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HISTOLOGICAL STUDIES ON EMBRYONIC DEVELOPMENT OF THE RABBIT HEART

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Two experiments were performed to evaluate the normal development of the rabbit heart. In the first experiment the most intensive period of heart development was determined in rabbit embryos. The second experiment studied the most intensive period of heart development, determined in the first experiment, by concentrated sampling at 8-hour intervals. After cutting open the uterine wall opposite the discoid placenta, rabbit embryos were removed from the ampullae of the uterus using capillary tubes, under stereomicroscope at fivefold magnification. The embryos were subsequently placed into 4% formalin solution for 24 h. After fixation, slides stained with haematoxylin and eosin were made for histological examination. In the first experiment 51 embryos were examined, while during the second experiment a total of 113 embryos, representing different stages of development, were collected. Finally the data obtained on rabbits were compared with the well-known development of the heart in humans and mice.

Key words: Embryonic development, heart, rabbit, histological studies, species comparison

In human medicine ‘surgical treatment of the fetus is now possible’ (Harrison et al., 1991), owing to the rapid development of diagnostic and surgical techniques and thorough knowledge of the normal development of human embryos.

Veterinary medicine also makes claims on understanding the aetiology and pathogenesis of congenital diseases and on making a correct diagnosis. Nevertheless, the veterinary literature is deficient in data on developmental embryology, because it analogises the development of animals with that of humans in approximately 95%. The members of all vertebrate classes pass through an identical evolutionarily conserved ‘phylotypic’ stage. It was assumed that this theory was correct and that there was a particular stage of development that was identical to all vertebrates. Only later in development would specific differences appear (Haeckel, 1874). The question arises whether we have any argument for drawing a correlation between human and animal development, considering that

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the length of intrauterine life differs by species and that the form of embryos in the same developmental stages varies widely. This fact reminds us that despite the above similarities, differences are also important (Richardson et al., 1997), and that at the time of their birth different species exhibit different levels of development. This is well illustrated by the example of autophagi and insessorials.

As the anatomy of the heart and great vessels varies widely in different species, on grounds of expedience we formed a table which can help in making comparisons between Streeter's developmental horizons (Streeter, 1942; Streeter, 1948), which are still being used in humans and at certain stages also in different species of animals.

The main goal of the present experiments was to describe the normal development of the canine heart. However, it is very difficult to collect embryos from dogs. To decrease the number of dogs to be used for experimentation, the rabbit was chosen as a model animal.

The basis of our choice was that in humans the embryonic period lasts from day 0 to 56 (Moore and Persaud, 1998), in rabbits from day 0 to 14 (Evans and Sack, 1973) and in dogs from day 0 to 28 (Latshaw, 1987) of gestation. Thus in dogs the embryonic period is half as long as in humans and twice as long as in rabbits. The developmental status of the nervous system is similar in newborn rabbits and dogs. Both species are insessorial. The developmental stage of the nervous system is important because of its parallel development with the circulatory system. The rapid growth of the nervous system causes the cardiogenic area and future pericardial sac to become ventral to the neural tube and causes the central portion of the cardiogenic plate and the developing pericardiac intraembryonic coelomic cavity to move from its rostral position to a ventral and caudal position (Sadler, 1999). The development of the heart in rabbit and dog embryos will be compared in future studies.

Materials and methods

The objective of the first experiment was to determine the most intensive period of heart development in rabbit embryos. The second experiment studied the most intensive period of heart development, determined in the first experiment, by concentrated sampling at 8-hour intervals.

Experiment I

Twenty New Zealand White rabbits were used. The rabbits were accommodated in the experimental animal house of the Department of Animal Breeding, Nutrition and Laboratory Animal Science of the Faculty of Veterinary Science of Szent István University, in a room of regulated temperature (20–22 °C, relative humidity 75%), in individual metabolic cages (non-corrodible steel, grid

