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# Prognostic potential of amniotic fluid analysis at birth on canine neonatal outcomes

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**ABSTRACT**

Glucose, lactate and cortisol concentrations in amniotic fluid were measured at birth in 95 pups and related to neonatal viability based on Apgar scoring and to neonatal mortality. Neither amniotic parameters nor neonatal mortality were associated with the Apgar score. Stillborn pups showed high lactate ($P<0.001$)and cortisol ($P<0.05$) but low glucose amniotic concentrations ($P<0.001$). No amniotic fluid differences were observed between normal and malformed pups. Amniotic glucose ($P<0.001$),lactate ($P<0.05$) and cortisol ($P<0.05$) concentrations were higher in pups delivered by vaginal parturition than by Caesarean section. Birth weight was higher in live pups than in pups dying within 48 h ($P<0.05$). Although these are preliminary results, the analysis of amniotic fluid collected at birth could be a valuable predictor of neonatal outcomes in dogs.

**ARTICLE**

The very first minutes after birth represent the most critical phase in neonatal animals and perinatal factors that provide early detection of fetal distress have long been pursued both in human and veterinary medicine. Specific biomarkers could assist in discriminating between healthy pups and those requiring obstetrical assistance and so help to reduce neonatal

mortality. In humans, amniocentesis has been a valuable tool and is still used to assess fetal well-being during pregnancy and to clinically manage neonatal patients(Underwood et al., 2005). Despite its obvious potential, amniocentesis has not yet been applied in the canine species.

This study was conducted on 24 healthy pregnant purebred owned bitches from which a minimum volume of 1.5 mL of amniotic fluid (AF) for each puppy was collected at delivery using a sterile 5 mL syringe and 21-G needle. Amniotic glucose and lactate(Accutrend Plus, Roche) and cortisol (MiniVidas, BioM√©rieux) were measured and microbial analysis performed as previously described(Groppetti et al., 2012). Apgar score (Groppetti et al., 2010), birth weight, malformations and mortality within 48 h were also recorded for all pups. Surgical and anaesthetic procedures were routinely performed in bitches subjected to Caesarean section (CS)(Groppetti et al., 2010).

All data were analysed using a commercial statistical program for a descriptive and inferential evaluation (SPSS 21.0, IBM). For statistical purposes pups were stratified in groups according to maternal weight(small sized breed, <10 kg; medium sized breed, 10-34 kg; large sized breed, >34 kg. The 24 bitches whelped 108 pups. Of these, 13 were excluded from the study due to inability to collect amniotic fluid. A total of 95 pups with different fates and their AF samples were analysed. Three were stillborn and 11 (from brachycephalic breeds) were born alive but died within 48 h; five were euthanased immediately after birth on account of malformations, while 76 pups were still alive 48 h after birth (Table 1). Malformations were observed in 8/95 pups (Table 1); all were live-born but three died spontaneously within 48 h of birth. Apgar score was not associated with neonatal mortality at or within 48 h of birth. No differences in amniotic glucose, lactate and cortisol concentrations were recorded with respect to Apgar scoring (Table 2). Amniotic glucose values below the limit of detection (20 mg/mL) were replaced by 20/,àö2. Neonatal mortality was evaluated in a total of 90 pups, excluding those euthanased at birth (Table 2). AF lactate ($P<0.001$) and cortisol ($P<0.05$) concentrations showed high values in stillborn pups, whereas amniotic glucose concentrations were low

in pups dying within 48 h ($P<0.001$). Amniotic parameters did not differ between normal and malformed pups(Table 2). All amniotic biomarker concentrations were higher in pups delivered by vaginal parturition than by CS ($P<0.05$) (Table 2). Neonatal mortality was not related to the type of parturition. Apgar score and amniotic parameters were not influenced by breed size. In this study, every amniotic specimen collected tested negative for microbiological culture, which prevented any comparative analysis of AF parameters in case of intra-amniotic infection.

Amniotic glucose concentrations in humans at birth are reported to be about 22 mg/dL (Stefos et al., 2003). In neonatal dogs, amniotic glucose concentrations have not been reported, but in the present study were about 14 mg/dL in pups dying within 48 h, and about 20 mg/dL in live pups. This difference could be of relevant clinical significance in identifying hypoglycaemic conditions. Consistently with human evidence (Marom et al., 2010), significantly higher concentrations of amniotic glucose were recorded in pups born by vaginal parturition rather than by CS. High concentrations of umbilical lactate have been associated with canine neonatal mortality (Groppetti et al., 2010). In the present study, the highest values of amniotic lactate (>18 mmol/L) were observed in stillborn pups. Consistent with human evidence (Borruto et al., 2008), the lowest concentrations of amniotic lactate were detected in pups born by elective CS rather than in those born by emergency CS, and the highest concentration was found in pups born by vaginal delivery. It is known that uterine activity during labour induces hypoxic-ischaemic effects on placental vessels and peripheral tissues with consequent hyperlactaemia and fetal acidosis at birth (Bakker et al., 2007.

Several studies have shown that spontaneous vaginal delivery is more stressful for human neonates than CS (Gitau et al., 2001). Our results confirmed these data, as amniotic cortisol concentrations were significantly higher in pups born by vaginal parturition than by CS. AF cortisol levels were also related to neonatal viability,with significantly high values in stillborn pups. Surprisingly, amniotic cortisol

concentration was low in pups dying within 48 h.

