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Amniotic cortisol and glucose as markers of perinatal viability of pups delivered by caesarean section#

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Abstract

The identification of new, reliable amniotic fluid markers for evaluating neonatal maturity is crucial in canine clinical practice to optimise perinatal outcomes. The current study assessed the impact of elective caesarean section (ELCS) and emergency caesarean section (EMCS) on amniotic fluid glucose and cortisol concentrations in relation to perinatal outcomes in different breeds of dogs. Twelve high-risk pregnant bitches underwent ELCS (n = 6) or EMCS (n = 6), resulting in a total of 53 pups. Amniotic fluid was collected from 24 pups at delivery and analysed for glucose and cortisol. Survival at birth and neonatal mortality within 48 h were recorded. The proportion of liveborn pups was higher in ELCS (87.50%) than EMCS (86.21%), with stillbirth rates of 12.50% and 13.79%, respectively. Live pups showed significantly higher amniotic glucose (43.81 ± 4.68 mg/dL) and lower cortisol (21.12 ± 2.49 nM/L) compared to stillborn pups (25.22 ± 2.43 mg/dL glucose; 48.51 ± 8.66 nM/L cortisol; p<0.05). Mode of delivery influenced amniotic glucose levels among liveborn pups, with significantly higher levels in ELCS compared to EMCS, while cortisol levels did not differ significantly. No neonatal mortality occurred within 48 h in the ELCS group, whereas it was 8% in the EMCS group. An advantage of planned ELCS in high-risk pregnancies was reflected in better live birth rates compared to EMCS. The results suggest that higher amniotic cortisol and lower glucose are associated with perinatal mortality, indicating their potential as prognostic markers for identifying pups that may require special care.

Keywords: *Amniotic fluid, caesarean section, neonatal survival, dog*

In canine high-risk pregnancies, the incidence of maternal, foetal, and/or perinatal morbidity or mortality is significantly higher. Analysis of certain biochemical and endocrinological levels in dogs is increasingly recognised as a vital diagnostic and prognostic resource in veterinary obstetrics and neonatology. The initial moments following birth are the most crucial period for neonates, and the perinatal factors that enable early identification of foetal distress have been found in both human and veterinary medicine for a long time. Specific biomarkers may aid in distinguishing between

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healthy pups and those requiring obstetrical interventions, thereby contributing to a decrease in neonatal mortality. In human medicine, amniocentesis has proven to be an essential tool and continues to be utilised to evaluate foetal health during pregnancy and to manage neonatal patients clinically (Underwood et al., 2005). In canine obstetrics, bubble test represents a simple and reliable diagnostic tool for assessing foetal lung maturity by detecting the presence of surfactant in amniotic fluid (Suprith, 2020). Analysing components such as lecithin, sphingomyelin and surfactant protein A (SP-A) in amniotic fluid is essential for assessing foetal lung maturity and overall readiness for delivery. This information is crucial for planning elective caesarean sections and estimating neonatal viability, especially when the timing of gestation is uncertain. The concentrations of glucose, lactate and cortisol found in amniotic fluid at the time of birth have been associated with neonatal survival rates and immediate outcomes (Plavec et al., 2022). These biomarkers can serve as early in canine, enabling veterinarians to identify pups that may require closer observation or intervention (Reddy et al., 2024).

In canine pregnancies, amniotic glucose reflects the newborn's energy reserves and supports viability at birth, influenced by maternal and foetal glucose exchange and foetal metabolic demands near term (Groppetti et al., 2015). Amniotic cortisol, which rises in late gestation, signals foetal lung maturation through surfactant production and may be influenced by maternal stress, potentially affecting placental function and foetal neurodevelopment. Studies indicate that evaluating amniotic fluid during birth or late pregnancy offers significant insights into the health, maturity, and immune status of canine neonates (Bolis et al., 2017). The collection of amniotic fluid in dogs can be safely conducted during a caesarean section, rendering it a minimally invasive procedure that does not interfere with the birth process. This direct collection method facilitates the development of diagnostic protocols that support clinical decision-making for canine pregnancies.

The current study was envisaged to compare the amniotic markers between live and stillborn pups, to examine their association with the neonatal survival of liveborn pups, and to determine the influence of delivery type on these markers.