floor). Feed and drinking water were provided *ad libitum* (self-feeder, self-watering system, commercial rabbit diet). The rabbits were treated with PMSG in a dose of 0.5 ml/animal to induce oestrous (40 IU/0.5 ml, Werfaser inj. A.U.V. 1000 IU, Werfft-Chemie). After two days of oestrus induction the does were treated with GnRH in a dose of 0.5 ml/animal to induce ovulation (0.8 µg/0.5 ml, Receptal inj., Hoechst GmbH, Munich), after which they were inseminated immediately. Semen was obtained from Labnyúl Ltd. (Gödöllő). The semen was diluted fivefold with Horváth's yolk diluent (Vetési, 1980). Before embryo collection, the does were treated with a combination of ketamine and xylazine in a dose of 25 mg/kg ketamine and 5 mg/kg xylazine (1 g/10 ml, SBH-Ketamin inj. A.U.V., SelBruHa Ltd., 2 g/10 ml Rompun 2% inj. A.U.V., Bayer) to induce narcosis before euthanasia. After the 9th day the gravid uterus was palpated before treatment of the female rabbits. As the formation of the cardiac tube coincides with that of the embryonic disc and elongation of the embryo, embryos from the visible measuring range were collected. Since the time of onset of embryonic disc formation was not known, collection of embryos was started already from the 2nd day of gestation.

In our opinion there was no need to collect embryos after the 15th day of gestation, as the 14th gestational day means the end of the embryonic period in rabbits (Evans and Sack, 1973).

After cutting open the uterine wall opposite the discoid placenta, rabbit embryos were removed from the ampullae of the uterus using capillary tubes, under stereomicroscope at fivefold magnification. The embryos were subsequently placed into 4% formalin solution for 24 h. After fixation, slides stained with haematoxylin and eosin were made for histological examination, and blocks were made for scanning electron microscopy. The morphological landmarks of the embryos and the number of somites were also examined.

During the first experiment samples were collected from 20 New Zealand White rabbits. Overall, 51 embryos representing different stages of development were examined, in the distribution shown in Table 1.

Experiment II

In this experiment 10 gravid female New Zealand White rabbits were used, which were bred and supplied by Labnyúl Ltd. (Gödöllő) as females guaranteed to be pregnant. Because of the great morphological changes observed in the previous experiment, samples were collected at 8-hour intervals in this period. Morphologically there are no large differences between species, and therefore this sampling frequency was sufficient. Thus, embryos were examined from the 10th day of gestation every 8 hours. During the experiment a total of 113 embryos were collected by the same method as in the first experiment (Table 2).

Table 1

Samples collected during the experiment performed to determine the most intensive period of heart development

Gestational day	Rabbits used for sampling	Visible embryos	Corpus luteum	Ampullated uterus
2	1	–	–	–
3	1	–	–	–
4	1	–	9	–
5	1	–	7	–
6	1	–	6	–
7	1	–	8	–
8	1	–	9	+
9	1st	–	–	–
	2nd	8	9	+
10	1	4	4	+
11	1st	–	–	–
	2nd	9	9	+
12	1st	–	–	–
	2nd	8	8	+
13	1st	–	–	–
	2nd	6	6	+
14	1st	–	–	–
	2nd	8	8	+
15	1st	–	–	–
	2nd	8	8	+

Table 2

Samples taken at 8-hour intervals in the most intensive period of heart development

Gestational day	Hour	Sign	Number of embryos
10	0	10/0	11
10	8	10/8	9
10	16	10/16	12
11	0	11/0	14
11	8	11/8	11
11	16	11/16	13
12	0	12/0	9
12	8	12/8	12
12	16	12/16	10
13	0	13/0	12

Results

Experiment I

On the 9th gestational day of the rabbit the embryonic disc appears, on the 10th day the single cardiac tube is formed, on the 11th day the bulboventricular loop is formed, and the heart consists of three chambers. On the 12th day the septation of atria and ventricles is close to its end, on the 13th day the heart consists of four chambers, and on the 14th day the developmental stage of the heart is very similar to that seen in the newborn. In rabbits the most intensive development of the heart takes place in the period between the 10th and the 13th day.

