SZENT ISTVÁN UNIVERSITY

Veterinary Doctoral School

COMPARATIVE MORPHOLOGICAL STUDY OF NORMAL AND PATHOLOGICAL DEVELOPMENT OF THE HEART IN RABBIT, DOG, PIG AND SHEEP

Theses of Doctoral Dissertation

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CONTENTS

1. Antecedents and objectives of the study

Veterinary practitioners tend to come across an increasing number of cardiac anomalies, the recognition of which is facilitated by the great progress that has taken place in the examination and diagnostic methods in recent years.

The objective of this research was to gain a deeper insight into the development of the canine heart, in view of the fact that the exact chronological course of that development is yet unknown. For these studies the rabbit was used as model animal.

The question arose whether the developmental stages of different species could be compared with a view to body conformation, gestational day and heart development. This directly prompted the question whether a correlation could be established on the basis of the length of gestation and embryonic life, with the help of which correlation the most intensive period of heart development could be determined and divided into stages also in other species of animals, without further experiments.

According to my hypothesis, a close parallelism can be drawn between the development of the canine and the human heart. Different species cannot be simultaneously compared with each other and with humans, as – while body conformation is comparable but still shows marked differences – major differences may exist between species in the length of embryonic life and in the most intensive periods of development of the individual organ systems as related to the entire length of gestation. At the time of birth, insessorial and autophagous animal species show substantial differences in the developmental status of their nervous system, the development of which is closely correlated with the development of the heart.

Another objective of the research was to study the pathological effects exerted on the developmental process by a single large dose of a substance known to be embryotoxic but having no special effects, when this substance was administered at a specific stage of development. I studied what pathological course of development and, consequently, what types of developmental anomalies could arise which could be diagnosed after birth, and I also studied the stages of development to which these abnormalities could be linked. To answer these questions, I performed the third rabbit experiment during which the embryos were treated with a teratogenic substance.

Namely, if the development of cardiac anomalies in dogs can be determined and linked to specific time-points, then with the help of information collected during gestation it will be possible to draw retrospective conclusions on their cause as well. The knowledge of canine data would enable us to get a closer insight into the development of human pathological entities, since dogs live together with humans and are exposed to the same effects; therefore, a close correlation can be drawn between the cardiac anomalies described in dogs and humans, as regards both their clinical manifestations and their incidence.

A further question was whether in the porcine species there exist substantial differences in the morphology of the heart between individuals of the same species but of different breed (genotype), differences which could have an impact on their life performance. I wished to determine whether a given pig breed could be genetically more resistant to developmental anomalies than other breeds. Such data could prove valuable for animal breeders in the selection of pig breeds and varieties.

In addition, studies were conducted on heart development in sheep and dogs, i.e. the two animal species exposed to the highest risk of developmental abnormalities. Description of the abnormalities found enabled us to draw conclusions on the state of gestation at which the individual was exposed to the embryotoxic effect.

1.1. Objectives

1. To review the veterinary literature with a view to the normal and pathological development of the heart. Such a review has so far been lacking in the veterinary literature.
2. To get to know the chronological course of heart development in canine and rabbit embryos.
3. To determine whether the developmental stages of different species can be compared simultaneously according to body conformation, gestational day and heart development.
4. To establish correlations, if any, between the length of the gestation period, the length of embryonic life and the most intensive period of heart development, with the help of which the development of the heart could be divided into stages also in other species of animals without performing further experiments.

5. Linking the appearance of different developmental anomalies with specific time-points or stages of embryonic life in rabbits and dogs.
6. To study whether there are substantial differences between pigs of the same species but of different genotype with a view to cardiac morphology or development, which differences could influence the life performance of the animals, and to determine whether there exist any differences between genotypes in genetic resistance to anomalies of cardiac development.
7. Occurrence and description of cardiac anomalies in sheep and dog, the two animal species exposed to the highest risk of developmental abnormalities.