Materials and methods

The study was conducted in 12 healthy dogs of various breeds, including six Beagles, one each of French Bulldog, English Bulldog, American Bully, Shih Tzu, Dachshund and Rottweiler, aged between one and five years. Based on the criteria by Johnson (2008), the female dogs included in this study were selected for their high-risk pregnancy status. These included dogs that have experienced dystocia, previous caesarean sections, high stillbirth rates from prolonged labour, advanced maternal age, brachycephalic breeds, pelvic deformities, vaginal strictures, or unusually small or large litter sizes should be classified as high-risk pregnancies. Properly planned elective caesarean section (CS) is considered a safe, effective, and justified intervention in such cases.

Caesarean sections

The animals under the study were divided into two groups. Bitches identified as having a high-risk pregnancy were selected for the study. Dogs confirmed pregnant on sonography at 25 to 30 days of gestation had their Inner Chorionic Cavity (ICC) measurements recorded, and the expected delivery date (EDD) was calculated according to Luvoni and Grioni (2000). Animals were monitored closely during the last week of pregnancy, and an elective caesarean section (ELCS) was performed in six bitches when a decline in serum progesterone concentration to below 2 ng/mL was detected at time of presentation (Group I). Another six dogs presented with signs of dystocia, such as green vaginal discharge without delivery of puppies and unresponsive to medical management, ineffective contractions lasting over 30 minutes, signs of foetal distress (heart rate < 120 bpm), a constricted birth canal, foetal obstruction, and uterine inertia, were subjected to emergency caesarean section (EMCS) and formed Group II as reported by Cramer and Nothling (2020).

Animals were premedicated with Glycopyrrolate at 0.01 mg/kg body weight intramuscularly followed by an intravenous administration of Butorphanol at 0.2 mg/kg body weight. Standard operating procedures for surgery and general anaesthesia were followed, with propofol administration at 3.5 mg/kg body weight and maintenance with 2% Isoflurane during the CS. APGAR scores, assessed using the modified for canine neonates described by Veronesi et al. (2009), were recorded at birth, 30 minutes, and 60 minutes in both groups. The survival rate at birth was documented, and neonatal survivability was monitored for 48 hours.

Sample collection and analysis

Amniotic fluid samples were collected from the amniotic sacs of 24 pups (two samples from each dog) before opening the foetal bags, including 17 samples from live pups and seven from stillborn pups. Minimum of 3 mL of amniotic fluid was drawn using an 18-G needle from the area near the hind limb of the foetal bag. The harvested amniotic fluid was then centrifuged at 1000 x g for 20 minutes, and the supernatant was collected for analysis of glucose and cortisol levels. Glucose was measured using a semi-automatic analyser. Cortisol levels in the amniotic fluid were determined with a Chemiluminescent Immunoassay (CLIA) using a commercial kit (Mindray Biomedical, China), with a reference range of 0.4 µg/mL to 60 µg/mL.

Statistical analysis

The potential impact of the delivery method (ELCS or EMCS) on amniotic glucose and cortisol concentrations, as well as their relationship with perinatal outcome, was evaluated using independent t-test.

Results and discussion

The present study assessed the effects of elective and emergency CS on puppy outcomes. Also, it evaluated the diagnostic and prognostic significance of amniotic biomarkers, glucose and cortisol, and their relation to delivery type (elective or emergency CS), puppy outcome (live born or stillborn), APGAR score and neonatal survival up to 48 h in high-risk canine pregnancies. In this study, out of 12 dogs, seven were primiparous and five were pluriparous and the mean (±SE) age of dams undergoing ELCS was 3.25 ± 0.48 years, while those underwent EMCS were, 2.75 ± 0.54 years, with no significant difference ($p > 0.05$). A total of 53 pups were delivered by caesarean section, comprising 24 from ELCS and 29 from EMCS; of these, 32 were male and 21 were female.