Experiment II

At 0 hour of the 10th gestational day the cardiac tube develops rapidly, forcing it to fold upon itself, the centrally located sinoatrial junction begins to shift to the right (Fig. 1A), and perforation appears in the dorsal mesocardium. In the 8th hour on the 10th day the atrium is common, the bulboventricular loop is formed, the septum primum appears (Figs 1B, 1C). In the 16th hour on the 10th day (Fig. 1D) – 0 hour on the 11th day the ventricular septum appears, the ventricles begin to dilate (Fig. 2A), the endocardial cushions appear, the precursor of the pulmonary vein appears. In the 8th hour on the 11th day the truncal swellings appear, the common atrium is divided into two by a septum. In the 16th hour on the 11th day – 0 hour on the 12th day the perforation in the septum primum is forming (Fig. 2B). In the 8th to 16th hour on the 12th day the bulboventricular flange begins to recede, the atrioventricular canal gains a 'dog's bone' appearance (Fig. 2D), the ostium secundum is formed by the free edge of septum primum, the sinoatrial junction has shifted completely to the right (Fig. 3A), and the heart is three chambered. In the 16th hour on the 12th day the foramen ovale is formed (Fig. 4D), the conus septum is shifted (Figs 3D and 4A, 4B), and the cranial and caudal endocardial cushions are fused (Figs 4B, 4C). At 0 hour on the 13th day the ventricular septum starts to grow and the coronary sinus is formed. On the basis of the results of the first experiment, at the end of the 13th day the interventricular canal is completely obliterated, and the heart is four chambered. On the 14th day the outflow tracts are completely separated.

Discussion

In human cases there are three criteria for establishing age: (1) the length of the embryo, (2) the number of somites, and (3) the morphologic landmarks.

In animals it is very complicated to use these criteria. In early developmental stages the embryos are straight and their measurements indicate the greatest length (GL). The older embryos are curved, and therefore the crown-rump length

(CRL) (O'Rahilly and Müller, 1987) is most frequently used. However, no anatomical marker clearly indicates the crown or rump, and these vary widely in different species, also between individuals, and the length of embryos may differ even in the same animal at the same stage of development.

The number of somites can be used as a criterion only in the early developmental stages.

Finally the Carnegie Embryonic Staging System was selected for measuring the embryos, because it is based on morphologic developmental landmarks of the embryo (Streeter, 1942), its use enables comparisons to be made between the findings of one species and those of another, and because this method is used internationally. Each stage consists of a two-day (ranges: 1 to 4 days) interval of embryonic development. In humans there are 23 stages, which means 56 days; this is the end of the embryonic and the beginning of the fetal gestational period.

The well-known development of human and murine embryos was compared with the development of rabbit embryos on the basis of the present experimental findings (Table 3).

Table 3

Comparison of heart development in human, mouse and rabbit embryos

Developmental stages	Gestational day/hour (from the point of view of heart development)		
	Human (Streeter, 1942, 1948)	Mouse (Evans and Sack, 1973)	Rabbit (Present study)
10	23	8/0	10/0
11	24	8/16	10/0
12	25, 26	9	10/8
13	28, 29, 30	10/0–10/8	10/16–, 11/0–, 11/8–11/16
14	31, 32	10/8–10/16	11/16–12/0
15	33, 34, 35, 36	10/16–11/0–11/8	12/0–, 12/8–12/16
16	37, 38, 39, 40	11/8, 11/16–12/0	12/16–13/0
17	41, 42, 43	12/0, 12/8–12/16	12/16–13/0
18	44, 45, 46	12/16, 13/0– middle of the 13th day	13/0– middle of the 13th day
19	47, 48		
20	49, 50, 51		
21	52, 53	13–15	13–14
22	54, 55		
23	56		

The 10th Carnegie Stage, the 23rd gestational day of humans corresponds to the beginning of the 8th gestational day of mouse, and to 0 hour of the 10th gestational day of rabbit. At this stage there are 4–12 somites, the heart is beating, the embryo is slightly curved, the neural tube is formed but open at the rostral and caudal neuropores, the primordia of eye and ear are present, the single cardiac tube is convoluted, and the bulboventricular loop is forming (Fig. 1A).

