among the upregulated genes, the expression of microphtalmia-associated transcription factor (MITF) increases about 6-fold in the lactating crop. This gene is activated by two signal mechanisms: the mitogen-activated protein kinase (MAPK) and the "wingless" (WNT) signal mechanisms.

MITF has a determining role in melanogenesis, during which its protein product activates seven genes (by upregulation): among them wnt signal receptor, frizzled homologue-3 and β-catenine. Since neither the inactive nor the active crop mucosa contains melanocytes, with its wide spectrum of effects, MITF probably acts on the basal layer of the epithelial stratum through extracellular matrix receptors, as a general initiator with a strong mitogenic effect. Rapid cell proliferation causes hypoxic stress in the epithelial tissue, which initiates antioxidant mechanisms (peroxiredoxin-1 and 3 heat shock protein-gene) and the expression of immune genes. A large part of the formed antioxidant and the immune proteins gets into the parakeratinised and lipid vacuole-containing epithelial cells, which later detach, forming an important ingredient of the crop milk. The upregulation of certain immune genes, such as platelet factor-4 and CD-36 (a membrane-bound glycoprotein, which assists platelet factor attachment) is also typical. The expression of the stomatostatin-coding gene, which modulates the function of the digestive system, also increases.

Increased lipid synthesis and accumulation in the parakeratinising epithelial cells provide the most typical ingredient of the crop milk. In birds, typically two acyl-CoA synthase get upregulated during this process. These genes code fatty acid oxidising enzymes in the parakeratinising epithelial cells, which result in the synthesis of neutral unsaturated triglycerides (Dumont, 1965). The precursor materials (VLDL) are produced by the liver and the adipose tissue, the blood stream carries them into the the mucosa of the crop lining, where they get into the differentiating epithelial cells by endocytosis. Triglycerides synthesised from these lipids in the epithelial cells get wrapped in micro-droplets by the Golgi complex and then transferred into the growing lipid vacuoles of the parakeratinising epithelial cells by the dynein microtubular system (Gillespie et al., 2011).

Incubation behaviour and actual circumstances of the incubation play an important role in the regulation of prolactin secretion in a reciprocal way. However, the modulatory effect of corticosterone can also be significant. Tactile stimuli from the eggs can be fundamental for the plasma prolactin level increase. Among others, this was suggested by the results of an experiment conducted on yellow-eyed penguin (*Megadyptes antipodes*). Placing an artificial egg in the nest increased prolactin concentration in females, while view of the egg in itself did not affect the level of prolactin. On the other hand, placing the artificial egg into the nest triggered incubatory behaviour in both males and females (Massaro et al., 2007). Removing the nest decreased plasma prolactin levels in bantam hens, and when incubating birds were removed from the nest and later united with hatchlings, which they adapted as their own, the prolactin concentration also decreased in these females (Sharp et al., 1988). The increase of plasma prolactin level is an important regulating factor of chich rearing, and in columbiformes prolactin treatment increases regurgitation of crop milk and pulp-like food, and also increases offspring-protective behaviour (Wang and Buntin, 1999).

The current body condition of the incubating female (body mass and the amount of fat deposits), as well as egg loss by predation during breeding as a proximal factor are also known to modulate parental behaviour and can also have hormonal effects, influencing corticosteroid and prolactin levels. In common eider (*Somateria mollissima*), when females do not feed during the 25 days of incubation, the size of the brood increases stress-stimulated corticosterone level caused by capture, but decreasing body weight does not affect stimulated corticosteroid levels. Plasma prolactin level is also tightly correlated with the decrease in body weight, and in those individuals where the incubation time was shortened body weight remained higher and higher prolactin levels were detected. On the other hand, increasing baseline corticosterone level by manipulation decreased plasma prolactin level (Criscuolo et al., 2002, 2006).

During **brooding and chick rearing**, **corticosterone**, as a stress-, and metabolic-hormone, can be in a reciprocally synergistic relationship with plasma prolactin level. Hyperphagia and related chick feeding are typical parental behaviours. The orexigenic effect of prolactin is determining in the initiation of hyperphagia, and it occurs through increased corticosterone secretion. Repeated intracerebroventricular injection of prolactin increases corticosterone plasma level in non-breeding doves, resulting in hyperphagia. However, if it was administered into the ventricle together with the glucocorticuoid antagonist RU38486, the stimulating effect of prolactin did not manifest (Koch et al., 2004).

The effects of **acute stress** on prolactin level were studied in Manx shearwater (*Puffinus puffinus*) during different stages of incubation and chick rearing. The plasma corticosterone-increasing effect of acute stress decreased plasma prolactin level during incubation and at the beginning of chick rearing, but increased prolactin level when the fed chicks were older, and this higher prolactin level permanently remained. The effect of prolactin on maintaining incubatory behaviour also manifested with the modulation of corticosterone (Riou et al., 2010). The relationship between breeding-chick rearing and the change in plasma corticosterone and prolactin levels was studied in black-legged kittiwake (*Rissa tridactyla*). Compared to non-breeding pairs, chick-rearing males and females had higher concentrations of both hormones. There was no correlation between hormonal levels and body condition. The stress-response corticosterone level increase was the same in chick-rearing and non-breeding pairs. On the other hand, the decrease of prolactin concentration following stress was negligible (9%) in individuals rearing chicks, while in males and females that were not caring for offspring it reached 41%. The ability for corticosterone secretion to increase in birds that were feeding their offspring supports foraging behaviour and the stability of the prolactin level ensures the maintainance of partental behaviour (Chastel et al., 2005).

The **corticosterone-prolactin synergism** can also ensure successful breeding through their effect on metabolic stability and their ability to create adaptive plasticity. The breeding activity of wandering albatross (*Diomedea exculans*) increases with age for a while. However, the primary reason for this is not age, but previous successful breeding events. Meanwhile baseline corticosterone level also increases continuously, reaching its maximum around the sixth breeding event. Plasma prolactin level increases in a similar way, but only in males. This hormonal development can ensure higher breeding success from the sixth breeding (Angelier et al., 2006).

In some extreme cases, the equilibrium between corticosterone and prolactin plasma levels ensures the survival of the parent. For examples, incubation and chick rearing keeps penguins tied to the nest and the chick for weeks, and this period can be critical for the energy replenishment of the parent. In some

cases, the parent abandons the egg or the hatchling and returns to the sea to feed. At this time, studied king penguins (*Aptenodytes patagonicus*) were in a stage of deep starvation, with increasing protein catabolism. This state primarily occurred during an early phase of breeding, and not before the expected successful conclusion of breeding. This state was characterised by a 4-5-fold increase in corticosterone level and a substantial decrease in prolactin, showing a very significant shift in the balance of the two hormones (Groscolas et al., 2008).