2. Materials and methods

2.1. Design and sequence of the experiments

Model experiments performed on rabbits were built on one another. The objective of the first experiment was to determine the most intensive period of heart development. Built upon the findings of the first experiment, the second experiment studied that most intensive period, broken down into 8-hour stages, in order to determine the exact chronological sequence of events occurring during normal development of the heart. During the third experiment performed on rabbits, doe-rabbits were treated with a single large dose of an embryotoxic substance and the resulting pathological course of development was studied, depending on the time of administration. As a continuation of the first two studies on rabbits, I also studied the normal embryonic development of the heart in canine embryos. In connection but not in close association with this, I carried out a postnatal morphologic survey on the hearts of pigs belonging to different genotypes, in order to answer the question whether there are developmentally important anatomical structures in the heart which can influence the life performance of a given genotype, and whether there exist differences between genotypes in genetic resistance to the development of cardiac anomalies. Subsequently, neonates of different species of animals were examined that died at a different age.

The main objective of the experiments was to describe the development of the canine heart; however, the collection of embryos is difficult in the canine species, due to the seasonal and long oestrus and individual variation in the time of conceptive ability; oestrus induction and ovulation detection

are difficult and costly, and maturation of the oocyte may take as many as 5 days depending on the individual. These facts markedly hamper the evaluation of results.

In order to decrease the number of animals used for experimentation, in compliance with the Act on the Protection and Welfare of Animals, a model animal had to be selected. For that purpose the rabbit proved to be the most suitable.

2.1.2. Criteria of model animal selection for studying the normal development process

(1) The embryonic period is half as long in the dog as in humans but twice as long as in rabbits. (2) The developmental status of the nervous system is also similar in the newborn dog and in the newborn rabbit. (3) Being a prolific and multiparous animal, the rabbit is suitable for the collection of a large number of samples. Its oestrus is independent of season and easily inducible; ovulation can also be induced by the administration of pharmaceuticals; in addition, rabbits can be fertilised easily. Maturation of the oocyte lasts for 0 to 1 day and, thus, interferes with the evaluation of results much less than the even 5-day variations typical of the canine species.

2.1.3. Sample collection and sampling

Twenty New Zealand White does were used in the first experiment, 10 New Zealand White does in the second experiment and 10 Californian White does in the third experiment. The rabbits were housed in a temperature-controlled animal room (20–22 °C temperature, 75% relative humidity) at the Department of Animal Breeding, Nutrition and Laboratory Animal Science, Faculty of Veterinary Science, Szent István University, in individual metabolic cages having stainless steel slatted floor. Feed and drinking water were supplied *ad libitum* (self-feeders, self-waterers, commercial rabbit diet). The fourth experiment included four Beagle dogs originating from the Beagle stock of the Pharmaceutical Research Institute Co. Ltd. The dogs were kept at the Dunakeszi premises of the Pharmaceutical Research Institute Co. Ltd., where Dr. Éva Kiss and Dr. István Novák provided assistance with the experiments. The dogs were kept in individual boxes (2 m^2 indoor area, 5 m^2 outdoor run, asphalt floor) and received a rationed food (commercial dog food) twice a day. Drinking water was supplied *ad libitum.*

In the first experiment artificial insemination of the rabbits was done by myself, while in the second and third experiments I examined embryos from doe-rabbits that were guaranteed to be pregnant by Labnyúl Ltd. of Gödöllő. In the fourth experiment conducted on dogs, hand mating was used: the male was placed beside the bitch in the box, and the animal tenders continuously supervised the oestrus of the bitches and the mating. After a single mating the male was removed from the box. In this way the time of conception could be documented precisely.

Experiment 1: The doe-rabbits were examined from the 2nd day of gestation. Thus, during the first experiment a total of 51 embryos were collected from different stages of development.