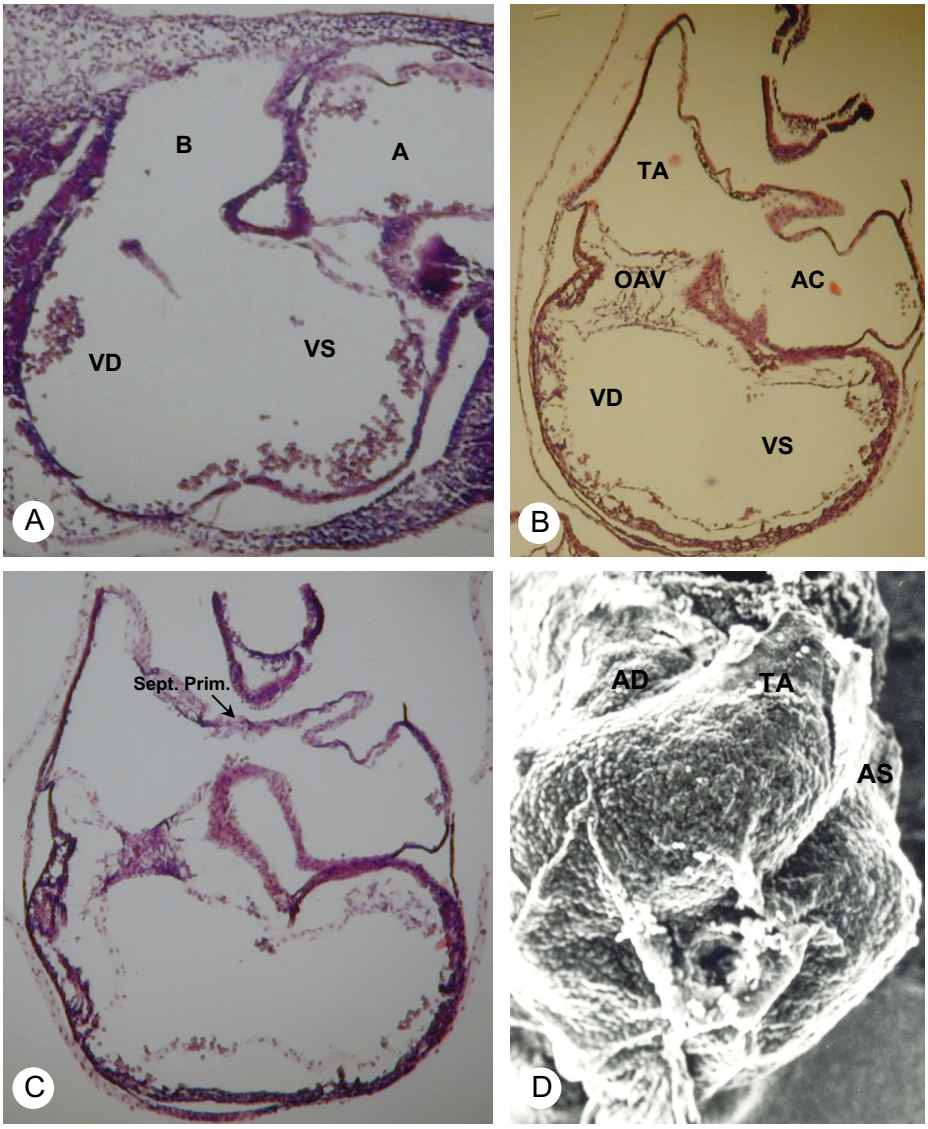

Fig. 1. Development of the heart on the 10th gestational day. **(A)** 10th gestational day, 0 hour: bulboventricular loop is forming, sinoatrial junction begins to shift to the right (× 32), **(B)** 10th gestational day, 8th hour: bulboventricular loop is formed, common atrium present, septum primum appears (× 32), **(C)** 10th gestational day, 8th hour: endocardial cushions appear (× 32), **(D)** 10th gestational day, 16th hour: bulboventricular loop is formed (× 201.6). A: atrium, AC: atrium communis, AD: atrium dexter, AS: atrium sinister, B: bulbus, OAV: ostium atrioventriculare, Sept. Prim.: septum primum, TA: truncus arteriosus, VD: ventriculus dexter, VS: ventriculus sinister