The mean (± SE) litter size in the ELCS group (3.83 ± 0.65) and the EMCS group (4.83 ± 0.95) did not differ significantly (Table 1). Out of 53 pups born, the percentage of liveborn pups was higher in the ELCS group, which recorded 87.50% (21/24), compared to the EMCS group, which had 86.21% (25/29), with correspondingly lower stillbirth rates in ELCS (12.50%) than in EMCS (13.79%). In the ELCS group, one stillborn pup exhibited foetal anasarca, while all other pups were free of congenital malformations. Neonatal mortality was observed for a period of 48 h. There was no mortality in ELCS (21/21), while two pups died in the EMCS group (23/25). Mean birth weight was slightly lower in ELCS with a mean (±SE) of 261.45 ± 24.13 g compared to EMCS with a mean (±SE) of 284.52 ± 32.84 g and a variation in birth weight was noticed with no significant difference ($P > 0.05$). This closer birth weight can be due to the uniformity of breeds between the groups.

An increased survival rate of pups in the ELCS group during high-risk pregnancies aligns with the observation that planned surgical intervention, timed using ICC measurements and performed before the onset of foetal distress, can improve neonatal viability by minimising hypoxic or traumatic events during delivery. A higher perinatal puppy survival rate at birth observed in the ELCS group in this study supports the findings of Rosset and Buff (2008), who reported no foetal deaths during elective caesarean procedures and a higher neonatal survival rate (34/37) at postoperative day 15 compared to 25/40 for emergency caesarean section (EMCS). Similarly, Moon et al. (2000) noted that the likelihood of all foetuses being alive at birth after EMCS is only 30% of that when surgery is planned, and Moon and Erb (2002) reported neonatal puppy mortality rates of 3.6% for ELCS *versus* 12.7% for EMCS.

An elective caesarean section (CS) is considered as a safe and effective procedure, offering improved survival outcomes for both the dam and the foetuses (Cramer and Nothling, 2020). In contrast, emergency caesarean sections (EMCS) are typically performed in response to dystocia or foetal distress, conditions that often result in reduced survival rates due to prolonged labour and heightened foetal stress. In this study, the higher stillbirth proportion observed in the EMCS group (13.79%) is consistent with the observations of Alonge and Melandri (2019), who linked emergency interventions to adverse neonatal outcomes associated with delayed delivery and intrapartum hypoxia.

The APGAR scoring system has been extensively modified for neonatal puppies to evaluate their immediate postnatal health and forecast early survival rates (Veronesi et al., 2009). At birth, neonates in both groups exhibited low APGAR scores, with a mean of 2.4 (Range: 0–4). By 30 minutes, a marked improvement was observed, with mean scores rising to 6.7 (Range: 5–9). At 60 minutes, the majority had reached optimal vitality, reflected in a mean APGAR score of 10.6 (Range: 10–14).

In the present study, the initial scoring of puppies occurred within five minutes after delivery, which might have contributed to the low APGAR scores at birth; however, the scores improved substantially at 30 and 60 minutes. The vitality at birth and the subsequent rise in APGAR scores showed no apparent differences between the two groups. This remarkably high rate of pups attaining elevated scores at both later time points could be attributed to the rapid clearance of gaseous anaesthetics from circulation (Ryan & Wagner, 2006). The gradual improvement observed aligns with earlier findings that neonatal puppies often exhibit low scores at birth, which improve as respiratory and cardiovascular adjustments stabilise (Mila et al., 2017).

Amniotic glucose and cortisol concentrations

The levels of amniotic glucose and cortisol were compared between live and dead pups. Cortisol concentration was significantly higher in dead pups,

Table 1. Maternal and neonatal parameters in dogs that underwent elective and emergency caesarean section

Mode of delivery	Dam age (years)	Litter size (n)	Live pups N (%)	Stillbirth N (%)	Survival upto 48 hr N (%)
ELCS	3.25 ± 0.48	3.83 ± 0.65	21 (87.50)	3 (12.50)	21 (100)
EMCS	2.75 ± 0.54	4.83 ± 0.95	25 (86.21)	4 (13.79)	23 (92)