Experiment 2: As samples collected during the first experiment revealed large morphological differences among the individual stages, in Experiment 2 a concentrated sampling approach was used. This meant that samples were taken at 8-hour intervals. During that experiment I collected embryos at 8-hour intervals from 0 h on the 10th gestational day up to 0 h on the 13th gestational day; thus, a total of 113 embryos were collected.

Experiment 3: In the 20th hour of the 9th day of gestation 3 mg ethyl alcohol per kg of body weight was administered intravenously to a doe-rabbit. Subsequently, one doe each received the same treatment at 8-hour intervals. Embryos were examined 4 hours after the administration of alcohol. A total of 36 embryos were collected during the experiment.

Experiment 4: During this experiment I studied the development of the cardiovascular system between day 15 and day 30 after conception, by taking samples at 5-day intervals. A total of 20 embryos were collected during this experiment.

Experiment 5: Samples collected at the Herceghalom slaughterhouse from 20 meat-type pigs and from 20 pigs of the Mangalica breed were compared on the basis of measurable anatomical structures.

Experiment 6: In the framework of this experiment, I examined heart samples from 50 lambs that died after birth and originated from sheep farms in Somogy county, heart samples from 113 newborn Beagle dogs that died after premature delivery at the Dunakeszi animal facility of the Pharmaceutical Research Institute Co. Ltd., and those from 15 dead dogs (aged 0–6 months) submitted to our Department.

2.1.4. Sample processing

The embryos were removed after making an incision on the ampullae of the uterus on the side opposite the discoid placenta in the rabbit experiments and beside the placenta zonaria in the case of dogs, using a capillary tube,

under stereomicroscope using 5-fold magnification. Subsequently the embryos were fixed in 4% formaldehyde solution for 24 hours. After fixation, 5 μm serial sections were made, using HE staining, along the entire length of the embryo. All slices thus obtained were examined. From samples obtained from the second experiment performed on rabbits blocks were also made for scanning electron microscopic (SEM) examination. The morphological characteristics of the embryos and the number of somites were examined.

Because of the nature of studies performed in the first four experiments there was no need for statistical analysis.

The results obtained during the experiment conducted on pigs were evaluated by the use of an SPSS statistical programme. Statistical analysis was carried out using the Mann-Whitney test.

In the case of studies conducted on lambs and newborn dogs, there was no need for statistical analysis because of the nature of the studies.

3. Results

3.1. Results of the first model experiment: determination of the most intensive period of heart development in rabbits

On the 10th day of gestation the single cardiac tube is formed, while on the 13th day of gestation the interventricular canal is completely obliterated and the heart consists of four chambers.

3.2. Results of the second model experiment: study of the most intensive period of heart development by concentrated sampling at 8-hour intervals

At 0 hour on the 10th day of gestation the cardiac tube develops rapidly, forcing it to fold upon itself, the centrally located sinoatrial junction begins to shift to the right, and perforation appears in the dorsal mesocardium.

In the 8th hour on the 10th day of gestation the atrium is common (common primordial atrium), the bulboventricular loop is formed, and the septum primum appears.

In the 16th hour on the 10th day – 0 hour on the 11th day of gestation the ventricular septum appears, the ventricles begin to dilate, the endocardial cushions appear, and the precursor of the pulmonary vein appears.

In the 8th hour of the 11th gestational day the septum primum divides the common atrium into two parts.

In the 16th hour of the 11th day – 0 hour of the 12th day of gestation the perforation of the septum primum is forming.

In the 8th to the 16th hour of the 12th gestational day the bulboventricular flange begins to recede, the atrioventricular canal gains a characteristic "dog's bone" appearance, the free edge of the septum primum forms the foramen primum, the foramen secundum is also formed, the sinoatrial junction has shifted completely to the right, and the heart is three chambered.

In the 16th hour of the 12th day of gestation the foramen ovale is formed, the conus septum is shifted, and the cranial and caudal endocardial cushions are fused.

At 0 hour on the 13th gestational day the ventricular septum starts to grow and the coronary sinus is forming.

