

## Original Research Article

# Neonatal blood lactate as a predictor of early survival in puppies delivered by emergency cesarean section

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## ABSTRACT

This study evaluated blood lactate as a biomarker of neonatal viability in puppies delivered by emergency cesarean section. A total of 28 bitches and 118 neonates were assessed for weight, temperature, plasma lactate and glucose levels, and Apgar scores immediately after birth. Eighteen neonates (15.25 %) did not survive: 14 (11.86 %) were stillborn, and 4 (3.39 %) died within 30 min postpartum. A moderate negative correlation between neonatal lactate and Apgar scores ( $p < 0.005$ ) indicated that higher lactate levels were associated with increased neonatal stress and reduced viability. Lactate was positively correlated with glucose ( $p < 0.005$ ) and negatively correlated with body temperature at birth ( $p < 0.05$ ). No significant association was found between maternal and neonatal plasma lactate, but a negative correlation ( $p < 0.01$ ) was observed between neonatal lactate and maternal glucose. A lactate threshold of 9.96 mmol/L was identified, significantly associated with Apgar scores above seven at one and 5 min postpartum, suggesting improved neonatal viability. These findings support the use of neonatal blood lactate as an early marker of metabolic stress and perinatal hypoxia. However, its predictive value should be interpreted alongside other physiological parameters to optimize neonatal care.

## 1. Introduction

Parturition is a natural event that presents a challenge for both the mother and the neonates. In dogs, neonatal viability depends on a successful transition from intrauterine to extrauterine life, a process that requires rapid adaptations in respiration, circulation and metabolism [1, 2]. To supervise the moment of delivery, several parameters may be assessed, including changes in body temperature, decreasing progesterone levels, or the presence of clear signs of labor. These indicators help distinguish between normal delivery and dystocia, which is crucial for minimizing fetal and maternal stress and reducing maternal and neonatal mortality [3].

Neonatal mortality in dogs varies between 5 % and 35 % [1,2,4], depending on multiple factors that can influence survival before, during, and after birth [5]. While perinatal mortality is influenced by factors such as litter size, mode of delivery, and maternal health [4], neonatal viability at birth is primarily determined by the neonate's ability to transition from intrauterine to extrauterine life. This transition is particularly critical in altricial species such as canines, meaning they are born in a vulnerable state with limited thermoregulatory capacity and

an immature respiratory system [1,6]. Identifying early indicators of neonatal viability in the first moments after birth is essential to provide immediate support and improve survival rates [7].

In human medicine, the mode of delivery has been shown to influence neonatal adaptation, particularly in terms of pulmonary function. Infants born via cesarean section exhibit impaired lung function due to reduced pulmonary fluid reabsorption, which can impact their respiratory transition at birth [8]. Similar findings have been observed in dogs, where neonates born naturally show better cardiorespiratory and metabolic adaptation than those delivered by cesarean section. Additionally, bitches that undergo vaginal delivery experience lower stress levels compared to those undergoing cesarean section [8]. Beyond these immediate effects, the type of delivery plays a fundamental role in both neonatal and maternal physiological and metabolic changes. Furthermore, complications arising during the first week of life have been linked to the duration of labor, with prolonged labor and dystocia being associated with higher rates of adverse outcomes, including increased neonatal mortality [8].

One of the most widely used methods for evaluating neonatal viability at birth is the Apgar Score, which assesses five parameters:

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heart rate, respiratory rate, reflex irritability, mucosal coloration, and mobility [1,9–11]. While this tool provides a rapid and practical assessment, it remains subjective, as it relies on observer interpretation. Additionally, the Apgar score does not provide direct metabolic information, making it necessary to integrate complementary biomarkers to improve neonatal assessment [12]. However, for the accurate application of the Apgar score, certain breed-specific adaptations have been necessary. For instance, modifications have been made for breeds such as the French and English Bulldog, whereas differences have been observed in breeds like the Chihuahua. Nevertheless, authors emphasize the need for further research to explore the Apgar score’s variability across different dog breeds and refine its application accordingly [11, 12].