suggesting acute intrauterine or peripartum stress prior to death. Elevated foetal cortisol was significantly associated with hypoxia, prolonged labour, and compromised placental function. The amniotic cortisol concentration was significantly higher ($p<0.05$) in dead pups (48.51 ± 8.66 n*M*/L) compared to live pups (21.12 ± 2.49 n*M*/L). These results are in concordance with findings reported by Reddy et al. (2024) in amniotic fluid, where dead pups showed 131.39 ± 11.12 n*M*/L and live pups 29.81 ± 2.48 n*M*/L. Bolis et al. (2018) suggested that cortisol levels in amniotic fluid measured at birth could serve as predictors for short-term survival in newborn pups. Similarly, Plavec et al. (2022) found that pups with lower amniotic cortisol concentrations generally had higher Apgar scores, indicating better neonatal health. This information may be vital for the early identification of pups needing special monitoring within the first 48 h after birth (Veronesi et al., 2018). The current research demonstrates significantly increased amniotic cortisol levels in dead pups, highlighting its potential as a prognostic marker for detecting pups susceptible to early mortality. This allows for timely postnatal observation and intervention aimed at improving survival rates during the crucial first 48 hours.

In this study, the concentration of amniotic glucose was significantly higher in live pups (43.81 ± 4.68 mg/dL) compared to dead pups (25.22 ± 2.43 mg/dL) ($p < 0.05$). The glucose values in this study exceeded those documented by Groppetti et al. (2015), which indicated 20.4 mg/dL for live pups and 14.2 mg/dL for deceased ones. In the study by Reddy et al. (2024), amniotic glucose concentrations in dead and live foetuses were reported as 22.67 ± 5.44 and 61.76 ± 6.28 mg/dL, respectively. All research has indicated that live pups have elevated amniotic glucose levels when compared to deceased pups, although the specific values differed among studies. Such variation between studies may be partly due to differences in maternal diet, with the high carbohydrate diet fed to the dams in the present study possibly contributing to the higher glucose concentrations observed. A significant reduction in glucose levels observed in stillborn pups indicated that diminished amniotic fluid glucose may correlate with adverse neonatal outcomes. This could be caused by inadequate nutrient supply, compromised foetal metabolism, placental insufficiency or pathological conditions. The marked reduction in amniotic glucose levels in stillborn pups from the present study reinforces its potential as an indicator of compromised foetal condition, suggesting that monitoring amniotic glucose at birth could help identify neonates at higher risk of adverse outcomes and in need of immediate supportive care.

When analysing the correlation between the mode of delivery and levels of amniotic markers in live-born pups, no significant difference was observed in cortisol levels. However, glucose concentrations differed significantly between the ELCS and EMCS groups (Table 2). Mean cortisol levels were 19.22 ± 2.96 n*M*/L in ELCS and 23.01 ± 4.10 n*M*/L in EMCS ($p = 0.468$). In contrast, mean glucose levels were 53.25 ± 7.30 mg/dL in ELCS and 34.36 ± 3.46 mg/dL in EMCS, which was statistically significant in live-born pups ($p = 0.037$).

In this study, the influence of mode of delivery was evident, with live pups from the ELCS group showing lower cortisol and higher glucose levels compared to those from the EMCS group. Similarly, Plavec et al. (2022) found lower amniotic cortisol in ELCS (3.83 ± 0.44 ng/mL) compared to EMCS (14.01 ± 1.10 ng/mL). Similarly, Fusi et al. (2021) observed amniotic cortisol levels of 5.85 ± 2.96 ng/mL in ELCS and 10.7 ± 4.15 ng/mL in EMCS. In contrast, Groppetti et al. (2015) reported that the amniotic cortisol concentration during ELCS (4.7 ± 3.4 ng/mL) was higher compared to EMCS (3.5 ± 1.4 ng/mL). A significant difference ($p<0.05$) was observed in the amniotic glucose of live pups in ELCS (53.25 ± 7.30 mg/dL) compared to EMCS (34.36 ± 3.46). Greghi et al. (2023) reported that the median glucose concentrations in the amniotic fluid of pups born *via* elective and emergency caesarean sections were 19 mg/dL and 11 mg/dL, respectively.

In the ELCS group, all pups survived, with amniotic glucose levels averaging above 50 mg/dL and cortisol around 19 n*M*/L, indicating a biochemical environment favourable for neonatal survival. In the EMCS group, two pups, one from each of two different dogs, died

Table 2. Comparison of amniotic cortisol and glucose levels with respect to survival status and mode of delivery.

