Placental barrier permeability to Ca, P, and Mg in the diagnosis of canine hip dysplasia

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The experience carried out in two groups of female dogs of different gestational age on 35-th day (first period), 45-th day (second period), 55-th day (third period), and 65-th day (fourth period). The weight of animals ranged from 21-27 kg, and age from 2.4 to 4.5 years. We selected the animals of different breeds, in which the ultrasound detected at least five fetuses. Diet of control group consisted of natural foods (cereals, meat, vegetables), involved–with Bosch Production and vitamin-mineral complex Caniletten (tablets) or Canipulver (powder) according to the dosage recommended by the manufacturer. The experiments were conducted for five years in clinically healthy pregnant dogs, who had healthy puppies (control) and subclinical patients with the development in the postnatal period have they had fathered puppies of dysplasia or hip elbow (experience). At the same time the overall clinical status of all born pups up to 3 months of age determined. All pregnant animals were determined by morphological and biochemical composition of venous blood from pups of different age obtained at caesarean section, collected the umbilical cord, puncture of membranes – amniotic fluid and alantoin, after their autopsy, the liver, hip and elbow joints. Blood analysis and examination of fetuses and organs removed from them were carried out by conventional methods, mineral composition of blood, provisional organs and joint tissues by means of flame spectrophotometry. In the blood of pregnant female dogs we determined the content of Ca, P and Mg in the direction of a blood of mother – the fetal part of the placenta – the blood of the umbilical vein, the liver of fetal – tissue puppies – and alantoin amniotic fluid. There were no regular changes in the number of blood elements in the studied animals, but the number of red blood cells, the average volume, percentage, and absolute width of their distribution are lower in experimental animals compared to the control. The morphological analysis of differences in the cytological composition of blood and leucogramma did not revealed any variation, while the biochemical analysis found a significant increase in activity of AST and alkaline phosphatase for the experimental animals. We found that the fetal part of placenta accumulates Ca, P and Mg in concentrations much higher than in the mother blood. Main share of Ca from the mother blood in control group entered the fetal part of placenta during the second study period, share of P – during the first and third periods, and share of Mg gradually increased from the first to the fourth periods. The level of minerals in the blood of female dogs of the control group was critical during the second to third periods, from 45 to 55 days of pregnancy, and acted as a potential bioindicator of preventive diagnosis of canine joint dysplasia. During this period of pregnancy it is necessary to apply the adjustment rations by providing the animals with minerals.

Key words: Intrauterine development; Fetus; Pregnancy; Provisional organs; Liver

Introduction

Dysplasia is an abnoraml development and formation or anatomical defect of the bone and joint apparatus, which leads to impaired static and dynamic function of the extremities. (Velychko et al., 2001; Stevenson et al., 2009; De Pellegrin & Moharamzadeh, 2010; Teng et al., 2012; Mehdizadeh et al., 2019). Most commonly found among dogs, it develops as a result of the endogenous and exogenous influence on the body in the prenatal and postnatal ontogeny. Some authors believe that dysplasia belongs to inherited diseases of multifactorial and polygenic nature, which cause functional failure of the structural elements of the skeleton (Kolinski, 2003; Arti et al., 2013), others argue for its predominantly genetic origin, while in human orthopedics they also pay attention to autoimmune factors caused by the affect of toxins, bacteria and helminth. (Kolinski, 2003). In the juvenile period excessive high-calorie protein feeding of puppies facilitates the intensive growth of the musculoskeletal system and the development of dysplasia. (Mitin & Jagnikov, 2005). In most cases, hip dysplasia occurs, whereas the defects of elbow joints are less frequent. Though the importance of providing the body with macro- and microelements has been established (Mitin & Jagnikov, 2001), no specific experimental evidence with regards to joint dysplasia, sensitivity of pregnancy periods, and bioindicators for preventive pathology diagnosis has been developed. In case of joint dysplasia, it is necessary to determine the blood components of pregnant animals, which could be potential bioindicators of the critical course of pregnancy and the correction of their feeding to provide the fetus with all mineral substances essential for the formation and growth of the bones during intrauterine development.