3.3. Results of the third model experiment: development of the heart in embryos of alcohol-treated doe-rabbits

Between 0 hour and the 16th hour of the 10th gestational day the embryos of does treated with alcohol 4 hours earlier died.

At 0 hour of the 11th gestational day the bulboventricular loop is formed, the bulboventricular flange is well visible, the atrium is common, the wall of the ventricles is thin, the septum primum is missing, and the atrioventricular endocardial cushions are not visible.

In the 8th hour of the 11th gestational day the bulboventricular flange is expressed, the atrium is common, the endocardial cushions appear, the wall of the ventricles is thin, and the ventricular septum is absent.

In the 16th hour of the 11th gestational day the atrial septum is insufficiently developed, the atrium is still common and dilated, the sinoatrial junction is located on the left, the wall of the ventricles is thin and starts to dilate, the primordium of the ventricular septum appears.

At 0 hour of the 12th gestational day the atrium is divided into two by a sufficiently developed septum primum, the sinoatrial junction has shifted to the right, the venous valves are developed, the endocardial cushions are expressed, the wall of the ventricles is trabeculated, the muscular ventricular septum is pronounced and the truncal ridges appear.

In the 8th hour of the 12th gestational day the septum primum is expressed, the foramen secundum is formed, the sinoatrial junction is located on the right, and the truncal and conal ridges appear.

In the 16th hour of the 12th gestational day the atrial septum is well developed, the foramen ovale appears, the right and left venous valves are formed and the ventricular septum is well developed.

At 0 hour of the 13th gestational day the atrioventricular orifice is expressed and separation of the truncus arteriosus has progressed.

3.4. Results of experiments conducted on dogs: chronological sequence of the normal heart development process

On the 15th day of gestation there is no appreciable embryo.

On the 20th day of gestation the loop of the cardiac tube is formed, the bulboventricular loop is expressed and the bulboventricular flange is well visible. The ventricles and the atria have a single chamber and the endocardial cushions are not yet visible.

On the 25th day of gestation the wall of the ventricles is expressly dilated and trabeculated, and the interventricular septum is forming. The endocardial cushions in the atrioventricular orifice are well developed, in the atrium the septum primum is formed and the septum secundum has started to form, the ostium sinoatriale is located on the right side, and separation of the truncus arteriosus starts.

On the 30th day of gestation the interventricular septum is well developed, in the atrium the septum secundum is well developed and the foramen ovale is formed, and the spatium interseptale between the septum primum and the septum secundum is well visible. The atrioventricular endocardial cushions are well visible, the primordia of the valves are visible, and the separation of the truncus has progressed.

3.5. Postnatal morphological comparative study of the heart in meat-type and Mangalica pigs

Marked differences can be found between meat-type and Mangalica pigs in the morphological indices of the heart. The numerical data measured on the heart of Mangalica pigs were markedly lower. The morphological examinations revealed atrial septal defect in 5 meat-type pigs (25%) while in the Mangalica pigs no morphological abnormalities were found.

3.6. Congenital cardiac abnormalities found in samples collected from dogs and lambs

3.6.1. Heart of a lamb that died 12 hours after birth

The trunk of the pulmonary artery originates from the right ventricle, the pulmonary opening is small, and the semilunar valves are present and movable. The pulmonary trunk is short, it is constricted after 2.5 cm and its lumen disappears. The aorta originates from the right ventricle. Five vessels originate from the aortic arch. The lumen of the aorta is markedly constricted. At the level of the septum membranaceum there is a large defect, situated high. The supraventricular crest is normal. The septal leaflet of the tricuspid valve is cleft and attached to the margin of the defect with a few chordae tendineae. In the atrium a large foramen ovale is visible, while on the right side of the septum secundum the septum primum is small and rudimentary.

3.6.2. Heart of a lamb that died 72 hours after birth

The pulmonary artery originates from the right ventricle and is connected with the aorta by the wide ductus arteriosus. The aorta arises from the left ventricle, and a single vessel originates from the aortic arch. Both the right atrium and the left atrium are normally developed. The interventricular septum is intact.