The presumed effect of prolactin to trigger brooding behaviour has still not been clearly proven. First Riddle et al., (1935) reported that mammalian prolactin treatment triggered broodiness in laying hens. While this study was repeated several times, its results could not be reproduced. Nevertheless, other results directly or indirectly reinforce Riddle's observation. For instance, treating laying hens and turkeys with different doses of ovine prolactin resulted in the atrophy of large yellow ovarian follicles, but did not induce incubation behaviour (Opel and Proudman 1980). However, when ovariectomised turkeys pretreated with estrogen were administered progesterone, they showed breeding behaviour. When these estrogen+progesterone treated birds were given ovine prolactin, they showed persistent incubation behaviour with increased prolactin and decreased plasma LH levels (El Halawani et al., 1986). Recently some positive results have been reported when American kestrels (*Falco sparverius*) were implanted with an osmotic minipump that started to function after laying the second egg of the brood, resulting in a continuous infusion. In this experiment ovine prolactin was administered in three different concentrations, and the results showed that prolactin treatment increased incubation behaviour without affecting the number of eggs in the brood (Sockman et al., 2000).

Passive immunisation experiments using turkey prolactin antibody also confirmed the incubation behaviour-initiating effect of prolactin. None of the 15 treated turkey hens showed incubation behaviour, while over 50% of the control group became broody. The plasma prolactin level of anti-prolactin antibody-treated female turkeys was significantly lower compared to control individuals, and they also laid more eggs. However, prolactin antibody treatment did not affect plasma LH and estradiol levels, its effect only manifested by decreasing prolactin level (Crisostomo et al., 1997). Laying turkeys actively immunised for VIP also showed inhibition of brooding behaviour, along with decrease in plasma prolactin level (El Halawani et al., 2000).

Prolactin probably plays a role in the **regulation of moult** in birds. Nevertheless, prolactin is not the sole responsible factor for moult regulation, but an important element of the neuroendocrine "cocktail" that regulates moult as it develops as part of the annual cycle. (The complex regulation of moult will be introduced later in details). It has already been demonstrated in the 1950s that

prolactin treatment triggers moulting in chickens, and at the same time it inhibits egg laying. In castrated roosters prolactin also triggers moulting (Harris and Juhn, 1956, Juhn and Harris, 1958). In photogonado-stimulated turkeys the level of prolactin continuously increases, reaching its maximum at the beginning of incubation, and starts to decrease when the following period of photorefractorness and postnuptial moult start (Mauro et al., 1989). In the case of starlings actively immunised with VIP-conjugated neuropeptide, photostimulated birds showed gonadal growth, and while the plasma prolactin level stayed low in some of the birds, in the rest of the treated group in increased only to 1/4 or 1/3 of the levels observed in the control group. In those treated starlings where the photostimulative increase of prolactin did not occur at all, moult did not happen either, and in invidividuals with a small amount of prolactin increase only two primary feathers got replaced (Dawson and Sharp, 1998). Dawson (2006) studied postnuptial moult during the photorefractory period after breeding in photogonodo-stimulated starlings. He found no effect of photostimulation and gonadal regression on the date of moult initiation. There was a strong correlation between plasma prolactin levels and the initiation of postnuptial moult. The comparison of several experimental systems showed that the initiation of moult is always preceeded by high prolactin plasma level. The initiation of moult, however, was not correlated with the height of the prolactin peak, but with its timing. Very similar results were seen in free-living dark-eyed juncos in experiments conducted during spring-early summer. Postnuptial moult initiated at the beginning of the photorefracter stage, and was related hormonally to the timining of the annual prolactin peak, which developed somewhat earlier (Deviche et al., 2000). Plasma prolactin level decrease and moult initiating at the same time were interpreted in a different context by Dawson et al., (2009) who studied mute swan (*Cygnus olor*). They compared non-breeding individuals, breeding females and chick-rearing males and found that moult initiated at different times in these individuals, and it was determined by critically low values of decreasing prolactin levels. Therefore, the initiating factor of moult is not the high prolactin value that occurs earlier, but its sharp decline. This assumption is confirmed by results obtained from all other study species, finding that formally high prolactin levels are substantially decreasing when moult initiates. However, the question remains: what mechanisms are initiated by decreasing prolactin levels that lead to the shedding of old feathers? The lagged stimulatory effect of a high hormonal level can be much more of a trigger effect as opposed to the lack of a hormone.

Sexual steroids, thyroid hormones and prolactin contribute to the hormonal regulation of the formation of **brood patch**. Prolactin plays a crucial role in the feather loss of the pectoral and abdominal area (special moult regulation) and in the loosening of the epidermis in the brood patch. The increasing number of blood vessels (mostly the amount of capillarisation) primarily results from the

peripheral effect of estrogens. Bare skin makes more intensive heat conductance possible towards the eggs. The structure and function of brood patch will be detailed in the section about ecophysiology.

The role of prolactin seems very probable in the multihormonal regulation of **bird migration**. Meier et al., (1969) first described in the 1960s that the diurnal rhythm of prolactin production is an important regulating factor of lipid metabolism in migratory white-throated sparrow (*Zonotrichia albicollis*). Lipid metabolism is the energetic base of muscle function during long-distance flight. The very high diurnal value of hypophyseal prolactin production before migration leads to a so-called premigratory lipid hyperproduction and the formation of subcutaneous adipose depots („premigratory fattening"). However, during spring migration, prolactin shows maximum plasma level during early morning, and this high plasma level results in strong lipid catabolism in the adipose depots, and a rapid increase in fatty acid oxidation. The circadian rhythm of prolactin change similarly affects carbohydrate metabolism, but plays a more subordinate role in the energy production mechanism of birds. To supply energy for long-distance flight, prolactin works together with corticosterone, thyroid hormones and glucagon (Meier and Farner, 1964, Meier et al., 1969, Meier and Martin, 1971).

These early results were later confirmed in dark-eyed junco. The prolactin level in this migratory bunting (technically in the USA it's a sparrow), similar to other species, shows consistent seasonal variation, increasing in the spring (photostimulatory period), reaching a high value during and right after the breeding season (the beginning of photorefractory period: breeding and postnuptial moult). Nevertheless, plasma prolactin level shows a substantial increase during spring migration, idenpendently of the seasonal changes, and this increase co-occurs with the increase in plasma corticosterone concentration. The simultaneous increase of plasma prolactin and corticosterone levels is the result of increasing daylength during spring. Later, during photostimulatory experiments on captured juncos, it was found that with increasing photoperiod, as a function of the number of days that passed, birds increased their food uptake, subcutaneous fat depots, night locomotor activity and their plasma prolactin and corticosterone levels continuously increased. This data suggests that the changed secretion of both prolactin and corticosterone increases lipid metabolism, providing the energetic background to migration. Migratory restlessness (Zugunruhe) develops probably as a results of direct and indirect stimulatory effects of the two hormones (Holberton et al., 2008). The above results are only valid to form a working hypothesis for spring migration, they do not explain the different physiological stage that is responsibe for the triggering of autumn migration.

