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Amniotic Fluid Analysis at Birth as a Predictor of Canine Neonatal Survival

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ABSTRACT

Establishing dependable indicators for neonatal survival is essential in canine practice. Historically, amniotic fluid has not received attention as a diagnostic tool in dogs. This study analyzed amniotic fluid obtained at whelping from 36 dogs that underwent elective caesarean section (CS) at term to evaluate its potential as a predictor of neonatal survival. Glucose and cortisol concentrations in amniotic fluid were measured at birth in 72 puppies delivered by elective CS. These biochemical parameters were subsequently analyzed to relate puppy survival at birth and neonatal viability, as assessed by APGAR scoring, and to neonatal mortality. The results revealed that stillborn puppies exhibited significantly higher ($p<0.001$) cortisol and lower ($P<0.001$) glucose concentrations compared to puppies that survived beyond 48 h post-birth. Although no significant differences in amniotic glucose and cortisol levels were observed across varying APGAR scores, a linear positive trend was noticed between amniotic glucose levels and APGAR scores while the relation between amniotic cortisol and APGAR score was linear and negative. From a clinical perspective, the assessment of amniotic cortisol and glucose concentrations immediately following delivery might serve as a valuable tool for identifying neonates that require intensified monitoring during the first 48 h of life.

Keywords : Amniotic Fluid, Glucose, Cortisol, APGAR Scores, Neonatal Survivability.

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INTRODUCTION

The evaluation of amniotic fluid has become an essential tool in assessing fetal well-being and predicting neonatal outcomes in human medicine. Among the various components of amniotic fluid, glucose, and cortisol levels hold particular significance due to their direct reflection of the fetal metabolic and stress status. Amniotic glucose is a crucial indicator of foetal energy availability and metabolic function. Glucose levels in amniotic fluid can provide insights into the metabolic environment in utero, potentially signalling issues such as foetal hypoglycaemia

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or hyperglycaemia, both of which are associated with adverse neonatal outcomes, including metabolic disorders. Amniotic cortisol, a hormone produced by the foetal adrenal glands, is a vital marker of the foetal stress response. It plays a significant role in the maturation of fetal organs and the preparation for extrauterine life. Elevated or diminished levels of amniotic cortisol could indicate an abnormal stress response, which might correlate with complications such as preterm birth, growth restriction, and other developmental challenges. By assessing the levels of glucose and cortisol in the amniotic fluid, clinicians can gain valuable insights into the intrauterine environment and the potential risks to the foetus. This information is crucial for anticipating neonatal complications, allowing for timely interventions that can improve neonatal outcomes and enhance the overall management of pregnancies at risk. Therefore, a fundamental study in dogs was conducted to measure amniotic glucose and cortisol levels at birth, aiming to correlate with puppy survival, neonatal viability using APGAR scoring and neonatal mortality

MATERIALS AND METHODS

The study was conducted in 36 healthy dogs of different breeds subjected to elective CS. Before opening the foetal bags, a minimum of 3 mL of amniotic fluid was aspirated using an 18-G needle from the foetal bag at the level of the hind limbs (Fig 1). Glucose in amniotic fluid was measured by a semi-automatic analyzer and cortisol was measured employing Chemiluminescent immunoassay (CLIA) using a commercial CLIA kit (Mindray Biomedical, China). Survival rate at birth, APGAR score (Groppetti *et al.*, 2010), and neonatal mortality within 48h were recorded. Standard operating procedures for surgery and general anaesthesia induced with propofol @ 3.5 mg/kg b.wt , 2% Isoflurane maintenance was employed while performing CS.

Samples were collected from amniotic sacs of 72 puppies including four that were stillborn and two puppies that died within 48 h post-surgery. Cortisol and glucose in amniotic fluid were compared for neonatal viability (table 1) using an independent t-test.

RESULTS AND DISCUSSION

In the present study, a significant difference in the levels of amniotic glucose and cortisol was observed between viable and dead puppies. Despite the small number of samples from dead or stillborn puppies (n=6), a statistical comparison with surviving puppies amniotic fluid was conducted to explore potential markers for early prediction of newborn outcomes or metabolic imbalances, as this could be clinically valuable.

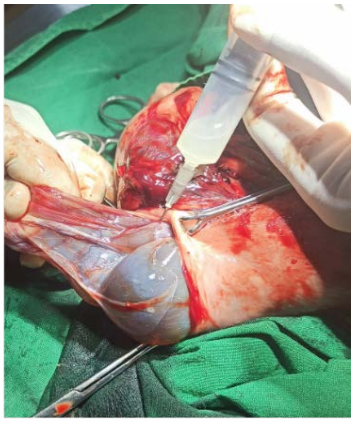

Fig. 1: Collection of amniotic fluid during CS

The amniotic glucose recorded in the present study in dead and live puppies were 22.67 ± 5.44 and 61.76 ± 6.28 mg/dL respectively. The values exceeded those reported by Groppetti *et al.* (2015), which were 20.4 mg/dL for live puppies and 14.2 mg/dL for dead ones, as well as by Bolis *et al.* (2018), who found 24.5 ± 1.38 mg/dL for live puppies and 18.3 ± 4.44 mg/dL for dead ones. The results obtained in this study were in concurrence with the findings of works mentioned above stating amniotic glucose concentrations were lower in puppies dead within 48 h after birth compared to live puppies in the same litter. Low amniotic glucose concentration in non-surviving puppies could be a cause or effect of metabolic imbalance at the time of birth leading to metabolic acidosis and thus mortality of newborns (Greghi *et al.*, 2023). The low amniotic glucose levels in puppies born dead corroborate the findings of Plavec *et al.* (2022) who related unusually low glucose levels at a specific gestational age to possible complications, including placental insufficiency or hindered fetal growth. The differences in amniotic glucose concentrations observed among the dogs in this study may also be linked to the dietary variations of the dams, as high carbohydrate diets can influence amniotic glucose levels. However, exploring this relationship was not within the scope of the current research.