3.6.3. Morphology of the heart of a 6-week-old German Shepherd dog

The pulmonary artery opens from the right ventricle. The aorta arises from the left ventricle, then the aortic arch turns right and assumes a retro-oesophageal location, while the descending aorta runs parallel to the right

vena azygos on the right side of the vertebral column. The ductus arteriosus connects the right aortic arch with the pulmonary artery, thus forming a vascular ring around the oesophagus. The atria and the vessels running into them, the ventricles, the valves and the ventricular septum are normally developed.

3.6.4. Morphology of the heart of a 3-month-old mongrel dog

The pulmonary artery and the aorta are located normally. The aorta shows marked supravalvular dilatation. The vena cava cranialis is doubled, the left vena azygos flows into the left vena cava cranialis and then it runs into the right atrium through the sinus coronarius. The right vena azygos opens into the larger right vena cava cranialis. On the left side of the ventricular septum a defect can be seen on the dorsal smooth septum, which on the right side can be found under the septal cusp of the tricuspid valve; the defect is not connected to the surrounding structures. The septal defect is larger than the tip of a forceps. The mitral, tricuspid and pulmonary valves are thicker than normal while the aortic valve is normal. The heart is hypertrophic in its entirety, and hypertrophy is especially marked in the infundibular region.

4. Conclusions, new results, recommendations

(1) Preparation of a gap-filling review of the veterinary literature on the normal and pathological development of the heart.

(2) Description of the chronological course of heart development in rabbits and dogs, by the use of 8-hour sampling intervals in rabbits and 5-day sampling intervals in dogs.

(3) The developmental stages of different species cannot be compared simultaneously according to body conformation, gestational day and heart development.

(4) Among the length of the gestation period, the length of embryonic life and the most intensive period of heart development there exists no correlation with the help of which the development of the heart could be divided into stages also in other species of animals without performing further experiments.

(5) Linking the appearance of different developmental anomalies with specific 8-hour developmental stages in rabbits, drawing relevant conclusions in dogs.

(6) There are substantial differences in cardiac morphology and development between pigs of different genotypes; the Mangalica breed is genetically more resistant to the development of cardiac anomalies.

(7) Description of complex cardiac developmental anomalies in sheep and dogs with embryological explanations and determination of the time of their appearance.

5. Scientific papers published on the subject (or officially accepted for publication)

1. Balogh E., Sótonyi P.:
Multiple cardiac anomaly in sheep: A case study and review of the literature.
Accepted for publication: September 12, 2002
Acta Vet. Hung. **51** (1) (2003)
2. Balogh E., Sótonyi P.:
Histological studies on embryonic development of the rabbit heart.
Accepted for publication: September 12, 2002
Acta Vet. Hung. **51** (1) (2003)
3. Balogh E. (2002): A szív pathológiás fejlődése kutyában I. A pitvarok fejlődési rendellenességei (Pathological development of the heart in the dog I. Developmental anomalies of the atria). Kisállatpraxis **4,** 2–9.
4. **Balogh E.:**
A szív pathológiás fejlődése kutyában II. A kamrák fejlődési rendellenességei (Pathological development of the heart in the dog II. Developmental anomalies of the ventricles).
Accepted for publication: September 10, 2002
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5. Balogh E.:
A szív pathológiás fejlődése kutyában III.: A nagyerek fejlődési rendellenességei (Pathological development of the heart in dogs III. Developmental anomalies of the great vessels).
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Recieved publications

1. **Balogh E.,** Sótonyi P.:
The alcohol expressure in embryonic heart in rabbits.
Acta. Vet Hung
2. **Balogh E.,** Sótonyi P.:
Histological studies on embryonic development of the dog heart.
Acta. Vet Hung
3. **Balogh E.,** Sótonyi P.:
multiple cardiac anomaly of dog heart: Case study.
Acta. Vet Hung