5.10. The role of the inhibin-activin system in the regulation of gonadal function

The gonads express several biologically active extracellular signal molecules that are in dimeric form, containing a 25 kDa glycoprotein, which belongs to the transforming growth factor (TGF) group and are important autocrine-paracrine and endrocrine regulating factors of the ovary and the testis. This is the inhibin-activin system. The anti-Mullerian hormone, bone morphogenetic proteins and tumour-growth factors belong to the same TGF group. Inhibins are inhibitory regulating factors of the adenohypophyseal FSH production, while their effect of LH secretion is negligible. Activins on the other hand are stimulating elements of FSH production and mechanism of action. Both proteins have a wide spectrum of activity in the gonads and other organs.

Inhibins and **activins** are cysteine-rich peptides, dimeric molecules bound to each other by disulphide bridges, with their α element binding to one of the two β subunits (β-A or β-B), forming the two inhibins, inhibin-A or inhibin-B, respectively. The tree activins are homo- or heterodimeric molecules of similar structure, bound by disulphide bridges, which however, lack the α subunit. Activin has β-A or β-B subunits forming activin-A (β-A+β-A) or activin-B (β-B+β-B) or activin-AB (β-A+β-B) (Lovell et al., 2001, Knight et al., 2005).

Activins and inhibins are synthesised as part of a large pro-activin molecule. The amino-terminal of the pro-activin molecule contains a signal sequence, and its carboxy terminal contains a β subunit. Its biosynthesis is regulated by a gene that determines five subunits (with some overlap). The expression of these genes can be detected in the ovarian theca and granulosa cells and in both the Sertoli and Leydig cells of the testis. In both the ovary and testis FSH and LH determine inhibin-activin synthesis through cAMP-linked mechanisms, and IGF-I also takes part in the regulation of their production. In chicken ovary their synthesis is most important in the large white and small yellow follicles (Lovell et al., 2001, Knight et al., 2004). The appearance of the inhibin-activin group shows sexual differences. Inhibin-A is found in the ovary and the blood of females while inhibin-B is in the testis and blood of males. Otherwise the two inhibins have the same biological activity, and their plasma levels are always in an inverse relationship to the current FSH concentration.

Inhibins and activins are produced in several endrocrine and non-endocrine organs. Therefore besides the gonads, they are found in the adenohypophysis, the adrenal gland, the bone marrow (regulating red blood cell development), the liver, the pancreas, the wall of the blood vessels (endothel and smooth muscle cells) and in the central nervous system. Besides their autocrine and paracrine effects, their presence in the blood plasma also suggests endocrine function (Schwall, 1999a,b).

Both inhibins and activins act through membrane receptors, and they are antagonistic in their effect on the target cell. The two types of activin (ActR-I and II) bind with membrane receptors, and there are three versions in birds: ActRI, ActRII-A and ActRII-B. All three receptors are monomer glycoproteins. Their amino terminal is the extracellular ligand-binding domain, which is linked to a simple transmembrane part, which ends intracellularly in a serine/threonine-kinase C terminal. Activin binds to the type I receptor, however, for a perfect binding it necessitates the presence of receptor II, and for the signal effect to work, its ligand needs the presence of both II-A and II-B receptors.

Inhibins, on the other hand, bind to activin type II receptors, but their binding activity is about 20 times lower compared to activins. In the case of inhibin binding, this prevents the participation of the type I receptor in the formation of the receptor-ligand complex, and through this mechanism, inhibin can inhibit the effect of activin. The TGF-β-III receptor of the target cell membrane can also participate in this process, which increases the binding and acting of inhibin through a betaglycan molecule, which acts as a receptor coactivator (Knight et al., 2005).

***α*2-macroglobulin** is a modifying element of the activin effect that non-specifically binds to several other plasma elements. Its role is little known (Schwall, 1999a, 1999b, Gilfillan and Robertson, 1999).

At the level of the adenohypophysis, inhibins decrease the transcription of the FSH-β gene, which also manifests through basic as well as activin-stimulated secretion. Inhibin, secreted in the hypophysis, can also take part in these processes, as well as inhibin present in the blood plasma that originated in other organs (besides the gonads). Activin, which stimulates FSH production, acts similarly, through paracrine and endocrine pathways in the adenohypophysis. Activin-B is probably the primary regulating factor of FSH secretion, which increases the expression of the β-B subunit and stimulates the synthesis of the activin receptor. Compared to the gonadotropin stimulating effect of GnRH, activin acts slower, its effect is more prolonged and it does not influence LH secretion.

Inhibin and activin molecules mostly affect the gonads in a paracrine way. In the ovary, inhibin accelerates oocyte maturation, increases LH-stimulated androgen synthesis in the theca cells and results in follicular growth. Activin stimulates granulosa cell proliferation (by increasing DNA synthesis), increases the amount of FSH receptors, the number of FSH-stimulated LH receptors, and aromatase expression. Simultaneously, it decreases androgen production in theca cells and progesterone production in the granulosa. Inhibin synthesised in Sertoli and Leydig cells of the testis slows down the maturation of spermatogenic cells. Activin increases the division of spermatogonia, the connections between Sertoli cells and spermatogenic cells, and inhibin and transferrin secretion by Sertoli cells. It increases the number of activin receptors in the

Sertoli cells, however decreases FSH-induced aromatase activity, as well as the amount of androgen receptors (Schwall, 1999a, 1999b).

Follistatin is also a participant in the mechanism of action of the activin-inhibin complex. Follistatin is a 31–45 kDa polypeptide (the longer form consists of 344, the shorter 217 amino acids), which has six or more known isoforms. Typically, the glycosylated N-terminal of the molecule is very rich in cysteine. The follistatin gene contains six exons, and it shows a very high interspecific similarity. The first exon of the polypeptide codes for a 29 amino acid-containing signal sequence, exons 2-5 determine an EGF-like unit and exon 6 is responsible for the 27 amino acid-containing C-terminal.

Follistatin is primarily produced in the gonads and the adenohypophysis and expresses its (autocrine and paracrine) local effects there. In the adenohypophysis it mostly expresses in the gonadotropic cells. It interacts with the also locally synthethised inhibin-α and β-B elements, modifying the effect of activin-B and to a smaller degree also of inhibin-β on FSH production. Its effects manifest through binding to activin, and blocking it from binding to the activin receptor, thereby inhibiting/decreasing FSH release. Follistatin does not only change the amount of FSH produced, but also the pulsation of secretion. It decreases the amplitude of pulsation but does not affect its frequency. On the other hand, follistatin does not affect LH production.

In the ovary, follistatin is only synthesised in the granulosa cells, its production is stimulated by FSH and estrogens, while being essentially unaffected by LH. Follistatin inhibits aromatase activity and inhibin production in the granulosa cells, as well as their LH and FSH synthesis. It also inhibits oocyte development, blocking the meiosis-stimulating effect of activin.