It is notable that all pups dying within 48 h weighed significantly less than their surviving littermates, suggesting intrauterine growth retardation (IUGR). Adrenocortical immaturity leading to low cortisolconcentrations can be assumed in pups with IUGR as has been observed in preterm infants (Midgley et al., 1996). Considerable evidence of the role of birth weight in neonatal outcomes has been reported, with low weight being associated with high neonatal morbidity and mortality (Baibazarova et al., 2013). Due to the great variation in weight (from toy to giant) among canine breeds, we stratified our pups based on breed size. The results obtained are consistent with human findings; in fact, lower bodyweight was found in pups dying within 48 h after birth in medium sized breeds ($P<0.05$). Collecting appropriate volumes of blood from neonatal canine patients is difficult, so amniocentesis performed at delivery could be a viable alternative in diagnosing pup distress at birth. The measurement of amniotic glucose, lactate and cortisol concentrations at birth may provide useful information with respect to neonatal viability and mortality risk. Although these preliminary results show significantly clinical relevance, future large-scale studies in an evenly distributed population are necessary before the findings should be applied to neonatal practice.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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**Table 1**
Number of pups and their birth weights in relation to the type of parturition and breed size in alive, dead, normal and malformed pups.

| | Vaginal parturition | Emergency CS | Elective CS | Bodyweight (g) |
|---|---|---|---|---|
| All pups | | | | |
| Small breed | ND | 1 | 16 | 194.9 ± 30.3 |
| Medium breed | 12 | 10 | 45 | 323.1 ± 167.2 |
| Large breed | ND | 2 | 9 | 583.4 ± 78.9 |
| Overall | 12 | 13 | 70 | |
| Alive pups | | | | |
| Small breed | ND | 1 | 13 | 204.2 ± 20.9 |
| Medium breed | 11 | 9 | 32 | 358.3 ± 165.0 |
| Large breed | ND | 2 | 8 | 571.1 ± 71.0 |
| Overall | 11 | 12 | 53 | |
| Dead pups | | | | |
| All pups dying within 48 h | | | | |
| Small breed | ND | ND | 3 | 151.7 ± 32.6 |
| Medium breed | 1 | 1 | 8 | 212.3 ± 121.2 |
| Large breed | ND | ND | 1 | 707.0 |
| Overall | 1 | 1 | 12 | |
| Stillborn pups | | | | |
| Medium breed | 1 | 1 | 1 | 316.0 ± 164.7 |
| Normal pups | | | | |
| Small breed | ND | 1 | 16 | 194.9 ± 30.3 |
| Medium breed | 12 | 10 | 37 | 341.1 ± 167.4 |
| Large breed | ND | 2 | 9 | 583.4 ± 78.9 |
| Overall | 12 | 13 | 62 | |
| Malformed pups | | | | |
| Medium breed | ND | ND | 8 | 192.9 ± 96.6 |

CS, Caesarean section; ND, not detectable.

**Table 2**
Amniotic glucose, lactate and cortisol concentrations in relation to neonatal viability, mortality, malformation and type of parturition.

| | Glucose (mg/dL) | Lactate (mmol/L) | Cortisol (ng/mL) |
|---|---|---|---|
| Viability | | | |
| Severely stressed: Apgar score 0–4 ($n = 9$) | 17.9 ± 9.7 | 12.0 ± 7.2 | 4.1 ± 2.5 |
| Moderately stressed: Apgar score 5–9 ($n = 13$) | 17.3 ± 5.6 | 9.5 ± 3.6 | 4.7 ± 2.0 |
| Healthy: Apgar score 10–14 ($n = 73$) | 21.0 ± 9.5 | 9.3 ± 3.3 | 4.8 ± 4.2 |
| Mortality | | | |
| Alive pups ($n = 76$) | 20.4 ± 9.1[b] | 9.6 ± 3.6 | 4.8 ± 4.0 |
| All pups dying within 48 h ($n = 14$) | 14.2 ± 0 | 10.2 ± 4.9 | 3.7 ± 2.0 |
| Stillborn pups ($n = 3$) | 14.2 ± 0 | 18.3 ± 1.6[b] | 5.6 ± 2.0[a] |
| Malformation | | | |
| Normal ($n = 87$) | 20.4 ± 9.2 | 9.7 ± 3.8 | 4.7 ± 3.9 |
| Malformed ($n = 8$) | 18.3 ± 7.7 | 7.5 ± 2.3 | 5.3 ± 2.4 |
| Type of parturition | | | |
| Vaginal parturition ($n = 12$) | 29.0 ± 10.6[b] | 12.1 ± 5.1[a] | 6.5 ± 6.4[a] |
| Emergency CS ($n = 13$) | 15.2 ± 2.6 | 10.5 ± 2.8 | 3.5 ± 1.4 |
| Elective CS ($n = 70$) | 19.5 ± 8.4 | 8.7 ± 3.4 | 4.7 ± 3.4 |

CS, Caesarean section.
[a] Significant differences within columns referring to the same variable ($P < 0.05$).
[b] Significant differences within columns referring to the same variable ($P < 0.001$).