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Cortisol fetal fluids concentrations and newborn outcome in term pregnancy small-sized purebred dogs

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Despite the high perinatal mortality in dogs, knowledge about canine perinatology is limited compared to humans. Because newborns blood samples analysis are challenging, the collection of fetal fluids at birth is safe for the puppies, and useful in research\textsuperscript{(1)}. Fetal fluids are easily collectable during cesarean section (CS), frequently performed as elective surgery in bitches at high risk for dystocia, such as small-sized purebred bitches. Cortisol plays important roles in the final fetal multi-organ maturation and in the neonatal adaptation. In order to provide knowledge for a better neonatal assistance to newborn dogs, this study was aimed to assess the cortisol amniotic (CAM) and allantoic (CAL) concentrations in relation to newborn outcome and to some, reported below, maternal and neonatal parameters, in dogs at term of pregnancy (defined on the base of ovulation day estimation coupled to fetal ultrasonographyc measurement of both the inner chorionic cavity and biparietal diameter, and by blood progesterone concentrations monitoring\textsuperscript{(1)}) and submitted to elective CS before the onset of labor. The aims of the study were: 1) to assess possible differences between the CAM and CAL concentrations; 2) to verify possible correlations between CAM and CAL concentrations, and also between CAM and CAL concentrations and maternal age and parity, litter-size, neonatal gender, birth weight, Apgar score and neonatal outcome at 24 hours and 7 days of age. The study was performed on 19 purebred small-sized bitches (body weight <10 kg), at risk of dystocia, 2-6 years old, 12 multiparous and 7 primiparous, undergoing elective CS at term of pregnancy. The amniotic and allantoic fluids were collected from each puppy, at the opening of fetal sacs with a sterile siringe without the needle, without interfering with neonatal assistance, and stored at -20°C, until cortisol analysis by RIA. At birth, newborns were evaluated for viability by Apgar score\textsuperscript{(2)}, gender, absence of gross physical malformations and weighed before nursing. Litter size was recorded. A total of 50 normal puppies, 28 males and 22 females were obtained from litters ranging between 1 and 6. Mean (±SD) birth weight was 160±46.02 g and according to Apgar score, 47 puppies were scored as ≥7, 1 was scored as 4-6, and 2 were scored as <4. At 24 hours after birth, 4 out 50 puppies (8%) died and within 7 days of age, one additional puppy was found dead. In all cases, necropsy did not show evidences of gross malformations, trauma or infections. The overall median value, 1\textsuperscript{st} and 3\textsuperscript{rd} quartile for CAM and CAL concentrations assessed as reported above on 46 amniotic and 41 allantoic samples were 7.4 ± 5.7 ng/ml and 8.4 ± 4.88 ng/ml. Significantly (p<0.01) higher median, 1\textsuperscript{st} and 3\textsuperscript{rd} quartile CAL than CAM levels were found (3.7, 2.3 and 11.1 ng/ml; 6.7, 4.0 and 11.6 ng/ml, respectively) and a high (p<0.0001), positive correlation (R=0.83) between CAM and CAL concentrations detected. Highly significant (p<0.05) CAM concentration were found in puppies that die, in comparison to those surviving at 24 hours after birth (median value; 1\textsuperscript{st} and 3\textsuperscript{rd} quartile: 11.2, 7.2 and 12.2 ng/ml vs 3.9, 2.8 and 11.1 ng/ml, respectively), but no difference were found for mortality at 7 days of age, as well as for all the other maternal or neonatal studied parameters reported above. In conclusion, among the studied parameters, only high CAM levels at birth were associated to short-term survival and could be useful to recognize puppies that need special assistance and surveillance in the first 24 hours of birth.