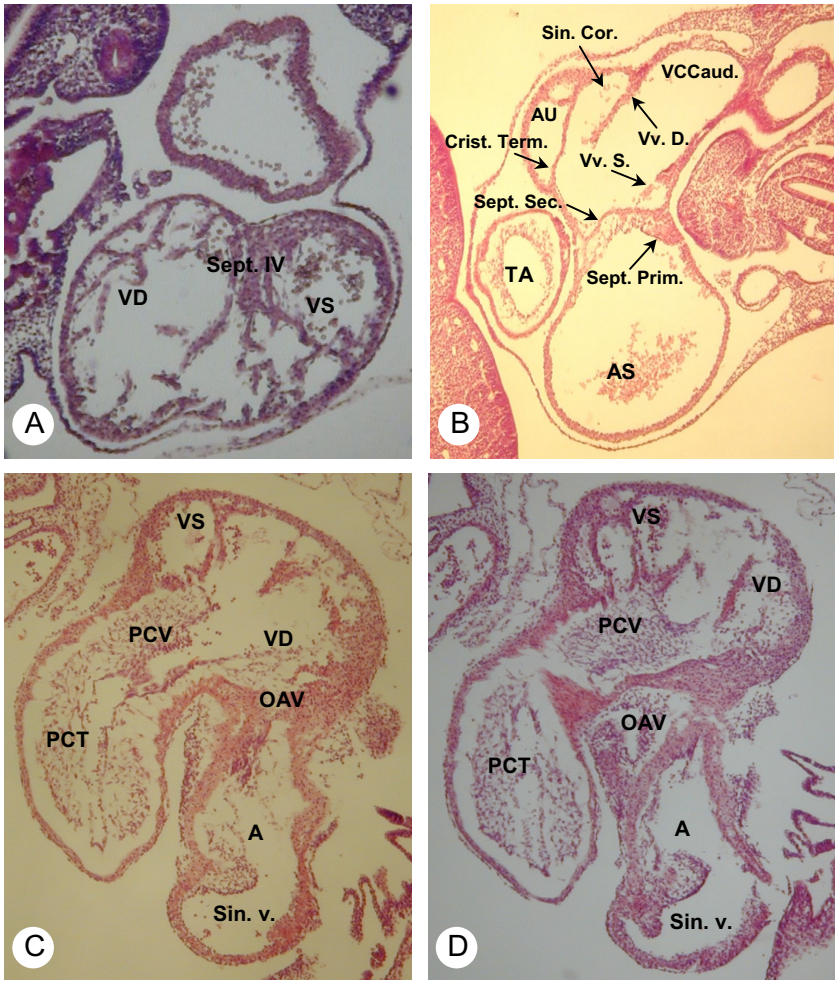

Fig. 2. Development of the heart on the 11th gestational day and on the beginning of the 12th gestational day. **(A)** 11th gestational day, 0–8th hours: dilating ventricles, the fold of the ventricular septum appears (× 32), **(B)** 11th gestational day, 16th hour – 12th gestational day, 0 hour: ostium secundum is forming (× 32), **(C)** 12th gestational day, 0–8th hour: transverse sinus of the pericardium begins to form (× 32), **(D)** 12th gestational day, 0–8th hour: common atrioventricular opening has a special form (× 32). A: atrium, AS: atrium sinister, AU: auricula, Crist. Term.: crista terminalis, OAV: ostium atrioventriculare, PCT: panniculus conotruncalis, PCV: panniculus conoventricularis, Sept. IV.: septum interventricularis, Sept. Prim.: septum primum, Sept. Sec.: septum secundum, Sin. Cor.: sinus coronarius, Sin. v.: sinus venosus, TA: truncus arteriosus, VCCaud.: vena cava caudalis, VD: ventriculus dexter, VS: ventriculus sinister, Vv. D.: valvula venosa dextra, Vv. S.: valvula venosa sinistra