The placenta as an organ created by the embryo consists of the maternal and fetal parts and organizes actively an environment from which it absorbs required substances and energy (Mitin et al., 2003).
The nutrients are transported from a mother to a fetus through the both placental parts. The maternal part of the placenta is a barrier to the flow of maternal blood to the fetus from the mother. Its function is clearly compared to the work of the valve, that is, it passes to the fetus only those substances which are vital for the latter. The fetal part of the placenta is a barrier on the fetus’ side, and it performs, among other things, two basic functions: the first, which is conventionally compared to the valve, passes all substances from the fetus to the mother; the second one is a barrier function, that is, it also regulates the flow of substances, but, in this case, from the maternal part of the placenta to the fetus, and also functions as a valve (Mitin & Jagnikov, 2005). Phases of animal ontogeny in prenatal fetal development include fetal, pre-natal, fetal (early and late) growth and the period of newborns. According to this classification, approximately 1/5 of the domestic animals’ pregnancy is the period of the fetal formation and growth. It has been experimentally established that during mammalian organogenesis, there are relatively short time when even a slight influence of various agents can impair the normal development of the embryo and the appearance of pathological changes in its organs. These critical phases of ontogeny occur during the change in the type of metabolism between the embryo and the maternal organism caused by high sensitivity of embryonic cells to the influence of external agents during embryogenesis. Problems regarding placental function and permeability of the placental barrier for various substances of exogenous and endogenous origin have been widely discussed in the scientific literature during the 60-70 years of the last century (Davydov, 2007). The results of the studies presented in them have not acquired further in-depth experimental study and still remain basic. The attention of this research is drawn to the question of the placental barrier functioning in cows in conditions of their maintenance in the territories of different biogeochronochemical provinces, further contaminated with radionuclides due to the accident at the Chernobyl AU, and in mares in the steppe zone of Ukraine. The aim of the present research was to study the placental barrier permeability to Ca, P and Mg during the canine pregnancy. The research objectives were to determine the critical cutoffs of prenatal development of puppy fetus during the pregnancy and to reveal the indicators for the diagnosis of canine joint dysplasia.

