CONTENT OF RETINOL AND RETINYL ESTERS IN BLOOD PLASMA, LIVER, KIDNEY AND REPRODUCTIVE ORGANS OF JAPANESE QUAILS

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Due to its importance in many physiological processes such as cell proliferation and differentiation, vitamin A plays a key role in reproduction. The present study examines the content and distribution of retinol and retinyl esters in the blood plasma, liver, kidney, ovary and oviduct (infundibulum, magnum, isthmus and uterus) of the laying Japanese quail. (1) The results show that the stage of egg laying had no influence on the level of vitamin A (retinol or retinyl esters) in plasma, kidney and liver. (2) The results further indicate that in the oviduct there are quantitative and qualitative differences in the concentration of retinol and retinyl esters, but that these differences are not altered by the stage of egg formation. (3) The highest levels of vitamin A in the isthmus and uterus were associated with a predominance of retinyl esters (palmitate and stearate); sections with lower total levels of vitamin A (infundibulum, magnum) had retinol as the more dominant form of vitamin A. (4) Changes in the ratio of retinol to retinyl esters in the various sections of the avian oviduct might point to metabolic differences. The storage of vitamin A might therefore be the predominant function of the uterus and isthmus; in the infundibulum and magnum, where vitamin A is predominantly present as retinol, vitamin A serves rather as a precursor for the modulation of the cellular metabolism of these structures.

Key words: Vitamin A, blood plasma, liver, reproduction, ovary, oviduct, Japanese quail

The importance of retinoids for many physiological processes has been established in numerous nutritional studies. Retinoids exhibit striking effects on cell proliferation and differentiation and are also essential for the maintenance of normal reproductive processes in mammals and birds.

It is well known that egg production (Moore, 1958; Hashish et al., 1984) and embryonic development (Thompson, 1969) require an adequate dietary pro-
vision of vitamin A. One initial consequence of vitamin A deficiency in adult birds is a rapid drop in egg production and hatchability. The egg must contain the required substances (e.g. vitamin A) in sufficient quantities in order for growth and development of the embryo to take place, because the egg yolk is the only supply for the embryo during the incubation period (Joshi et al., 1973). Using the vitamin A deprived embryonic avian model, it has been demonstrated that the quail embryo develops normally without an exogenous source of active vitamin A up until 18–24 h of incubation. After 24–28 h, however, the heart and the cardiovascular system fail to develop unless vitamin A is supplemented (Dersch and Zile, 1993).

Retinoids might be important not only for the developing embryo, but also for other processes related to egg formation. In recent studies using pigs, temporal and spatial changes in the distribution of vitamin A, its binding proteins and nuclear receptors were observed. These findings helped point to the value of vitamin A in early preimplantation steps of reproduction in general (Schweigert et al., 1999; Schweigert and Siegling, 2001). Such results are useful, but little is known about the reproductive tract of birds. Studies have shown, however, that vitamin A is involved in oestrogen-induced cell proliferation but not in cytodifferentiation of the chicken oviduct (Ninomiya et al., 1996) and that a deficiency of vitamin A causes morphological changes in these structures (Ganguly et al., 1983).

The aim of the present study was therefore to evaluate the possible spatial and temporal changes in retinol and retinyl ester content in reproductive structures during egg formation in Japanese quail and to note possible differences that might be indicative of specific needs during egg formation. Plasma, kidney and liver were investigated to evaluate possible changes in systemic vitamin A metabolism associated with egg laying as indicators of mobilisation, storage or excretion.