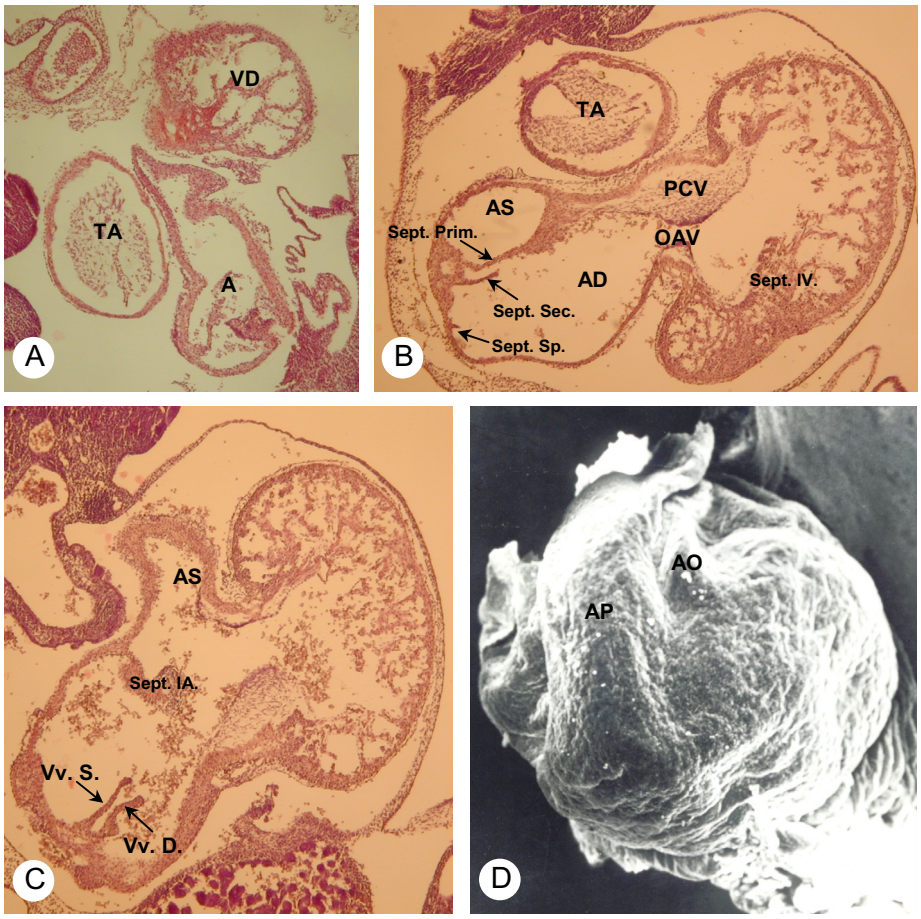

Fig. 3. The development of the heart from the 2nd third of the 12th to the beginning of the 13th gestational day. **(A)** 12th gestational day, 0–8th hour: sinoatrial orifice has shifted completely to the right (× 32), **(B)** 12th gestational day, 16th hour: cranial and caudal endocardial cushions are fused (× 32), **(C)** 12th gestational day, 16th hour – 13th gestational day, 0 hour: the venous valves are forming in the coronary sinus (× 32), **(D)** 12th gestational day, 16th hour – 13th gestational day, 0 hour: the great vessels (aorta and pulmonary artery) of the truncus arteriosus are forming (× 100.8). A: atrium, AD: atrium dexter, AO: aorta, AP: arteria pulmonalis, AS: atrium sinister, OAV: ostium atrioventriculare, PCV: panniculus conoventricularis, Sept. IA.: septum interatriale, Sept. IV.: septum interventriculare, Sept. Prim.: septum primum, Sept. Sec.: septum secundum, Sept. Sp.: septum spurium, TA: truncus arteriosus, VD: ventriculus dexter, Vv. D.: valvula venosa dextra, Vv. S.: valvula venosa sinistra