Materials and Methods
The experiments were performed during five years at different gestation times of clinically healthy female dogs from which the healthy puppies (control) were born and subclinically ill puppies with hip or (and) elbow joint dysplasia in post-natal development (experimental group). Simultaneously the general clinical condition of all puppies up to 3 months was determined. Morphological and biochemical composition of venous blood was studied in all pregnant animals. After the puppy fetuses of different ages were obtained by caesarean section, the cord was disjoined, amniotic and allantoic fluids were taken by means of the puncture and the liver, hip and elbow joints were removed in the result of the autopsy. Blood, fetuses and the organs extracted from them were examined using conventional methods, and the mineral composition of blood, transitional organs and joint tissues was performed by flame spectrophotometry. The experiments were done at four periods of canine pregnancy: 35th day (first period), 45th day (second period), 55th day (third period), and 65th day (fourth period, Table 1). The weight of the animals, aged from 2.4 to 4.5 years, ranged from 21 to 27 kg. Only the animals with at least 5 fetuses diagnosed with an ultrasound were used for the experiment. The food ration of the control group consisted of natural products (porridge, meat, vegetables) (Clarke, 2014), whereas the food intake of the experimental one included Bosch Reproduction, vitamin and mineral complex Caniletten (tablets) or Canipulver (powder) in accordance with the dose recommended by the manufacturer. In the blood of clinically healthy pregnant bitches, which gave birth to the puppies with joint dysplasia, the amount of Ca, P, and Mg was determined along the gradient ‘the maternal blood – the fetal part of the placenta – the blood of cord veins – the fetal liver – puppy fetuses’ joint tissues – amniotic and allantoic fluids’. No regular changes in the number of blood cells and their derivatives in the animals of the control and experimental groups have been established. At the same time, the number of red cells, the average volume, the percentage and the absolute width of their distribution is lower in the experimental group, compared with the control one, but does not go beyond the physiological indicators. All other indicators (Table 1) are higher in the experimental group than in the control one, but they also do not go beyond physiological limits. The composition of leukograms in both healthy dogs and those with dysplasia fluctuated within physiological limits (Table 2). Biochemical blood test showed a significant increase in the activity of aspartate aminotransferase and alkaline phosphatase (Table 3) in dogs of the experimental group ($P < 0.05; P < 0.01$). It is obvious, that the rate of P is higher in the fetal part of canine placenta in control group than in the experimental one, compared to the levels of Ca and Mg, which reflects its barrier and regulatory functions. The level of other liver (ALT, GGT, total protein, albumin, bilirubin), renal (urea, creatinine), pancreatic (amylase) parameters and glucose, potassium, sodium concentration varied within physiological limits. The high activity of AST is evident in many tissues of the fetus (Clarke, 2014), whereas the food intake of the experimental one included Bosch Reproduction, vitamin and mineral complex Caniletten (tablets) or Canipulver (powder) in accordance with the dose recommended by the manufacturer. In the blood of clinically healthy pregnant bitches, which gave birth to the puppies with joint dysplasia, the amount of Ca, P, and Mg was determined along the gradient ‘the maternal blood – the fetal part of the placenta – the blood of cord veins – the fetal liver – puppy fetuses’ joint tissues – amniotic and allantoic fluids’. No regular changes in the number of blood cells and their derivatives in the animals of the control and experimental groups have been established. At the same time, the number of red cells, the average volume, the percentage and the absolute width of their distribution is lower in the experimental group, compared with the control one, but does not go beyond the physiological limits. The composition of leukograms in both healthy dogs and those with dysplasia fluctuated within physiological limits (Table 2). Biochemical blood test showed a significant increase in the activity of aspartate aminotransferase and alkaline phosphatase (Table 3) in dogs of the experimental group ($P < 0.05; P < 0.01$). Therefore, it is possible to assume that the increase in AST activity in the blood of the dogs with dysplasia is caused by pathological changes alongside with the static-dynamic apparatus disorders of the limbs and all muscles of the body and by the effect on the hepatocytes of the metabolism products thus formed. The P and Mg levels in the blood of the dogs with joint dysplasia are at the low limits, whereas the rate of Ca below it indicates that the course of metabolism is accompanied by the intensive excretion from the body, or by obstacles in the course of their assimilation, which causes the imbalance between them (2.5: 1: 3.7 respectively). Much of calcium, phosphorus and magnesium is localized in the skeleton. Crystalline hydroxyapatite Ca$_{10}$ (PO$_4$)$_2$(OH)$_2$ contributes to mechanical strength of the bones and is a reservoir of calcium and phosphorus. The metabolism of calcium, phosphorus and magnesium is affected by estrogens, glucocorticoids, parathyroid hormone, vitamin D, calcitonin, growth hormone, glucagon, and triiodothyronine. The negative effects of these agents are extremely rare in animals (Mitin & Jagnikov, 2005).

Results and Discussion
We revealed (Table 1), that the hemoglobin level in the blood of dogs from the experimental group (125.3 ± 12.1 G L$^{-1}$) is lower by 12.9% than that in animals from the control group (144 ± 13.65 G L$^{-1}$). We also have found (Table 4) that the concentrations of Ca, P and Mg accumulated in the fetal part of the placenta are much higher than in the mother’s blood. Therefore, the fetal part of the placenta, a depot of minerals, provides penetration of the latter in the direction ‘umbilical veins - liver - joints - amniotic fluid of the fetus’. Analysis of the dynamics of minerals in the blood during the canine pregnancy allows to distinguish the periods, when their level decreases or does not change in the control group dogs. Thus, the concentration of Ca in blood decreases from 0.114 ± 0.04 to 0.111 ± 0.003 between the 45th and the 55th day of pregnancy, phosphorus remains unchanged (0.069 ± 0.002 – 0.069 ± 0.003).

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Table 1. Blood morphological indicators in healthy dogs and dogs with the joints dysplasia, M±m, n=12