**Materials and methods**

Mature (9 months old) Japanese quail hens (*Coturnix coturnix japonica*) were kept on the commonly available diet that met or exceeded NRC requirements and contained ~10,000 IU/kg feed vitamin A. The birds were divided into three groups (n = 7) on the basis of their egg formation period (developing egg in magnum, calcifying egg in uterus and immediately after the laying). Whole blood was collected into heparinised test tubes and the plasma was separated by centrifugation. In each given phase of egg formation the birds were killed by decapitation and the liver, kidney and ovary with the oviduct were removed, weighed and stored at –20 °C until examined. Prior to the analysis of vitamin A (retinol and retinyl ester) content in the reproductive tract, the oviduct was further divided into four sections: infundibulum, magnum, isthmus and uterus. In the ovaries, the four largest (F1-F4) maturing follicles were studied individually;
the other developing, immature follicles were pooled together for further analyses. The content of retinol and retinyl esters was determined in the plasma, liver, kidney and individual sections of the reproductive tract by rp-HPLC as described previously (Schweigert et al., 1998). Due to the high percentage of lipids in egg yolks, the organic extract was saponified. An aliquot of the organic extract (300 µl) was first dried and then saponified under nitrogen with 1 ml of ethanolic KOH (5 g KOH + 5 ml H₂O + 50 ml ethanol) for 30 min at 70–75 °C (Frolik and Olson, 1984). After two further organic extractions with 2 ml of n-hexane (0.05% BHT), the combined organic extract of the sample was dried under nitrogen and then reconstituted. Finally, the reconstituted samples were directly injected onto rp-HPLC.

Data are reported as means ± SD. Data of the sections of reproductive tract were compared by Student’s t-test.

**Results**

Table 1 presents retinol and retinyl ester levels in plasma at different stages of egg formation, in the liver and in the kidney. Neither retinol nor retinyl ester levels were affected by the stage of egg formation. Therefore, the results from all 21 quails are summarised and treated as one group.

<table>
<thead>
<tr>
<th>Vitamin A (retinol, retinyl esters) concentration in the blood plasma (µg/ml), liver and kidney (µg/g wt) of quails (mean ± SD)</th>
</tr>
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<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Plasma</td>
</tr>
<tr>
<td>Egg in magnum</td>
</tr>
<tr>
<td>Egg in uterus</td>
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<tr>
<td>Empty</td>
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<tr>
<td>Liver</td>
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<td>Kidney</td>
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</tbody>
</table>

Table 2 displays the results for retinol and retinyl ester levels from ovaries and developing eggs. In our investigation, no significant differences were observed in (F1-F4) vitamin A content between the maturated follicles (four to six large yolk-filled follicles). The predominant form of vitamin A found in the follicle (F4) was retinol.

Despite obvious morphological differences in the sections of the reproductive tract at distinct stages of egg formation, there was no variation in regard to the content and distribution of retinol and retinyl esters between the individual sections of the oviduct. Therefore, Table 3 presents the individual sections and
all animals are treated as one group. In the four distinguishable sections of the oviduct that were examined, not only significant quantitative differences (P < 0.001) were observed, but also a change in the ratio of retinol to retinyl esters occurred. In the infundibulum and the magnum, retinol was the dominant form, representing 84.1 ± 23.4 and 85.8 ± 13.7%, respectively. In the isthmus, there was a higher percentage of retinyl palmitate and stearate and in the uterus retinol was again the main form of vitamin A (Fig. 1). The differences between the isthmus, infundibulum and magnum (P < 0.001) and the uterus and isthmus (P < 0.05) were significant for retinol and retinyl esters.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Retinol</th>
<th>Retinyl palmitate</th>
<th>Retinyl stearate</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4</td>
<td>7.97 ± 2.77\textsuperscript{a}</td>
<td>0.49 ± 0.21</td>
<td>not detectable</td>
</tr>
<tr>
<td>F1\textsuperscript{1}</td>
<td>6.96 ± 1.43\textsuperscript{a}</td>
<td>––</td>
<td>––</td>
</tr>
<tr>
<td>Egg\textsuperscript{2}</td>
<td>6.56 ± 0.85\textsuperscript{a}</td>
<td>––</td>
<td>––</td>
</tr>
</tbody>
</table>

\textsuperscript{1}after esterification using ethanolic KOH at 70–75 °C; \textsuperscript{2}after esterification in different stages of the oviduct during egg formation; \textsuperscript{a}values followed by the same letter are not significantly different.