In humans, lactate measurement is a key parameter for assessing fetal distress and the degree of hypoxia during delivery, as elevated lactate levels indicate increased anaerobic metabolism, suggesting potential tissue hypoperfusion and cellular hypoxia [13–15]. In veterinary medicine, lactate has been studied as a potential indicator of neonatal viability, particularly in equine neonates, where it has been associated with perinatal hypoxia and survival outcomes [16,17]. In dogs, there is no consensus regarding the specific lactate thresholds predictive of neonatal survival, and its reliability as an independent viability marker remains uncertain. Previous studies have suggested that elevated lactate levels (>13 mmol/L) are associated with increased neonatal mortality within the first 24 h of life, whereas normal lactate levels range around 5 mmol/L [1]. However, these measurements must be taken as soon as possible, ideally within the first 5 min after birth, to provide relevant prognostic information [1,2].

In addition to lactate, other biochemical and physiological parameters have been studied as indicators of neonatal viability, including glucose levels, temperature and birth weight [7]. Neonatal glucose is a critical factor, as hypoglycemia is one of the leading causes of neonatal mortality. Blood glucose concentrations below 40 mg/dL have been associated with poor Apgar scores [18], while levels below 37 mg/dL within the first 8 h of life have been linked to increased mortality [19]. Similarly, neonatal temperature plays a crucial role in survival, with hypothermic neonates being more susceptible to poor resuscitation outcomes and higher mortality rates [1]. Puppies with body temperatures below 35 °C in the first hour of life are considered hypothermic, and temperatures below 33.9 ± 1.2 °C in neonates younger than seven days have been associated with increased mortality [18].

Furthermore, maternal factors may also influence neonatal biochemical parameters at birth. Maternal hypoglycemia, metabolic status, and anesthetic protocols could potentially impact neonatal lactate concentrations, yet their specific influence remains poorly understood [20,21]. Additionally, anesthetic agents used during cesarean sections could affect neonatal viability by crossing the placental barrier and altering neonatal respiratory function [21–23]. While some studies suggest that alfaxalone may result in higher Apgar scores compared to propofol, its direct impact on neonatal lactate levels has not been fully elucidated [24,25].

This study aimed to assess whether neonatal blood lactate can serve as an early biomarker of viability in puppies delivered via emergency cesarean section, but not to assess lactate as a long-term predictor of neonatal survival. Additionally, we assess the relationship between lactate levels with Apgar scores, glucose levels, and temperature to determine its clinical utility. Finally, this study aimed to value whether different maternal factors—including maternal weight, glucose levels, lactate levels, and age—could influence neonatal lactate concentration.

2. Material and methods

2.1. Animals

This study included 28 bitches (*Canis lupus familiaris*) diagnosed with dystocia and admitted to the Reproduction Service of the Veterinary

Clinical Hospital (University of Las Palmas de Gran Canaria). A total of 118 neonates were recorded, of which 18 (15.25 %) did not survive (14 stillborn, 4 died within 30 min post-partum). Only dystocic bitches requiring emergency cesarean section were included in the study. The bitches weighed between 1.5 kg and 58 kg; both primiparous and multiparous females were included in the study. The population consisted of various breeds, including Chihuahua, American Bully, Teckel, Presa Canario, and mixed-breed dogs, among others. The age of the bitches ranged from 2 to 8 years, with the majority being between 3 and 4 years old.

Dystocia was diagnosed based on a history of labor for more than 1 h without fetal expulsion or a resting phase exceeding 5 h. On the other hand, ultrasonographic evidence of fetal distress (fetal heart rate <180 bpm) was also defined. Bitches diagnosed with severe systemic diseases (e.g., sepsis, metabolic disorders) or those undergoing elective cesarean sections were excluded from the study.

Data collection was carried out between 2023 and 2024. This study was conducted in accordance with the ethical guidelines established by the Bioethics Committee of the Veterinary Hospital of Las Palmas. Since all animals were clinical patients requiring emergency cesarean section, no additional ethical approval number was necessary. All procedures followed standard emergency protocols, and owners provided informed consent prior to any intervention.