<table>
<thead>
<tr>
<th>Experimental index</th>
<th>Measurement units</th>
<th>Rates</th>
<th>Control n = 6</th>
<th>Experimental n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>t L⁻¹</td>
<td>5.4-7.8</td>
<td>6.62 ± 0.4</td>
<td>6.42 ± 0.5</td>
</tr>
<tr>
<td>MCV</td>
<td>fl</td>
<td>64-74</td>
<td>70.9 ± 2.5</td>
<td>68.2 ± 2.39</td>
</tr>
<tr>
<td>RDW</td>
<td>%</td>
<td>11-16</td>
<td>13.7 ± 0.8</td>
<td>15.0 ± 0.91</td>
</tr>
<tr>
<td>HCT</td>
<td>L L⁻¹</td>
<td>0.37-0.51</td>
<td>0.41 ± 0.01</td>
<td>0.48 ± 0.02</td>
</tr>
<tr>
<td>PLT</td>
<td>G L⁻¹</td>
<td>160-430</td>
<td>244 ± 17.28</td>
<td>249 ± 19.47</td>
</tr>
<tr>
<td>MPV</td>
<td>fl</td>
<td>6.7-11.1</td>
<td>9.4 ± 0.5</td>
<td>9.5 ± 0.49</td>
</tr>
<tr>
<td>WBC</td>
<td>g L⁻¹</td>
<td>6-17</td>
<td>11.1 ± 0.7</td>
<td>14.5 ± 0.9</td>
</tr>
<tr>
<td>HGB</td>
<td>g L⁻¹</td>
<td>130-190</td>
<td>144 ± 13.65</td>
<td>125.3 ± 12.1***</td>
</tr>
<tr>
<td>MCH</td>
<td>pg</td>
<td>22-27</td>
<td>25.2 ± 1.3</td>
<td>23.3 ± 1.4</td>
</tr>
<tr>
<td>MCHC</td>
<td>g L⁻¹</td>
<td>340-360</td>
<td>346 ± 27.25</td>
<td>333 ± 27.31</td>
</tr>
<tr>
<td>LYMa</td>
<td>g L⁻¹</td>
<td>1-4.8</td>
<td>2.83 ± 0.2</td>
<td>3.64 ± 0.3</td>
</tr>
<tr>
<td>GRANa</td>
<td>g L⁻¹</td>
<td>3.5-12.0</td>
<td>7.25 ± 0.45</td>
<td>9.92 ± 0.61</td>
</tr>
<tr>
<td>MIDA</td>
<td>g L⁻¹</td>
<td>0.15-1.35</td>
<td>1.01 ± 0.12</td>
<td>1.07 ± 0.14</td>
</tr>
<tr>
<td>LYM</td>
<td>%</td>
<td>12-30</td>
<td>18.7 ± 1.7</td>
<td>21.4 ± 1.9</td>
</tr>
<tr>
<td>GRAN</td>
<td>%</td>
<td>60-83</td>
<td>73.0 ± 2.56</td>
<td>75.3 ± 2.78</td>
</tr>
<tr>
<td>MID</td>
<td>%</td>
<td>2-9</td>
<td>5.0 ± 0.32</td>
<td>5.7 ± 0.39</td>
</tr>
<tr>
<td>ESR</td>
<td>mm L⁻¹</td>
<td>0-22</td>
<td>14.7 ± 0.89</td>
<td>19.3 ± 1.8</td>
</tr>
</tbody>
</table>

Note: WBC - white blood cell count; LYMa - an absolute number of leukocytes; LYM% - a percentage range of lymphocytes; MIDA - an absolute number of monocytes; MID% - a percentage range of monocytes; GRAN% - a percentage range of granulocytes; RBC - a number of red blood cells; MCV - the average volume of the red cells; HCT - hematocrit; RDW% - percentage range of the red cell distribution; RDWabs - the absolute width of red cell distribution; HGB - the amount of hemoglobin in the blood; MCH - mean cell hemoglobin in one erythrocyte (color index of blood = 0.03хMCH); MCHC - mean corpuscular hemoglobin concentration; PLT - a number of thrombocytes; MPV - mean platelet volume. *** P < 0.001; ESR - erythrocyte sedimentation rate.

If the levels of minerals increase in the blood during the pregnancy of female dogs, it indicates the greatest need for them. To identify the amount of minerals transiting to the fetus, we have subtracted a smaller index (maternal blood) from the larger index (fetal portion of the placenta) at different periods of pregnancy. Therefore, the difference between the levels of mineral substances is an indicator of their penetration from the mother to the fetal part of the placenta. The growth of minerals in the blood of pregnant female dogs is a natural phenomenon, because the fetus’ needs them to rise with its growth. Thus, it is important to know at what concentration they accumulate in the fetal part of the placenta and come to fetuses during the pregnancy.

Table 2. Leukogram of healthy dogs and dogs with joint dysplasia, (M ± m, n = 12).