Table 3

<table>
<thead>
<tr>
<th></th>
<th>Retinol</th>
<th>Retinyl palmitate</th>
<th>Retinyl stearate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary\textsuperscript{3}</td>
<td>0.81 ± 0.25</td>
<td>0.44 ± 0.16</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Infundibulum</td>
<td>0.35 ± 0.19\textsuperscript{b}</td>
<td>0.05 ± 0.04</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>Magnum</td>
<td>0.18 ± 0.09</td>
<td>0.04 ± 0.02\textsuperscript{a}</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Isthmus</td>
<td>0.14 ± 0.07\textsuperscript{b}</td>
<td>0.70 ± 0.68\textsuperscript{c}</td>
<td>0.25 ± 0.26\textsuperscript{c}</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.22 ± 0.07</td>
<td>0.23 ± 0.21</td>
<td>0.07 ± 0.04</td>
</tr>
</tbody>
</table>

\textsuperscript{3}only with numerous small grey-yellow and white immature follicles; \textsuperscript{a,b,c}significantly different from the subsequent section of the reproductive tract at P < 0.001 (a), P < 0.01 (b) or P < 0.05 (c).

Discussion

Under physiological conditions, vitamin A in the plasma occurs primarily as retinol. This corresponds to results on chickens and quails (Kerti and Bárdos, 1999; Kerti et al., 1997) and is also similar to findings in mammals, in which the retinol level in plasma is homeostatically regulated (Blomhoff et al., 1990). In contrast to plasma, the liver, the main storage organ of vitamin A, has primarily retinyl esters present, which represent 88.5% of total vitamin A. In our study ab-
solute levels and the ratio of retinol to retinyl esters were in close agreement to previous findings (Schindler et al., 1987; Periquet et al., 1991). Only limited information is currently available about vitamin A in avian kidneys. Although the quantitative differences between the liver (306.6 ± 138.7 µg/g) and the kidney (4.2 ± 3.2 µg/g) corresponded to those in mammals (Blomhoff et al., 1990), much higher levels were reported in mallards (1067.5 and 867.5 µg/g, respectively), which might indicate dietary and/or species specific differences (Surai et al., 2000).

In contrast to a study conducted by Bárdos (1989) using laying hens during egg formation, we were unable to show a similar increase in plasma retinol while egg formation was taking place in the magnum. In close agreement, however, is the observation that plasma retinyl ester levels were not affected during the formation process. In hens, the increase in retinol and its plasma carrier retinol-binding protein (RBP) may be caused by the sexual steroid effect necessary for satisfying the retinoid needs of the ovaries and shell gland (avian uterus). Assuming a similar efficiency of vitamin A absorption during egg formation in quails, differences observed might be in part attributed to a faster metabolism and egg formation cycle found in quails than in hens. Further studies in which RBP can be monitored would be necessary to evaluate if these differences are species specific.

The functionally mature ovary of the hen is arranged in an obvious hierarchy of follicles. The position of the follicle within this hierarchy does not substantially influence vitamin content (primarily retinol) (Kerti and Bárdos, 1999). This indicates that there is a continuous transfer of vitamin A into the follicle during the period of fast maturation. The F1 (preovulatory) follicles contained...
the same amount of vitamin A as the eggs did, which indicates that the total amount of vitamin A in the yolk is already accumulated during follicular growth.