In the testis, follistatin is synthesised in the Sertoli cells, and its activin-inhibiting effect manifests in a paracrine way affecting dividing spermatocytes and spermatidae, as well as slowing down spermatogonium proliferation. It also decreases Sertoli cell proliferation in an autocrine way.

Follistatin molecules have two important binding sites, one making it possible to bind to activin or inhibin, and the other to the heparan-suphate molecule on the cell coat of the target cell, to form a stabilising bond. Given that activin can bind to two follistatins, while inhibin can bind to one, follistatin can modify the effect of inhibin to a lesser degree.

Follistatin synthesis has been detected in several other organs (liver, pancreas, bone tissue and central nervous system), where it also serves as a local (autocrine and paracrine) regulating factor. Additionally, substantial amounts appear in the blood plasma, which demonstrates its endocrine effect (Gifillan and Robertson, 1999).

The inhibin-activin system plays an important role in the embryonal, prepubertal and pubertal development of the gonads, and in the regulation of the functioning of the active testis and ovary. In male duck embryo, plasma inhibin

content decreases between Days 21 and 24 of incubation, then increases and stays high after hatching. Inhibin subunits (α, β-A and β-B) are synthesised in the developing seminiferous tubules. During this time, FSH levels change in the opposite direction, which suggests that the reciprocal characteristics of gonadal regulatory factors already appear during embryogenesis (Yang et al., 2005). In Japanese quail, testicular mass increases continuously and gradually during weeks 1-4, and then during maturation (weeks 5-7) the growth becomes more intensive. Plasma inhibin level increases significantly from hatching until week 5, and then decreases during puberty. Similar observations were made in chicken, where the increase in FSH during puberty occurs along with a decrease in inhibin level (Sedqyar et al., 2008, Johnson and Brooks, 1996). The follistatin and inhibin/activin β-B-content of testicular tissue is also higher in juvenile male duck and chicken compared to sexually mature individuals (Fu et al., 2001, Davis and Johnson, 1998). Plasma inhibin-A- and inhibin-B-content change differently in male birds during prepuberty and puberty: in roosters the level of inhibin-A stays low in weeks 6-12, and increases substantially in weeks 14-18. At the beginning, there is no correlation between inhibin-A and plasma FSH level, and significant negative correlation is only detectable from week 18. Inhibin-B is not detectable in the plasma until week 16, then it appears and its amount increases parallel with FSH and testosterone. The α subunit is found in higher concentration than the dimeric inhibins, and its level continuously increases with age (Lovell et al., 2000).

During the period of prepuberty and puberty definite sexual differences appear in roosters and hens in the initiation and amount of production of inhibin/activin subunits. In the testis, in weeks 3-18 (during prepuberty), mRNA of all inhibin/activin subunits are produced intensively, however in the ovary only β-A and β-B express before sexual maturation. The production of the α subunit in the ovary only initiates right before puberty. The amount of mRNA of all subunits changes with age in both sexes (Onagbesan et al., 2004).

In female Japanese quail, inhibin plasma level increases evenly up to prepuberty (week 6), and during weeks 7-8 it continues to increase steeply until the beginning of egglaying, parallel to the increase of the mass of the ovary. Inhibin-α subunit expression can be detected in all follicles of the active ovary with the exception of large white follicles. The largest amount is synthesised in the small yellow (F5) follicles and it gradually decreases with follicular maturation. The highest amount of inhibin-β-B subunit synthesises in the little yellow follicles and is absent in the F4-F1 follicles. The inhibin-β-A subunit on the other hand is produced in the largest follicles (Davis and Johnson, 1998, Sedqyar et al., 2008). The inhibin/activin system does not only contribute to the development of follicles, but through this process also plays a regulatory role in the ovarian cycle. Intrafollicular studies of laying hens showed that during follicular development, inhibin-B content changed the same way in the theca and

granulosa layers, but its amount was at least four times higher in the granulosa cells. The amount of inhibin-B continuously increases in the prehierarchical follicles from little white follicles of 1-4 mm size to the little large follicles of 7-9 mm, where it shows a peak value and starts to decrease sharply in the larger follicles. On the other hand, the amount of inhibin-A stays at a very low level in the prehierarchical follicles, and its sudden increase only starts in the F5-F3 categories, reaching a maximum amount in the F3 follicles. In the white follicles inhibin-A is only detectable in the theca layer, while in the F1 follicles 97% of its amount appears in the granulosa. After ovulation, the amount of both inhibin-B and inhibin-A strongly decreases in the postovulatory follicle. During follicular development, activin-A is practically only detectable in the theca layer of the prehierarchical follicles of 1-9 mm size, while in the preovulatory, rapid growth phase it also appears in the granulosa. Its maximal amount appears in the F4-F3 follicles. The amount of follistatin is highest in the prehierarchical follicles, decreases to an intermediate amount in the large yellow follicles, followed by a strong continuing decline in the F1 follicles (Lovell et al., 2003).

The elements of the activin/inhibin system are specifically arranged in the wall of the ovarian follicles. Activin-A is mostly located in the theca layer of the preovulatory follicles, while inhibin-A expresses mostly in the granulosa cells. Activin-A increases the synthesis of the β-B subunit in all follicle types, increases FSH receptor expression in the granulosa cells of all follicles types. It also increases the amount of LH receptors in the wall of the large yellow follicles, but has no effect in the little yellow follicles. Activin-A decreases granulosa cell proliferation in the follicular wall. However, it has no effect on the β-B subunit nor on the FSH and LH receptors, but slightly increases granulosa proliferation (Johnson et al., 2006). The activin-A produced in the theca layer of the F1-F2-F3 follicles has a local paracrine effect on the granulosa cells, modifying the baseline level of inhibin-A production in these cells, and increasing the secretion of gonadotropin-stimulated inhibin-A and progesterone (Lovell et al., 2002).

The functional relationship between steroid hormones and the inhibin/activin system is an important regulatory element of gonadal functioning. Under in vitro conditions, estradiol inhibits the proliferation of granulosa cell obtained from different generations of large yellow follicles, but stimulates mRNA expression of inhibin-α , inhibin-β-B and follistatin (Davis et al., 2000). Nevertheless, the ovarian production of follistatin shows a definite parallel with inhibin plasma level change (Sedqyar et al., 2008). Activin-A alone does not affect progesterone secretion, but substantially increases FSH-stimulated P4 production. At the same time, it hardly affects the LH-triggered increase in progesterone secretion. Activin is produced in the theca cells and affects progesterone production in the granulosa cells of F3-F2-F1 follicles in a paracrine way, proven by the large amount of activin-I-A, I-B and II-B receptors expressing in the granulosa (Lovell et al., 2002).