To establish a clear inclusion criterion, only bitches that met at least one of the following conditions were selected: (1) failure to deliver a fetus after at least 1 h of active labor, despite having a progesterone level below 1 ng/mL; (2) after delivering one or more fetuses naturally, the resting period exceeded 6 h without resumption of labor; (3) ultrasound examination showed at least one fetus with a heart rate below 180 bpm.

In cases where some neonates were born naturally, those neonates were excluded from the study, and only those born via cesarean section under the previously mentioned conditions were included. Additionally, the following cases were excluded from the study: (1) stillborn neonates (not counted in the study data); (2) bitches with uterine abnormalities (e.g., torsion, rupture, or poor uterine condition); (3) neonates with congenital malformations; (4) bitches diagnosed with other concurrent pathologies beyond dystocia; (5) cases of elective cesarean sections.

2.2. Experimental design

This study was designed as a prospective observational study, including bitches diagnosed with dystocia and requiring emergency cesarean section. The animals (Table 1) were randomly assigned into two groups based on the anesthetic protocol used for induction. Group 1 received alfaxalone (2 mg/kg IV), while Group 2 received propofol (4 mg/kg IV). Additionally, the bitches were categorized according to maternal weight, with two groups established: those weighing less than 10 kg and those weighing more than 10 kg. Litter size was also classified into three categories: small litters (<3 neonates), medium litters (3–5 neonates), and large litters (>5 neonates). All neonates were evaluated at three specific time points: immediately at birth (T0), 5 min post-partum (T5), and 60 min postpartum (T60) as part of routine neonatal monitoring. Blood samples were collected at each time point for glucose and lactate analysis. However, for this study, only the data obtained at T5 were considered for analysis (Table 2).

Table 1  
Groups classification for the experimental design.

Category	Subgroup	N (bitches)	N (Neonates)
Anesthetic group	Alfaxalone	14	60
	Propofol	14	58
Maternal weight	<10 Kg	12	69
	>10 Kg	16	49
Litter Size	Few (<3 neonates)	5	10
	Moderate (3–5 neonates)	13	52
	Many (>5 neonates)	10	56

**Table 2**

Apgar score for dog newborn viability evaluation.

PARAMETERS	SCORE		
	0	1	2
<b>Mucus colour</b>	Cyanotic	Pale	Pink
<b>Heart rate (bpm)</b>	<180	180–220	>220
<b>Reflex irritability</b>	Absent	Grimace	Vigorous
<b>Motility</b>	Flaccid	Some flexions	Active motion
<b>Respiratory efforts</b>	No crying/<6	Mild crying/6–15	Clear crying/>15

### 2.3. Preoperative evaluation and anesthetic induction

Upon admission, all bitches underwent a complete clinical examination, including heart rate (bpm), respiratory rate (breaths per minute), blood pressure (mmHg), and rectal temperature (°C) measurements. A venous blood sample was collected from the cephalic vein before anesthesia to assess maternal hematological and biochemical parameters, including hematocrit (%), total protein (g/dL), urea (mg/dL), and creatinine (mg/dL) (Catalyst Dx, IDEXX Laboratories, S.L, Spain). Glucose and lactate concentrations were measured using a Pet-Test Glucose Monitoring System (PetTest, USA) and a Cera Check Lactate Analyzer (RAL, Spain), respectively.

All bitches underwent a preoperative ultrasonographic examination using a microconvex transducer (C4-1 Curved Array, ZONE Sonography®, Mindray Zonare Z.One PRO) to evaluate fetal viability based on heart rate (bpm) and fetal movements. Fetal distress was defined as a heart rate below 180 bpm. Blood samples were taken to measure progesterone levels using the Speed™ Reader for Speed™ Progesterone (Virbac, Spain). Additionally, thoracic radiographs and an electrocardiogram were obtained as part of the routine pre-surgical protocol to assess anesthetic stability and to estimate the number of neonates, allowing for appropriate organization of the resuscitation team.