In this study, the oviduct was investigated for the first time regarding its general retinol and retinyl ester content. Additionally, the study explored the possible significance of location as well as the stage of egg laying. Our results showed that the stage of egg laying had no effect but that obvious differences were observed in relation to the total amount of vitamin A present and the ratio of retinol to retinyl esters in the individual sections of the oviduct investigated. This observation contradicts previous findings in mammals. In the uterine tissues of gilts, temporal and spatial changes in the levels of retinol and retinyl esters and in proteins related to the metabolism of vitamin A (i.e. retinol binding protein, cellular retinol binding protein I and retinoic X receptor) can be observed during the ovarian cycle and implantation. In mammals, spatial and temporal changes in the concentration of vitamin A, its cellular binding proteins and its nuclear receptors have been attributed to the co-ordination of ovulation, egg transport through the mammal oviduct and implantation (Schweigert et al., 1999; Schweigert and Siegling, 2001). These opposing observations of mammals and birds might be explained by differences in function. During egg transport and prior to implantation, the mammalian oviduct and uterus both secrete a fluid to supply the embryo with the components necessary for survival and development. The composition of the oviductal and uterine fluid is a result of the signalling between the embryo and the endometrium. In this sense, embryonic oestrogen synthesis greatly affects the major components of mammalian uterine fluid, namely retinol and its binding protein, retinol binding protein (Harney et al., 1994). Since the egg accumulates all of its vitamin A during yolk formation in the ovary (Schneider and Wolf, 1976) and virtually no vitamin A is found in the egg white itself (Plack, 1960), the oviduct of birds, in contrast to the oviduct and uterus of mammals, has no role in retinol secretion. Thus, observed differences between individual sections of the oviduct in quails might be solely attributed to the importance of retinoids in modulating cellular functions during egg formation. Additionally, studies show that vitamin A is involved in oestrogen-induced cell proliferation but not in cytodifferentiation of the chicken oviduct (Ninomiya et al., 1996); vitamin A deficiency causes morphological changes in these structures (Ganguly et al., 1983). It is likely that individual hormones (such as oestrogen and others, e.g. follicle-stimulating hormone) or growth factors (ovarian insulin-like growth factor) might influence the expressional pattern of the retinoic acid receptor (RARβ) gene during follicular development (gonadotropic growth) (Fu et al., 2001).

The observed differences in the total amount of vitamin A present and the ratio of retinol to retinyl esters in the individual sections of the oviduct might be attributed to the varying structural composition of the oviduct. Temporal and spatial variations have been found in gilts, but differences between individual tis-
sue structures have been observed not only in the total amount of vitamin A present but also in the ratio of retinol to retinyl esters.

In the endometrium, total vitamin A, predominantly found as retinol, was significantly higher (P < 0.01) than in the myometrium, where retinyl palmitate dominated. It has been hypothesised that increased levels of retinol in the endometrium are indicative of the importance of these structures in retinol secretion into the uterus, while retinyl ester levels in the myometrium are indicative of its storage.

Like the mammalian oviduct and uterus, the avian oviduct contains secretory structures such as the mucosa and smooth muscle. The first layers of albumen (extravitelline and outer layers of the yolk membrane) are produced in the infundibulum. The largest portion of the oviduct is the magnum, where the majority of albumen is formed. As the egg passes through the magnum, the ridges distend and within them the secretory cells discharge the components of egg white. In the region of the isthmus, both inner and outer shell membranes are formed. The shell gland (uterus) contains secretory cells and is surrounded by well-developed muscles (Etches, 1996; Johnson, 2000). The differences in the total amount of vitamin A as well as the ratio of retinol to retinyl ester in the oviduct of quails might therefore be influenced by both the function of specific structures of the oviduct during egg formation as well as the relationship of mucosa to muscular layers. In the infundibulum and magnum, an increased proportion of retinol might indicate that retinol serves as a precursor for the modulation of general metabolic aspects such as an intensive development of the egg and an increased metabolic need of albumen (egg white) secreting glands. The greater proportion of muscular structures in the isthmus and the uterus might account for the greater proportion of retinyl esters available for the storage of vitamin A.

In conclusion, the study shows that despite obvious differences between the functions of mammalian and avian oviducts, quantitative and qualitative changes in retinol and retinyl esters were observed that point to the importance of retinol and/or retinyl esters in the cellular differentiation of the avian oviduct during egg formation. Further studies are necessary to evaluate the interaction of dietary supplementation and laying success as well as molecular mechanisms that might be involved on the level of retinoic acid in gene expression during the formation of the egg white or the egg shell. Both aspects are not only of physiological but also commercial importance for the laying performance of quails and other birds.

Acknowledgements

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