The expression of activin receptors and the coreceptor betaglycan is regulated by the current level of FSH and LH. FSH decreases activin-II-B receptor mRNA synthesis in the granulosa cells of the little yellow follicles, while LH increases it. However, both FSH and LH increase the amount of activin-II-A and betaglycan mRNA in the granulosa of the little yellow follicles, but in the theca cells the two gonadotropins only increase the amount of betaglycan mRNA, they do not affect the amount of activin receptors. In F1 follicles the two gonadotropins increase the amount of activin receptor-I, activin-receptor II-B and betaglycan, but do not affect II-A mRNA. Supposedly, the expression of activin-receptors and betaglycan are regulated by gonadotropins in a different way, and these effects form the fine-tuned system of follicular maturation (Lovell et al., 2007).

References 5/8.

1. Akazome Y, Park MK, Mori T, Kawashima S (1994) Characterization of cDNA-encoding N-terminal region of the quail lutropin receptor Gen.Comp.Endocrinol., 95, 222-231.
2. Akazome Y, Shimizu F, Park MK, Mori T, Kawashima S (1996): Molecular characteristics of the N-terminal region of the quail follitropin receptor In Vivo, 10, 345-349.
3. Ando H, Ishii S (1994): Molecular cloning of complementary deoxyribonucleic acids for the pituitary glycoprotein hormone alpha-subunit and luteinizing hormone beta-subunit precursr molecules of Japanese quail (Coturnix coturnix japonica) Gen.Comp.Endocrinol., 93, 357-368.
4. Bona-Gallo A, Licht P, Papkoff H (1983): Biological and binding activities of pituitary hormones from the ostrich, Struthio camelus Gen.Comp.Endocrinol., 51, 50-60.
5. Burke WH, Papkoff H, Licht P, Gallo AB (1979): Preparation and properties of luteinizing hormone (LH) subunits from the turkey (Meleagris gallopavo) and their recombination with subunits of ovine LH Gen.Comp.Endocrinol., 37, 501-507.
6. Chase DJ (1982): Gonadotropin specificity of acute testicular androgen secretion in birds Gen. Comp.Endocrinol., 46, 486-499.
7. Follett BK, Scanes CG, Cunningham FJ (1972): A radioimmunoassay for avian luteinizing hormone J.Endocrinol., 52, 359-378.
8. Foster DN, Foster LK (1991): Cloning and sequence analysis of the common alpha-subunit complementary deoxyribonucleic acid of turkey pituitary glycoprotein hormones Poult.Sci., 70, 2516-2523.
9. Hartree AS, Cunningham FJ (1969): Purification of chicken pituitary follicle-stimulating hormone and luteinizing hormone J.Endocrinol., 43, 609-616.
10. Hsieh Y-L, Chatterjee A, Chien J-T (2001): Molecular cloning of the cDNAs for pituitary glycoprotein hormone alpha subunits of two species of duck and their gene regulation J.Mol.Endocrinol., 27, 339-347.
11. Johnson AL, Bridgham JT, Wagner B (1996): Characterization of a chicken luteinizing hormone receptor (cLH-R) complementary deoxyribonucleic acid, and expression of cLH-R messenger ribonucleic acid in the ovary Biol.Reprod., 55, 304-309.
12. Kikuchi M, Kobayashi M, Ito T, Ishii S (1998): Cloning of complementary deoxyribonucleic acid for the follicle-stimulating hormone-beta subunit in the Japanese quail Gen.Comp.Endocrinol., 111, 376-385.
13. Koide Y, Papkoff H, Kawauchi H (1996): Complete amino acid sequences of follitropin and lutropin in the ostrich, Struthio camelus Eur.J.Biochem., 240, 262-267.
14. Maseki Y, Nakamura K, Iwasawa A, Zheng J, Inoue K, Sakai T (2004): Development of gonadotropes in the chicken embryonic pituitary gland Zoolog.Sci., 21, 435-444.

15. Mikami S (1986): Immunocytochemistry of the avian hypothalamus and adenohypophysis Int. Rev.Cytol., 103, 189-248.
16. Mikami S, Yamada, S (1984): Immunohistochemistry of the hypothalamic neuropeptides and anterior pituitary cells in the Japanese quail J.Exp.Zool, 232, 405-417.
17. Mizutani T, Minegishi T, Nonobe Y, Abe Y, Hasegawa Y, Wakabayashi K, Kamiyoshi M, Miyamoto K (1998): Molecular cloning and functional expression of chicken luteinizing hormone receptor Biochim.Biophys.Acta 1397, 1-8.
18. Noce T, Ando H, Ueda T, Kubokawa K, Higashinakagawa T, Ishii S (1989): Molecular cloning and nucleotide sequence analisis of the putative cDNA for the precursor molecule of the chicken LH-beta subunit. J. Mol. Endocrinol., 3, 129-137.
19. Péczely P, Szokoly M (1969): The light and electron microscopic localisation of the ACTH cell in the pars distalis of the pigeon (Columba livia domestica) hypophysis Abstr. 6. Conf.Hung.Soc. Electron Microsc., Balatonszéplak, 104.
20. Proudman JA, Vandesande F, Berghman LR (1999): Immunohistochemical evidence that follicle-stimulating hormone and luteinizing hormone reside in separate cells in the chicken pituitary Biol.Reprod., 60, 1324-1328.
21. Scanes CG, Godden PM, Sharp PJ (1977a): An homologous radioimmunoassay for chicken follicle-stimulating hormone: observations on the ovulatory cycle J.Endocrinol., 73, 473-481.
22. Scanes CG, Harvey S, Chadwick A, Bolton NJ (1977b): Studies on the hypothalamic control of growth hormone and prolactin secretion in the chicken In: Proc.Soc.Endocrinol., 148 Meeting, J.Endocrinol., 73, 3.Suppl., 10P, VIII.
23. Shen ST, Yu JY (2002): Cloning and gene expression of a cDNA for the chicken follicle-stimulating hormone (FSH)-beta-subunit Gen.Comp.Endocrinol., 125, 375-386.
24. Silverin B, Sharp P (1996): The development of the hypothalamic-pituitary-gonadal axis in juvenile great tits Gen.Comp.Endocrinol., 103, 150-166.
25. Tixier-Vidal A, Follett BK (1973): The adenohypophysis In: DS Farner, JR King (eds) avian Biology, Vol. 3.,110-182. Academic Press New York
26. Williams J, de Reviers M (1981): Variations in the plasma levels of luteinizing hormone and androstenedione and their relationship with the adult daily sperm outpout in cockerels raised under different photoschedules Reprod.Nutr.Dev., 21, 1125-1135.
27. You S, Bridgham JT, Foster DN, Johnson AL (1996): Characterization of the chicken follicle-stimulating hormone receptor (cFSH-R) complementary doxyribonucleic acid, and expression of cFSH-R messenger ribonucleic acid in the ovary Biol.Reprod., 55, 1055-1062.
28. You S, Foster LK, Silsby JL, El Halawani ME, Foster DN (1995): Sequence analysis of the turkey LH beta subunit and its regulation by gonadotrophin-releasing hormone and prolactin in cultured pituitary cells J.Mol.Endocrinol., 14, 117-129.
29. You S, Kim H, Hsu CC, El Halawani ME, Foster DN (2000): Three different turkey luteinizing hormone receptor (tLH-R) isoforms, I: characterization of alternatively spliced tLH-R isoforms and their regulated expression in diverse tissues Biol.Reprod., 62, 108-116.