<table>
<thead>
<tr>
<th>Experimental index</th>
<th>Rates</th>
<th>Units</th>
<th>Control</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>1-10</td>
<td>%</td>
<td>1.67 ± 0.11</td>
<td>3.0 ± 0.19</td>
</tr>
<tr>
<td>Basophils</td>
<td>0-1</td>
<td>%</td>
<td>0</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td>%</td>
<td>3.0 ± 0.18</td>
<td>4.67 ± 1.12</td>
</tr>
<tr>
<td>Band cells</td>
<td></td>
<td>%</td>
<td>66.0 ± 5.45</td>
<td>64.2 ± 5.40</td>
</tr>
<tr>
<td>Segmented</td>
<td>54-73</td>
<td>%</td>
<td>22.2 ± 1.99</td>
<td>19.7 ± 1.91</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>9-27</td>
<td>%</td>
<td>7.2 ± 0.62</td>
<td>8.33 ± 0.7</td>
</tr>
</tbody>
</table>

We detected that the rates of minerals at different periods of pregnancy increase gradually in experimental (Ca: 0.288-0.370 – 0.338-0.435 g/kg; P: 0.146-0.175-0.307-0.210 g/kg; Mg: 0.179-0.193-0.220-0.245 g/kg) and control (Ca: 0.271-0.278-0.398-0.431 g/kg; P: 0.216-0.288-0.264-0.314 g/kg; Mg: 0.183-0.257-0.303-0.333 g/kg) groups (Table 4). Comparison of the results testified that the level of Ca in the animals of the experimental group is higher than that in the control one, whereas P and Mg rates, on the contrary, are lower. Such dynamics of Ca levels are justified not only by the composition of the diet consumed, but also by the regulative function of the maternal part of the placenta in accordance with the needs of the fetus throughout the prenatal development. The amount of Ca permeated from the mother’s blood to the fetus was determined by the difference between its indices in the separate pregnancy periods of the animals of the experimental and control groups (experimental – control: 0.288-0.271) – (0.370-0.278) – (0.388-0.333) – (0.435-0.431), while P and Mg rates were estimated by the difference between the control and experimental groups of the animals (control experiment: P (0.216-0.146) – (0.218-0.175) – (0.246-0.207) – (0.314-0.241; Mg (0.183-0.174) – (0.257-0.193) – (0.333-0.245). This difference in Ca levels from the first to the fourth pregnancy periods (Table 4) are 0.017-0.092-0.055-0.004 g/kg; and as for Mg its concentration is 0.009-0.064-0.083-0.088 g kg⁻¹. Therefore, the largest amount of Ca transiting from mother’s blood to the fetal part of the placenta (0.092 g kg⁻¹) penetrated during the second period of pregnancy, the period of growth, skeletal formation and calcification of the skeletal bones (Nimand & Suter, 2004).
The dynamics of Mg in the blood of pregnant dogs is characterized by the increase in its concentration and reduction in its absorption rates in control and experimental groups. Thus, the level of Mg in the blood in both groups increases with the progress of pregnancy, but its indices are different: in the control group the amount of Mg increases by 0.011 g/kg from the 35th to the 45th day; by 0.012 g/kg between the 35th and 45th day, and by 0.059 g/kg between the 55th and 65th day. The changes in Mg in the experimental group make up 0.021 g/kg (from 0.011 ± 0.004 to 0.012 ± 0.004), 0.031 ± 0.006 g/kg (from 0.020 ± 0.04 to 0.031 ± 0.012), and 0.025 ± 0.003 g/kg (from 0.011 ± 0.003 to 0.012 ± 0.003 g/kg).

Similarly, the given levels drop from 0.114 ± 0.004 to 0.111 ± 0.003 g/kg, which probably corresponds to the optimal needs of the fetus at this period.

We assumed that during this period the calcium-phosphorus ratio is 1.6:1 and is regarded as a symptom of mineral metabolism. Therefore, the gestation period from 45th to 55th days of pregnancy is critical and may be potential indicator for the Ca and P supply. Consequently, accumulation of the minerals in the fetal part has also been monitored in this study. As it can be seen (Table 4), the levels of Ca, P, and Mg in the fetal part of the placenta of the animals in the experimental group increase gradually in the dynamics of pregnancy. Ca levels in the fetal placental part of the animals in the control group also increased during pregnancy, though its rate was relatively lower than in animals of the experimental group. Levels of P and Mg in the fetal part of the placenta in dogs from the control group are higher than in experimental group during the entire pregnancy (from 0.100 ± 0.004 to 0.114 ± 0.004 g kg\(^{-1}\)) (Table 4) and they decrease from 55th to the 65th days of pregnancy (from 0.111 ± 0.003 to 0.125 ± 0.003 g kg\(^{-1}\)). Similarly, the given levels drop from 0.144 ± 0.004 to 0.111 ± 0.003 g kg\(^{-1}\) between 45th and 55th days. We consider this period to be the critical, because on the 65th day of pregnancy the Ca levels increase.