### 2.4. Surgical procedure

For anesthesia, all bitches received premedication with intravenous fentanyl (Fentadon 50 µg/mL, Eurovet Animal Health B.V., Netherlands; 5 µg/kg) diluted in lactated Ringer's solution. Induction was performed using: Alfaxalone (2 mg/kg, IV; Alfaxane 10 mg/mL, Jurox, Ireland) in Group 1; Propofol (4 mg/kg IV; Propofol, 10 mg/mL, Esteve, Spain) in Group 2. Anesthesia was maintained with sevoflurane (2 %) in 100 % oxygen (1–2 L/min) using mechanical ventilation. Throughout the procedure, continuous monitoring was performed, including electrocardiography (ECG), pulse oximetry (SpO<sub>2</sub>, %), capnography (end-tidal CO<sub>2</sub>, mmHg), and non-invasive blood pressure measurements at 5-min intervals. To minimize fetal exposure to anesthetic agents, the time from induction to fetal extraction was recorded and kept as short as possible (always less than 15 min).

All cesarean sections were performed by the same surgical team following a standardized protocol. A midline abdominal incision was performed, with the length adjusted according to uterine size; the uterus was exteriorized, and fetal extraction was performed via a single hysterotomy at the uterine body. The uterus was closed using a double-layer continuous suture technique with Monosyn 3/0 (B. Braun Surgical SA, Spain). The abdominal wall was closed routinely, and postoperative analgesia was provided with methadone at a dose of 0.2 mg/kg (Semfortan 10 mg/dL, Dechra Veterinary Products S.L.U, Barcelona, Spain).

### 2.5. Neonatal reanimation

Immediately after birth, each neonate was transferred to a resuscitation area, where a standardized protocol was implemented. Airway clearance was ensured by removing fetal membranes and aspirating fluids from the mouth and nose using a bulb syringe. Neonates were vigorously dried and stimulated to encourage respiration.

Neonatal viability was assessed using the Apgar scoring system (Table 2). The Apgar score, assessed 5 min after birth, included heart rate (stethoscope), respiratory rate (breaths per minute), mucosal color (tongue and perinasal region), mobility (spontaneous movements), and irritability reflex (gentle pad pressure). Neonates were classified as normal vitality (7–10 points), moderate vitality (4–6 points), or poor vitality (0–3 points). To assess metabolic adaptation, rectal temperature (°C), body weight (g), blood glucose (mg/dL), and plasma lactate (mmol/L) levels were recorded: T5 (5 min postpartum) and T60 (60 min postpartum).

Neonates classified as severely compromised (Apgar score <4 at T5) received extended resuscitation, which included: jugular catheter placement for rapid fluid administration, naloxone (0.02 mg/kg IV) in cases of opioid-related respiratory depression and heptaminol (2 mg/kg IV) in cases of persistent bradycardia. Neonatal survival was monitored for the first 24 h postpartum, and mortality within this period was recorded for further analysis. The decision to administer pharmacological treatment was based on both heart rate (less than 80 bpm) and the duration of resuscitation efforts. Heart rate was always assessed prior to drug administration to avoid any influence on the recorded values and ensure an objective evaluation of neonatal viability.

### 2.6. Parameter testing

For each neonate, Apgar score, lactate (Cera Check Lactato, RAL, Barcelona, Spain), glucose (PetTest Glucose Monitoring System, PetTest, USA), rectal temperature, and birth weight were recorded. Blood samples for glucose and lactate analysis were collected via capillary puncture from the digital pad using a calibrated handheld analyzer and rectal temperature was measured with a digital thermometer.

### 2.7. Statistical analysis

Statistical analyses were performed using SPSS AMOS 29.0 (SPSS Inc., Chicago, IL, USA). Categorical variables, including neonatal viability (live births, neonatal mortality), anesthetic protocol (alfaxalone, propofol), maternal weight categories (<10 kg, >10 kg), and litter size (<3, 3–5, >5 puppies), were expressed as frequency and proportion. Continuous variables, such as lactate levels and Apgar scores, were presented as mean ± standard deviation. Normality was assessed using the Shapiro–Wilk test. Neonatal viability was analyzed using general linear models (GLM), considering the effects of anesthetic protocol, maternal weight, and litter size, as well as their interactions. The correlation between lactate levels and Apgar scores was analyzed using Spearman's rho correlation test. A statistically significant relationship was considered when  $p < 0.05$ . Receiver operating characteristic (ROC) curves were generated to assess the predictive value of the Apgar score and lactate levels in neonatal risk identification. The optimal cut-off point was determined using Youden's index to improve classification accuracy. A  $p$ -value <0.05 was considered statistically significant.