References 5/9.

1. Angelier F, Shaffer SA, Weimerskirch H, Chastel O (2006): Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird:the wandering albatros Gen.Comp.Endocrinol., 149, 1-9.
2. Bédécarrats G, Guémené D, Kühnlein U, Zadworny D (1999): Changes in levels of immunoreactive prolactin isoforms during a reproductive cycle in turkey hens Gen.Comp.Endocrinol., 113, 96-104.
3. Buntin JD, Becker GM, Ruzycki E (1991): Facilitation of parental behavior in ring doves by systemic or intracranial injections of prolactin Horm.Behav., 25, 424-444.
4. Chastel O, Lacroix A, Weimerskirch H, Gabrielsen GW (2005): Modulation of prolactin but not corticosteron responsesto stress in relation to parental effort in a long-lived bird Horm.Behav., 47, 459-466.

5. Chen X, Horseman ND (1994): Cloning, expression, and mutational analysis of the pigeon prolactin receptor Endocrinology, 135, 269-276.
6. Christensen D, Vleck CM (2008): Prolactin release and response to vasoactive intestinal peptide in an opportunistic breeder, the zebra finch (Taenopygia guttata) Gen.Comp.Endocrinol., 157, 91-98.
7. Criscuolo F, Bertile F, Durant JM, Raclot T, Gabrielsen GW, Massermin S, Chastel O (2006): Body mass and clutch size may modulate prolactin and corticosterone levels in eiders Physiol.biochem. Zool., 79, 514-521.
8. Criscuolo F, Chastel O, Gabrielsen GW, Lacroix A, Le Maho Y (2002): Factors affecting plasma concentrations of prolactin in the common eider Somateria mollissima Gen.Comp.Endocrinol., 125, 399-409.
9. Crisostomo S, Guémené D, Garreau-Mills M, Zadworny D (1997): Prevention of the expression of incubation behaviour using passive immunisation against prolactin in turkey hens (Meleagris gallopavo) Reprod.Nutr.Dev., 37, 253-266.
10. Dawson A (2006): Control of molt in birds: association with prolactin and gonadal regression in starlings Gen.Comp.Endocrinol., 147, 314-322.
11. Dawson A, Perrins CM, Sharp PJ, Wheeler D, Groves S (2009): The involvement of prolactin in avian molt: the effects of gender and breeding success on the timing of molt in Mute swans (Cygnus olor) Gen.Comp.Endocrinol., 161, 267-270.
12. Dawson A, Sharp PJ (1998): The role of prolactin in the development of reproductive photorefractoriness and postnuptial molt in the European starling (Sturnus vulgaris) Endocrinology, 139, 485-490.
13. Deviche P, Wingfield JC, Sharp PJ (2000): Year-class differences in the reproductive system, plasma prolactin and corticosterone concentrations, and onset of prebasic molt in male dar-eyed juncos (Junco hyemalis) during the breeding period Gen.Comp.Endocrinol., 118, 425-435.
14. Dumont JN (1965): Prolactin-induced cytologic changes in the mucosa of the pigeon crop during crop-„milk" formation Z.Zellforsch.Mikrosk.Anat., 68, 755-782.
15. El Halawani ME, Silsby JL, Behnke EJ, Fehrer SC (1986): Hormonal induction of incubation behavior in ovariectomized female turkeys (Meleagris gallopavo) Biol.Reprod., 35, 59-67.
16. El Halawani ME, Silsby JL, Fehrer SC, Behnke EJ (1983): Effects of estrogen and progesterone on serum prolactin and luteinizing hormone levels in ovariectomized turkeys (Meleagris gallopavo) Gen.Comp.Endocrinol., 52, 67-78.
17. El Halawani ME, Whiting SE, Silsby JL, Pitts GR, Chaisea Y (2000): Active immunization with vasoactive intestinal peptide in turkey hens Poult.Sci., 79, 349-354.
18. Forsyth IA, Buntin JD, Nicoll CS (1978): A pigeon crop sac radioreceptor assay for prolactin J.Endocrinol., 79, 349-356.
19. Garrison MM, Scow RO (1975): Effect of prolactin on lipoprotein lipase in crop sac and adipose tissue of pigeons Am.J.Physiol., 228, 1542-1544.
20. Gillespie MJ, Haring VR, McColl KA, Monaghan P, Donald JA, Nicholas KR, Moore RJ, Crowley TM (2011): Histological and global gene expression analysis of the „lactating" pigeon crop BMC Genomics, 12, 452-461.
21. Goldsmith AR, Edwards C, Koprucu M, Silver R (1981): Concentrations of prlactin and luteinizing hormone in plasma of doves in relation to incubation and development of the crop gland J.Endocrinol., 90, 437-443.
22. Goldsmith AR, Nicholls TJ (1984a): Thyroxine induces photorefractoriness and stimulates prolactin secretion in European starlings (Sturnus vulgaris) J.Endocrinol., 101, R1-3.
23. Goldsmith AR, Nicholls TJ (1984b): Thyroidectomy prvents the developments of photorefractoriness and the associated rise in plasma prolactin in starlings Gen.Comp.Endocrinol., 54, 256-263.
24. Goldsmith AR, Nicholls TJ, Plowman G (1985): Thyroxine treatment facilitates prolactin secretion and induces a state of photorefractoriness in thyroidectomized starlings J.Endocrinol., 104, 99-103.
25. Groscolas R, Lacroix A, Robin JP (2008): Spontaneous egg or chick abandonment in energy-depleted king penguins: a role for corticosterone and prolactin? Horm.Behav., 53, 51-60.
26. Hanks MC, Alonzi JA, Sharp PJ, Sang HM (1989): Molecular cloning and sequence analysis of putative chicken prolactin cDNA J.Mol.Endocrinol., 2, 21-30.