The dynamics of Mg in the blood of pregnant dogs is characterized by the increase in its concentration and reduction in its absorption rates in control and experimental groups. Thus, the level of Mg in the blood in both groups increases with the progress of pregnancy, but its indices are different: by 0.011 g/kg from the 35th to the 45th day, by 0.004 g/kg from the 45th to the 55th day, and by 0.009 g/kg between the 55th and 65th day in the experimental group; and in the control group by 0.009, 0.004, and 0.026 g kg\(^{-1}\), respectively. It is noteworthy that from the 45th to the 55th day of pregnancy, the level of Mg increases by the same amount of 0.004 g/kg, which probably corresponds to the optimal needs of the fetus at this period.

In the fetal placental part the Ca levels increased by 0.021 g/kg between the 35th and the 45th day of pregnancy, by 0.052 g kg\(^{-1}\) from 45th to 55th day and by 0.012 g/kg from 55th to 65th day, whereas the growth in indices in the experimental groups accounted for 0.060, 0.060, and 0.082 g kg\(^{-1}\), respectively. Therefore, Ca rates in the fetal part of the placenta in animals of the control group are lower than those in the experimental group. The rates of P and Mg in the control and in the experimental groups are different: in the control group the amount of P decreases by 0.067 g/kg from 35th to the 45th day; by 0.040 g/kg from the 45th to the 55th day, and by 0.059 g/kg between the 55th and 65th day. The changes in P in the experimental group make up 0.029 g/kg, 0.032 g/kg, and 0.034 g/kg, respectively; the Mg concentration in the control group rises by 0.083 g/kg, 0.050 g/kg and 0.065 g/kg, respectively, and in the experimental group it increases by 0.030 g/kg, 0.031 g/kg and 0.034 g/kg, respectively.

Comparing the dynamics of mineral substances in the blood of animals of the experimental group, the levels of Ca (0.101 ± 0.002–0.119 ± 0.004–0.123 ± 0.003–0.158 ± 0.001 g/kg) and Mg (0.020 ± 0.04–0.031 ± 0.012–0.035 ± 0.006–0.044 ± 0.012 g/kg)
increase and P remains at the same level (0.050 ± 0.001 g/kg) during the pregnancy. Analysis and comparison of the levels of minerals in the blood of pregnant female dogs and in the fetal part of the placenta show that, in the fetal part of the placenta, in comparison to maternal blood, Ca, P, and Mg are deposited in higher concentrations throughout the whole pregnancy of animals of both groups. Thus, at the end of pregnancy, the amount of Ca in the fetal part of the experimental group is almost 3.8 times more (0.150 ± 0.003–0.593 ± 0.001 g/kg), in the control group it is more than four times (0.125 ± 0.005–0.556 ± 0.002 g/kg), as for phosphorus, it is five times (0.050 ± 0.001–0.291 ± 0.002 g/kg) and five times higher (0.078 ± 0.002–0.392 ± 0.004 g/kg), turning to magnesium, it is five times (0.044 ± 0.012 and 0.289 ± 0.002 g/kg) and six times higher (0.059 ± 0.005 and 0.392 ± 0.010 g/kg), respectively.

It is considered that the study of the permeability of the placental barrier in the dynamics of pregnancy of female dogs, starting with the maternal blood, in the direction of its physiological flow (‘fetal part of the placenta → umbilical cord → fetal liver → fetal joints → amniotic fluid → alantoic fluid’) is quite appropriate and really important in terms of the clinical aspect of the problem. It has been found out, that the concentration of Ca, P and Mg in the tissues of the joints of puppies in the control group from the 35th to the 45th day of pregnancy (Ca – 10.092 ± 0.033 and 10.92 ± 0.035 g/kg, P – 5.969 ± 0.02 and 5.969 ± 0.030, Mg – 5.971 ± 0.020 and 5.970 ± 0.031 g/kg, respectively) is the lowest, whereas their levels increase in the liver (Ca – 0.368 ± 0.005 and 0.406 ± 0.003, P – 0.246 ± 0.08 and 0.297 ± 0.001, Mg 0.247 ± 0.008 and 0.298 ± 0.002 g/kg, respectively) and in the fetal part of the placenta (Ca – 0.371 ± 0.003 and 0.392 ± 0.001, P – 0.207 ± 0.001 and 0.287 ± 0.002, Mg – 0.203 ± 0.001 and 0.286 ± 0.001, respectively).