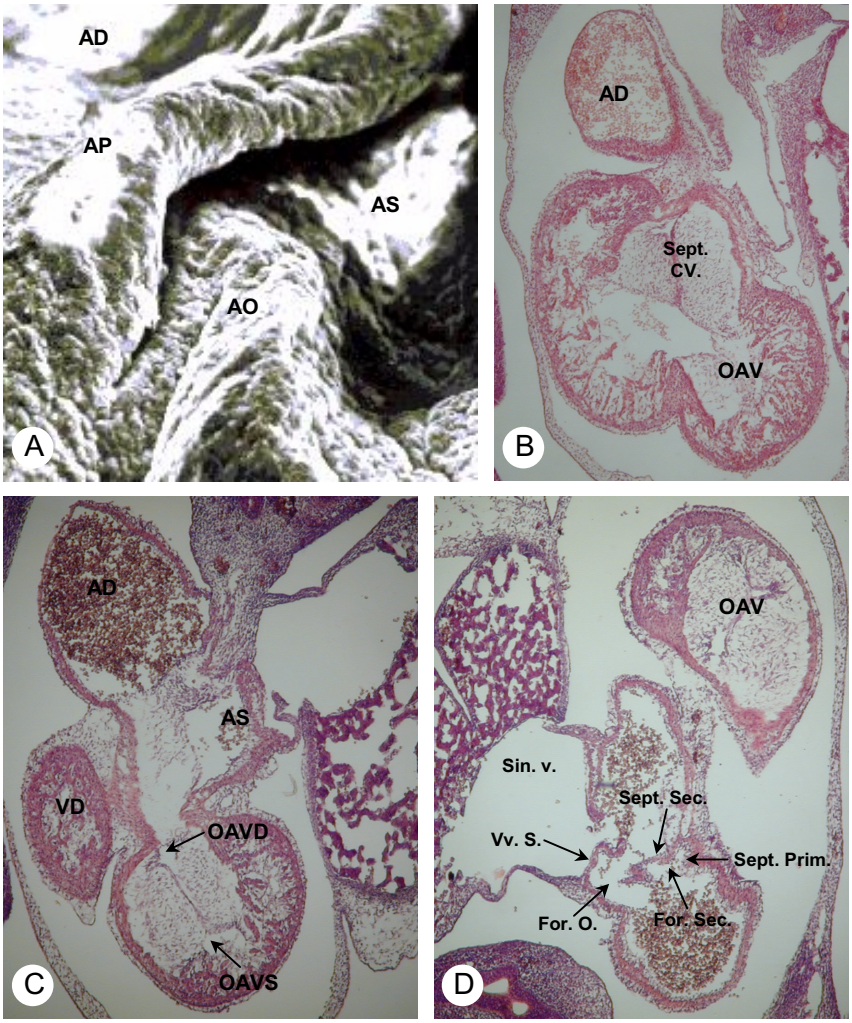

Fig. 4. Development of the heart from the end of the 12th to the beginning of the 13th gestational day. **(A)** 12th gestational day, 16th hour – 13th gestational day, 0 hour: separation of the truncus arteriosus (× 616), **(B)** 13th gestational day, 0 hour: the separation of the truncus arteriosus is forming, ventricular septum starts to grow (× 32), **(C)** 13th gestational day, 0 hour (× 32), **(D)** 13th gestational day, 0 hour: foramen ovale is formed (× 32). AD: atrium dexter, AO: aorta, AP: arteria pulmonalis, AS: atrium sinister, For. O.: foramen ovale, For. Sec.: foramen secundum, OAV: ostium atrioventriculare, OAVD: ostium atrioventriculare dexter, OAVS: ostium atrioventriculare sinister, Sept. CV.: septum conoventriculare, Sept. Prim.: septum primum, Sept. Sec.: septum secundum, Sin. v.: sinus venosus, VD: ventriculus dexter, VS: ventriculus sinister, Vv. S.: valvula venosa sinistra

The 11th Carnegie Stage, the 24th gestational day of humans corresponds to the end of the 8th gestational day of mouse, and to 0 hour of the 10th gestational day of rabbit. As embryos vary greatly even in the same interval of gestation (Sadler, 1999), 0 hour of the 10th gestational day of rabbit could be equal to either the 10th or the 11th Carnegie Stage. At this stage there are 13–20 somites, the embryo is curved in S-shape, the rostral neuropore is closed, the otic placodes are present, the optic vesicles are formed, and because of the looping of the heart the sinoatrial junction begins to shift to the right.

The 12th Carnegie Stage, representing the 25th–26th gestational day in humans, corresponds to the 9th gestational day of mouse, and to the 8th hour of the 10th gestational day of rabbit. At this stage there are 21–29 somites, the embryo is curved in C-shape, and the cervical curve is completed. The cranial limb buds appear, the caudal neuropore is closing, there are three pairs of branchial arches, otic pits are present, the liver, the pancreas and the spleen are beginning to differentiate. The heart prominence is distinct, the bulboventricular loop is formed, common atrium is present, and the septum primum appears (Figs 1B, 1C, 1D).