27. Harris PC, Juhn M (1956): Responses in molt and lay of fowl to progestins and gonadotrophins Proc.Soc.Exp.Biol.Med., 92, 709-711.
28. Holberton RL, Boswell T, Hunter MJ (2008): Circulating prolactin and corticosterone concentrations during the development of migratory condition in the Dark-eyed Junco, Junco hyemalis Gen.Comp. Endocr., 155, 641-649.
29. Horseman ND, Buntin JD (1995): Regulation of pigeon cropmilk secretion and parental behaviors by prolactin Annu.Rev.Nutr., 15, 213-238.
30. Hrabia A, Paczoska-Eliasiewicz H, Rzasa J (2004): Effect of prolactin on estradiol and progesterone secretion by isolated chicken ovarian follicles Folia Biol. (Krakow) 52, 197-203.
31. Juhn M, Harris PC (1958): Molt of capon feathering with prolactin Proc.Soc. Exp.Biol.Med., 98, 669-672.
32. Kledzik G, Marshall S, Gelato M, Campbell G, Meites J (1975): Prolactin binding activity on the crop sacs of juvenile, mature, parent and prolactin-injected pigeons Endocr.Res.Commun., 2, 345-355.
33. Koch KA, Wingfield JC, Buntin JD (2004): Prolactin-induced parental hyperphagia in ring doves: are glucocorticoids involved? Horm.Behav., 46, 498-505.
34. Kurima K, Proudman JA, El Halawani ME, Wong EA (1995): The turkey prolactin-encoding gene and its regulatory region Gene, 156, 309-310.
35. Li C, Kelly PA, Buntin JD (1995): Inhibitory effects of anti-prolactin receptor antibodies on prolactin binding in brain and prolactin-induced feeding behavior in ring doves Neuroendocrinology, 61, 125-135.
36. Liu Z, Shi ZD, Liu Y, Li MY, Huang YM, Yao BH (2008): Molecular cloning and charcterisation of the Magang goose prolactin gene Gen.Comp.Endocrinol., 155, 208-216.
37. Massaro M, Setiawan AN, Davis LS (2007): Effects of artificial eggs on prolactin secretion, steroid levels, brood patch development, incubation onset and clutch size in the yellow-eyed penguin (Megadyptes antipodes) Gen.Comp.endocrinol., 151, 220-229.
38. Mauro LJ, Elde RP, Youngren OM, Phillips RE, El Halwani ME (1989): Alterations in hypothalamic vasoactive intestinal peptide-like immunoreactivity are associated with reproduction and prolactin release in the female turkey Endocrinology, 125, 1795-1804.
39. Meier AH, Burns JT, Dusseau JW (1969): Seasonal variation in the diurnal rhythm of pituitary prolactin content in the white-throated sparrow, Zonotrichia albicollis Gen.Comp.Endocrinol., 12, 282-289.
40. Meier AH, Farner DS (1964): A possible endocrine basis for premigratory fattening in the white-crowned sparrow, Zonotrichia leucophrys gambelii (Nuttal) Gen.Comp.Endocrinol., 2, 584-595.
41. Meier AH, Martin DD (1971): Temporal synergism of corticosterone and prolactin controlling fat storage in the white-throated sparrow, Zonotrichia albicollis Gen.Comp.Endocrinol., 17, 311-318.
42. Mikami S, Kurosu T, Farner DS (1975): Light- and electron-microscopic studies on the secretory cytology of the adenohypophysis of the Japanese quail, Coturnix coturnix japonica Cell Tissue Res., 159, 147-165.
43. Mikami S, Yamada S (1984): Immunohistochemistry of the hypothalamic neuropeptides and anterior pituitary cells in the Japanese quail J.Exp.Zool., 232, 405-417.
44. Opel H, Proudman JA (1980): Failure of mammalian prolactin to induce incubation behavior in chickens and turkeys Poult.Sci., 59, 2550-2558.
45. Péczely P, El Halawani ME, Hargitai Cs, Mézes M, Forgó V, Jánosi Sz (1993): The photorefractoriness in domestic goose: effects of gonads and thyroid on the development of postbreeding prolactinemia Acta Biol.Hung., 44, 329-352.
46. Proudman JA (1998): Circulating prolactine levels at the end of the photophase and at the end of the scotophase throughout the reproductive cycle of the turkey hen Poultry sci., 77, 303-308.
47. Proudman JA, Siopes TD (2002): Relative and absolute photorefractoriness in turkeys hens: profiles of prolactin, thyroxine, and triiodothyroninee early in the reproductive cycle Poult.sci., 81, 1218-1223.
48. Proudman JA, Siopes TD (2005): Thyroid hormone and prolactin profiles in male and female turkeys following photostimulation Poult.Sci., 84, 942-946.
49. Proudman JA, Siopes TD (2006): Potential role of thyroid hormones and prolactin in the programming of photorefractoriness in turkey hens Poult.Sci., 85, 1457-1461.

50. Reddy IJ, David CG, Raju SS (2007): Effect of supression of plasma prolactin on luteinizing hormone concentration, intersequence pause days and egg production in domestic hen Domest.Animal Endocrinol., 33, 167-175.
51. Reddy IJ, David CG, Sarma PV, Singh K. (2002): The possible role of prolactin in laying performance and steroid hormone secretion in domestic hen (Gallus domesticus) Gen.Comp.Endocrinol., 127, 249-255.
52. Richard-Yris MA, Sharp PJ, Wauters AM, Guémené D, Richard JP, Forasté M (1998): Influence of stimuli from chicks on behavior and concentrations of plasma prolactin and luteinizing hormone in incubating hens Horm.Behav., 33, 139-148.
53. Riddle O, Bates RW, Lahr EL (1935): Prolactin induced broodiness in the fowl Am.J.Physiol., 111, 352-371.
54. Riou S, Chastel O, Lacroix A, Hamer KC (2010): Stress and parental care: Prolactin responses to acute stress throughout the breeding cycle in a long- lived bird Gen.Comp.Endocrinol., 168, 8-13.
55. Sharp PJ, Klandorf H, McNeilly AS (1986): Plasma prolactin, thyroxine, triiodothyronine, testosterone and luteinizing hormone during a photoinduced reproductive cycle in mallard drakes J.Exp.Zool., 238, 409-413.
56. Sharp PJ, Macnamee MC, Sterling RJ, Lea RW, Pedersen HC (1988): Relationships between prolactin, LH, and broody behaviour in bantam hens J.Endocrinol., 118, 279-286.
57. Shetty S, Hegde SN, Bharathi L (1992): Purification of a growth factor from pigeon milk Biochim. Biophys.Acta 1117, 193-198.
58. Shetty S, Salimath PV, Hegde SN (1994): Carbohydrates of pigeon milk and their changes in the first week of secretion Arch.Int.Physiol.Biochim.Biophys., 102, 277-280.
59. Shimada K, Ishida H, Sato K, Seo H, Matsui N (1991): Expression of prolactin gene in incubating hens J.Reprod.Fert., 91, 147-154.
60. Silver R (1984): Prolactin and parenting in the pigeon family J.Exp.Zool., 232, 617-625.
61. Sockman KW, Schwabl H, Sharp PJ (2000): The role of prolactin in the regulation of clutch size and onset of incubation behavior in the American kestrel Horm.Behav., 38, 168-176.
62. Sreekumar KP, Sharp PJ (1998): Ontogeny of the photoperiodic control of prolactin and luteinizing hormone secretion in male and female bantams (Gallus domesticus) Gen.Comp.Endocrinol., 109, 69-74.
63. Tixier-Vidal A, Follett BK (1973): The adenohypophysis In: DS Farner, JR King (eds) Avian Biology, Vol.3., 110-182., Academic Press New York
64. Van As P, Janssens K, Pals K, De Groef B, Onagbesan OM, Bruggerman V, Darras VM, Denef C, Decuypere E (2006): The chicken pituitary-specific transcription factor PIT-1 is involved in the hypothalamic regulation of pituitary hormones Acta Vet.Hung., 54, 455-471.
65. Wang J, Hou SS, Huang W, Yang XG, Zhu XY, Liu XL (2009): Molecular cloning of prolactin receptor of the peking duck Poult.Sci., 88, 1016-1022.
66. Watahiki M, Tanaka M, Masuda N, Sugisaki K, Yamamoto M, Yamakawa M, Nagai J, Nakashima K (1989): Primary structure of chicken pituitary prolactin deduced from the cDNA sequence. Conserved and specific amino acid residues in the domains of the prolactins J.Biol.Chem., 264, 5535-5539.
67. Wingfield JC, Goldsmith AR (1990): Plasma levels of prolactin and gonadal steroids in relation to multiple-brooding and renesting in free-living populations of the song sparrow, Melospiza melodia Horm.Behav., 24, 89-103.
68. Wong EA, Ferrin NH, Silsby JL, El Halawani ME (1991): Cloning of a turkey prolactin cDNA: expression of prolactin mRNA throughout the reproductive cycle of the domestic turkey (Meleagris gallopavo) Gen.Comp.Endocrinol., 83, 18-26.
69. Zhou JF, Zadworny D, Guémené D, Kuhnlein U (1996): Molecular cloning, tissue distribution, and expression of the prolactin receptor during various reproductive states in Meleagris gallopavo Biol.Reprod., 55, 1081-1090.