Parameters	Survival status			Mode of delivery		
	Live pup (n = 17)	Dead pup (n = 7)	P-value	ELCS (n = 10)	EMCS (n = 7)	P-value
Amniotic cortisol (n*M/L)*	21.12 ± 2.49	48.51 ± 8.66	0.024*	19.22 ± 2.96	23.01 ± 4.10	0.468
Amniotic glucose (mg/dL)	43.81 ± 4.68	25.22 ± 2.43	0.022*	53.25 ± 7.30	34.36 ± 3.46	0.037*

* Significance at 0.05 level

Table 3. Mean amniotic glucose and cortisol levels in dogs with and without neonatal mortality within 48 hours

Parameters	ELCS		EMCS	
	Surviving pups (n = 10)	Deceased pups (n = 0)	Surviving pups (5)	Deceased pups (n = 2)
Cortisol (n*M/L)*	19.22 (11.04 – 26.76)	-	23.01 (12.69 – 40.83)	47.13 (34.06 – 64.14)
Glucose (mg/dL)	53.25 (31.20 – 87.00)	-	36.40 (20.74 – 45.37)	36.28 (11.59 – 68.97)

within 48 h postpartum. significant, among these cases, the lowest amniotic glucose recorded was 11.59 mg/dL, while the highest amniotic cortisol concentration reached 64.14 n*M*/L compared to surviving littermates (Table 3). Across the two dogs, glucose values ranged from 11.59 mg/dL to 68.97 mg/dL (mean: 36.28 mg/dL), and cortisol levels ranged from 34.06 n*M*/L to 64.14 n*M*/L (mean: 47.13 n*M*/L). The combination of markedly low glucose and high cortisol in the amniotic fluid of these neonates may indicate an increased risk for early postnatal mortality.

These extreme values suggest a possible connection between hypoglycaemia, increased foetal stress, and early neonatal death. However, it was not possible to precisely determine the amniotic glucose and cortisol levels in individual pups that died, as matching each deceased pup to its specific amniotic sample proved difficult. Therefore, the average amniotic glucose and cortisol levels for each dam can serve as indicators for evaluating the risk of neonatal mortality within the litter (Reddy et al., 2024). The significant biochemical difference observed between surviving pups of ELCS and EMCS indicates that while the mode of delivery may influence neonatal stress hormone or glucose levels in pups that survive the perinatal phase, this effect is not statistically significant. Moreover, factors such as foetal distress or obstructive dystocia might have a more profound impact on these parameters. Consequently, amniotic glucose and cortisol levels can act as biomarkers to identify at-risk pups and assist in providing targeted care to improve neonatal survival.

Conclusion

In conclusion, the results of this study indicate that deceased pups had higher amniotic cortisol and lower amniotic glucose levels compared to live pups. These biochemical alterations exhibit significant prognostic value, indicating their potential as indicators for forecasting neonatal mortality and recognizing at-risk puppies during birth. These factors, especially concerning the mode of delivery, suggest that ELCS in high-risk pregnancies may offer clinical advantages by resulting in lower amniotic cortisol levels and higher live birth rates. The findings further show that all pups in the ELCS group survived, while neonatal deaths in the EMCS group were associated with lower glucose and higher cortisol levels, indicating a possible link between hypoglycaemia, increased foetal stress, and early neonatal death. Although the results highlight the diagnostic and prognostic value of analysing amniotic fluid for evaluating neonatal stress and viability, the current findings are limited by a small sample size. Future studies should include larger populations and explore correlations between amniotic fluid parameters and clinical factors such as gestational age, respiratory function, litter size, and birth weight. Particular focus should also be given to cases of early neonatal mortality, where identifying the exact amniotic glucose and cortisol levels in deceased pups remains difficult due to challenges in matching each pup with its corresponding sample. Additionally, advancements in canine amniocentesis methods, which are currently lacking in veterinary literature are crucial for a comprehensive understanding of the diagnostic and prognostic potential of amniotic fluid in relation to foetal monitoring.

Conflicts of interest

The authors declare no conflict of interest in the study.

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