In the present study, higher ($P<0.001$) amniotic cortisol concentrations were recorded in stillborn puppies in comparison to viable puppies. These results were concurrent with findings in amniotic fluid reported by Groppetti *et al.* (2015); Bolis *et al.* (2018) and Veronesi *et al.* (2018) that amniotic cortisol concentrations measured at birth could be prognostic for short-term survival in newborn puppies and could be very important for prompt detection of puppies that need special surveillance during first 48 h of birth. Plavec *et al.* (2022) reported that pup-

Table 1 : Comparison of glucose and cortisol in amniotic fluid between live and non-viable puppies

Parameter	Dead (n=6)	Live (n=66)	t-value	P-value
Cortisol (nM/L)	131.39 ± 11.12	29.81 ± 2.48	8.916**	<0.001
Glucose (mg/dL)	22.67 ± 5.44	61.76 ± 6.28	4.703**	<0.001

*** Significant at 0.01 level*

[+] *stillborn or died within 48 h post surgery*

Table 2 : Comparison of glucose and cortisol in amniotic fluid between puppies of different APGAR scores

Parameter	APGAR 1	APGAR 2	APGAR 3 and above	F-value	P-value
Cortisol (nM/L)	39.75 ± 2.63	31.11 ± 3.96	26.44 ± 3.25	1.347ns	0.275
Glucose (mg/dL)	59.67 ± 9.23	63.33 ± 8.87	71.69 ± 10.31	0.032ns	0.968

pies with lower amniotic cortisol concentrations tend to have higher APGAR scores, indicating better neonatal health. It could be inferred that the puppies that died at birth experienced greater stress, leading to higher cortisol levels compared to those that survived. Though significant differences in amniotic cortisol and glucose concentrations between surviving and non-surviving puppies were recorded.

APGAR scores were assessed for all the puppies, initially ranging between 1 and 3 immediately after. There was a significant increase in scores at 30 and 60 minutes post-surgery. A one-way ANOVA was conducted to compare glucose and cortisol levels in the amniotic fluid of live puppies with APGAR scores ranging from 1 to 3 and above (table 2). While no significant difference was noted, a declining trend in amniotic cortisol levels was observed as APGAR scores increased, whereas an opposite trend was seen with amniotic glucose levels.

Measuring the APGAR score at birth proved to be an effective method for quickly identifying low-viable newborns that required immediate neonatal care or assistance.

A positive linear relationship was observed between APGAR scores and amniotic glucose levels, while a negative linear relationship was found between APGAR scores and amniotic cortisol levels. These results suggest that combining amniotic parameter estimation with APGAR scoring could improve the monitoring of puppies.

The significant elevation of cortisol in non-viable puppies indicates that cortisol could be a potential predictor of neonatal mortality. Elevated cortisol might reflect intrauterine stress or adverse conditions leading to fetal demise. The significant decrease ($p<0.001$) in glucose levels in non-viable puppies suggests that lower amniotic fluid glucose may be associated with poor neonatal outcomes. It could indicate insufficient nutrient supply or metabolic dysfunction in foetuses that do not survive. The lack of significant differences in cortisol and glucose levels across different APGAR scores suggests that these parameters might be more indicative of extreme outcomes (survival vs. non-survival) rather than gradations of neonatal health in surviving puppies. Furthermore, it is advisable to examine the variations in amniotic glucose and cortisol concentrations between immature and mature foetuses to enhance understanding of its predictive value for assessing maturity.

Any assumption regarding the true cause-and-effect relationship between a greater cortisol content and low glucose in amniotic fluid to the mortality of puppies is prevented by the fact that non-surviving puppy numbers are too low.

The findings indicate that canine amniotic fluid has significant predictive value on neonatal outcomes. However, further research is needed to validate it as a reliable, minimally invasive indicator of neonatal maturity. Advances in canine amniocentesis techniques are crucial to fully explore the diagnostic and prognostic potential of amniotic fluid in dogs, enabling better planning for caesarean sections.

CONCLUSIONS

The findings suggest that high cortisol and low glucose levels in amniotic fluid might be important markers for predicting neonatal mortality in dogs delivered by elective CS. These parameters could be used to identify at-risk puppies during birth, allowing for more targeted interventions to improve neonatal outcomes. From a clinical perspective, the significant effect of higher amniotic cortisol and low glucose concentrations on negative outcome at 48 h of neonatal life seems to suggest that both cortisol and glucose measurement in amniotic fluid at birth could be useful for detection of puppies that need special surveillance during the first 24 h of age and should be coupled to the APGAR score. However, this finding is drawn from a small number of dead puppies and further focussed investigations on a larger number of subjects are needed.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the study

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