References 5/10.

1. Davis AJ, Brooks CF, Johnson PA (2000): Estradiol regulation of follistatin and inhibin alpha-and beta(B)-subunit mRNA in avian granulosa cells Gen.Comp.Endocrinol., 119, 308-316.
2. Davis AJ, Johnson PA (1998): Expression pattern of messenger ribonucleic acid for follistatin and the inhibin/activin subunits during follicular and testicular development in Gallus domesticus Biol.Reprod., 59, 271-277.
3. Fu Y, Niu D, Ruan H, Yu XP, Chen G, He GQ, Yang PX (2001): Expression pattern of mRNA for follistatin and inhibin/activin beta B-subunit during follicular and testicular development in duck (kínai nyelvû cikk) Yi Chuan Xue Bao, 28, 808-815.
4. Gilfillan C, Robertson DM (1999): Follistatin In: Encyclopedia of Reproduction (eds.: E Knobil, JD Neil) II., 396-406., Academic Press San Diego, London, Boston, New York, Sydney, Tokyo, Toronto
5. Johnson PA, Brooks C (1996): Developmental profile of plasma inhibin and gonadotropins from hatch to sexual maturity in male and female chickens Gen.Comp.Endocrinol., 102, 56-60.
6. Johnson PA, Woodcock JR, Kent TR (2006): Effect of activin A and inhibin A on expression of the inhibin/activin beta-B-subunit and gonadotropin receptors in granulosa cells of the hen Gen. Comp.Endocrinol., 147, 102107.
7. Knight PG, Gladwell RT, Lovell TM (2005): The inhibin-activin system and ovarian folliculogenesis in the chicken In: A Dawson, PJ Sharp (eds) Functional Avian Endocrinology, Narosa Publishing House, New Delhi, 324-337.
8. Lovell TM, Al-Musawi SL, Gladwell RT, Knight PG (2007): Gonadotrophins modulate hormone secretion and steady-state mRNA levels for activin receptors (type I, IIA, IIB) and inhibin co-receptor (betaglycan) in granulosa and theca cells from chicken prehierarchial and preovulatory follicles Reproduction, 133, 1159-1168.
9. Lovell TM, Gladwell RT, Groome NP, Knight PG (2002): Differential effects of activin A on basal and gonadotrophin-induced secretion of inhibin A and progesterone by granulosa cells from preovulatory (F1-F3) chicken follicles Reproduction, 124, 649-657.
10. Lovell TM, Gladwell RT, Groome NP, Knight PG (2003): Ovarian follicle development in the laying hen is accompanied by divergent changes in inhibin A, inhibin B, activin A and follistatin production in granulosa and theca layers J.Endocrinol., 177, 45-55.
11. Lovell TM, Knight PG, Groome NP, Gladwell RT (2000): Measurement of dimeric inhibins and effects of active immunization against inhibin alpha-subunit on plasma hormones and testis morphology in the developing cockerel Biol.Reprod., 63, 213-221.
12. Lovell TM, Knight PG, Groome NP, Gladwell RT (2001): Changes in plasma Inhibin A levels during sexual maturation in the female chicken and the effects of active immunization against Inhibin alpha-Subunit on reproductive hormone profiles and ovarian function Biol.Reprod., 64, 188-196.
13. Onagbesan OM, Safi M, Decuypere E, Bruggeman V (2004): Developmental changes in inhibin alpha and inhibin/activin betaA and betaB mRNA levels in the gonads during post-hatch prepubertal development of male and female chickens Mol.Reprod.Dev., 68, 319-326.
14. Schwall RH (1999): Activin and activin receptors In: Encyclopedia of Reproduction (eds.: E Knobil, JD Neil) I., 26-35., Academic Press San Diego, London, Boston, New York, Sydney, Tokyo, Toronto
15. Schwall RH (1999): Inhibin In: Encyclopedia of Reproduction (eds.: E Knobil, JD Neil) II., 832-839., Academic Press San Diego, London, Boston, New York, Sydney, Tokyo, Toronto
16. Sedqyar M, Weng Q, Watanabe G, Kandiel MM, Takahasi S, Suzuki AK, Taneda S, Taya K (2008): Secretion of inhibin in male Japanese quail (Coturnix japonica) from one week of age to sexual maturity J.Reprod.Dev., 54, 100-106.
17. Yang P, Medan MS, Arai KY, Jin W, Watanabe G, Taya K (2005): Secretion of inhibin and testicular expression of inhibin subunits in male duck embryos and newly hatched ducks Endocrine., 28, 171-179.
18. Wang Q, Buntin JD (1999): The roles of stimuli from young, previous breeding experience, and prolactin in regulating parental behavior in ring doves (Sterptopelia risoria) Horm.Behav., 35, 241-253.

INDEX

Scientific names

INDEX

English names