The 13th Carnegie Stage, which represents the 28th–30th gestational days in humans, is equivalent to the 10th gestational day of mouse, and to the end of the 10th day to the middle of the 11th day in rabbit by the morphology of embryos. There are 30–35 somites, the caudal neuropore is closed, the cranial limb buds flipper-like, caudal limb buds appear, the otic vesicles are present, the lens placodes are distinct, by the end of the period the primordia of the oral and nasal cavity appear. From the aspect of the developing heart the 28th gestational day of humans corresponds to the 16th hour of the 10th gestational day of rabbit. The 29th gestational day of humans corresponds to 0 hour of the 11th gestational day of rabbit. In that period, because of the dilating ventricles, the fold of the ventricular septum appears, the endocardial cushions are growing, the interatrial septum divides the common atrium into two cavities, the truncal swellings appear, and so the division of the conotruncus begins (Fig. 2A). The 30th gestational day of humans corresponds to the 8th hour of the 11th gestational day of rabbit. The perforation of the primary septum appears (Fig. 2B).

The 14th Carnegie Stage, which represents the 31st–32nd gestational days in humans, is equivalent to the middle of the 10th gestational day of mouse, and to the 16th hour of the 11th – 0 hour of the 12th gestational day of rabbit. The number of somites is not useful for measuring the embryos. The forelimb is paddle-shaped, the hindlimbs are flipper-like, there are three pharyngeal arches, and the mesonephric ridge appears. The lens pits and the nasal pits are visible. The ostium secundum is forming. The transverse sinus of the pericardium begins to form (Fig. 2C) because of the perforation of the dorsal mesocardium.

The 15th Carnegie Stage, representing the 33rd–36th gestational days in humans, is equivalent to the end of 10th–11th gestational day of mouse, and to the 0th hour of the 12th–16th hour of the 12th gestational day of rabbit. The car-

pal region is visible, the digital rays appear, the nasal pits are prominent, the hindlimbs are paddle-shaped. From the 8th hour of the 12th gestational day of rabbit until the 16th hour of the 12th day in the heart the bulboventricular fold is reduced, the common atrioventricular opening has a special form (Fig. 2D), and the ostium secundum is formed (in humans on the 33rd–34th gestational day). The sinoatrial orifice has shifted completely to the right (Fig. 3A) (in humans this occurs on the 35th–36th gestational day).

The 16th Carnegie Stage, which corresponds to the 37th–40th gestational days in humans, is equivalent to the 8th hour of the 11th day – 0 hour of the 12th gestational day of mouse, and to the 16th hour of the 12th gestational day of rabbit. The footplates are formed, the external ear appears. The foramen ovale is formed.

The 17th Carnegie Stage, corresponding to the 41st–43rd gestational days of humans, is equivalent to the 0 hour–8th hour of the 12th gestational day of mouse, and to the 16th hour of the 12th gestational day of rabbit. The digital rays are now visible, the external ear and the eye are prominent, and the cranial and caudal endocardial cushions are fused (Fig. 3B).

The 18th Carnegie Stage, corresponding to the 44th–46th gestational days of humans, is equivalent to the 16th hour of the 12th day – 0 hour of the 13th day of gestation in mouse, and to 0 hour of the 13th gestational day of rabbit. The cubital region is visible, the eyelids are forming, the ventricular septum starts to grow, and the coronary sinus is forming (Fig. 3C).

The 19th to 23rd Carnegie Stages, corresponding to the 47th–56th gestational days of humans, are equivalent to the 8th hour of the 13th gestational day – 0 hour of the 15th gestational days of mouse and to the middle of the 13th day – the end of the 14th gestational day of rabbit. The limbs, the eye and the ears are more developed. At the end of the 13th day of gestation in rabbit the interventricular canal is obliterated, the four-chambered heart appears, the separation of the truncus arteriosus is forming (Figs 3D and 4A, 4B, 4C), and the pulmonary veins are present. On the 14th day the separation of the truncus is completed.

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