We believe, that the period of pregnancy of female dogs from the 35th to 45th day, which correspond to the reduced deposition of Ca, P and Mg in the tissues of the hip and elbow joints from control group, is a potential bioindicator of the development of dysplasia and should be considered critical. During this period it is necessary to carry out preventive prevention of canine joint dysplasia. However, the results of these studies are not conclusive and complete. It is advisable to expand and deepen the scientific search aimed at determining the role of hormones and their correlation with the metabolism of minerals.

The function of the fetal part of the placenta as a barrier on the side of the fetus is aimed at the accumulation of nutrients, and the regulative function is aimed at their allocating at the request of the fetus into the blood stream of umbilical veins, from which they penetrate the fetal liver and partially outside it to other organs and tissues. In this case, minerals, in particular Ca, P and Mg, are deposited in the tissues of the joints, in the hip and elbow, performing important static and dynamic functions; in the dynamics of pregnancy they accumulate in concentrations higher than in the blood of the mother, that is, the fetal part of the placenta turns out to be the depot of mineral substances on the fetus' side.

Comparison of the levels of each mineral substance separately in the fetal part of the placenta has revealed that the initial blood level of Ca is higher in the experimental group of animals than in the control group, and the rates of P, on the contrary, are higher in the control group, while the levels of Mg are the same in both groups.

During the pregnancy, the concentration of minerals in the blood at certain periods varies: in the group of experimental female dogs Ca increases from the 35th to the 65th day (0.018–0.004–0.035), and in the control group it decreases (0.014–0.003). Consequently, the higher is the level of ME in the blood of pregnant female dogs, the greater is the reserve of their penetration through the placental barrier to their body. The decrease of the P and Mg rates in the blood of the female dogs in the control group from the 45th to the 55th day of pregnancy, compared with the 35th-45th days, indicates that the fetuses have the maximum need for them, because during this period the fetal weight significantly increases.

The established peculiarity determines the critical period of pregnancy in terms of the provision with Ca for the prenatal fetal development. The amount of Ca consumed by the fetuses of the female dogs in the experimental group is also the highest during this period. The P and Mg rates are almost the same in the blood of female dogs. From the 45th to the 55th day of pregnancy, the level of P in the animals of the control group does not change and is even higher than at the 35-day of pregnancy. Nevertheless, it decreases at the end of pregnancy, which indicates an increase and equal consumption of it by the fetuses. The level of P in the blood is the same throughout the pregnancy in the animals of the experimental group, which can be regarded as a result of the consumption of a balanced diet. The dynamics of Mg in the blood of pregnant dogs is similar to Ca and P and is characterized by the fact that the critical period for its level is also in between the 45th – 55th days of pregnancy, when its level is the highest. It should be mentioned that the dynamics of phosphorus levels in the mother’s blood and in the fetal part of the placenta are the same, but in the animals of the control group they are higher than in the experimental one. This is manifestation of the regulatory function of placenta fetal part.

Conclusion

We suggested that the fetal part of canine placenta is not only a barrier to the penetration of substances from the mother's blood, but also a depot in which the mineral substances accumulate in concentrations much higher than in the mother's blood. The minerals are deposited in puppies' liver at a concentration higher than in the fetal part of the placenta. We determined separate periods of the reduced accumulation of Ca, P and Mg in the tissues of the canine joints in puppies from control group from the 35th to the 65th day of pregnancy. We concluded that the period from the 35th to the 45th day of canine pregnancy with reduced deposition of Ca, P and Mg in the tissues of the hip and elbow joints in the control group should be a potential bioindicator of the development of dysplasia and must be considered critical. During this period it is necessary to carry out the preventive measures of canine joint dysplasia.

References


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