## 3. Results

A total of 28 bitches were included in the study and all survived the surgery. A total of 118 neonates were recorded; 14 (11.86 %) were stillborn and were not included in further viability analyses, as their exact time of death could not be determined. The remaining 104 live-born neonates were monitored for survival outcomes and 4 puppies (3.39 %) died within 15–30 min postpartum. All data analyses and reported results in this study were based on the measurements obtained at the 5-min postpartum evaluation.

Table 3 summarizes neonatal viability based on the anesthetic induction agent, litter size, and maternal weight. Regarding **dam's weight**, mortality was significantly higher in dams weighing **more than 10 kg (32.6 %)** compared to those **under 10 kg (2.9 %)** ( $p = 0.006$ ).

**Table 3**  
Neonatal mortality based in the anesthetic protocol, bitch weight and litter size.

		Total Neonatal mortality
Anesthetic Induction	Propofol	20.6 % (13/60) <sup>a</sup>
	Alfaxalone	9.1 % (5/58) <sup>b</sup>
Mothers's weight	<10 kg	2.9 (2/69) <sup>a</sup>
	>10 kg	32.6 % (16/49) <sup>b</sup>
Litter Size	1-2 neonates	10.0 % (1/10) <sup>a</sup>
	3-5 neonates	9.6 % (5/52) <sup>a</sup>
	>5 neonates	21.4 % (12/56) <sup>b</sup>

<sup>ab</sup>: Different letters in the same row and category denote significant differences ( $p < 0,05$ ).

Regarding **litter size**, the highest rate of mortality (**21.4 %**) was observed in litters **>5 neonates** ( $p = 0.031$ ), while few litters (**1–2 neonates: 10.0 %**, **3–5 neonates: 9.6 %**) showed lower mortality rates. **Table 4** presents the mean values ( $\pm$  standard deviation) of different neonatal physiological parameters based on the anesthetic induction protocol, excluding deceased neonates. No significant differences were observed in blood glucose (mg/dL), blood lactate (mmol/L), birth weight (g), or rectal temperature ( $^{\circ}\text{C}$ ) between groups. However, the Apgar score was significantly higher in neonates induced with alfaxalone ( $p = 0.0014$ ) compared to those induced with propofol.

**Table 5** categorizes neonatal blood lactate concentrations by maternal weight and litter size. While maternal weight did not significantly influence neonatal lactate levels ( $p = 0.03$ ), significantly lower lactate levels were detected in litters of 3–5 neonates compared to many litters ( $p = 0.023$ ). A cut-off value of 9.96 mmol/L for blood lactate concentration at 5 min postpartum was identified as a potential predictor of neonatal viability in emergency cesarean sections. This threshold was associated with Apgar scores  $>7$ , with an Area Under the Curve (AUC) of 0.732, indicating a good predictive capability. The sensitivity and specificity for this threshold were 79.3 % and 78.9 %, respectively (**Fig. 1**).

A significant moderate negative correlation was observed (**Table 6**) between neonatal lactate concentrations and Apgar scores (Spearman's  $\rho = -0.47$ ,  $p = 0.0008$ ), suggesting that higher lactate levels were associated with lower Apgar scores. A moderate positive correlation was observed between neonatal lactate and glucose levels (Spearman's  $\rho = 0.53$ ,  $p = 0.0001$ ), which may reflect metabolic adaptations to perinatal stress. Conversely, a moderate positive correlation was observed between lactate and neonatal glucose levels ( $p = 0.00003$ ). However, no statistically significant correlation was found between lactate levels and birth weight ( $p = 0.046$ ).

Maternal parameters were analyzed to determine their influence on neonatal lactate levels (**Table 7**). A weak, non-significant positive correlation was observed between maternal and neonatal lactate

**Table 4**  
Apgar scores, birth weight, temperature and plasma levels of glucose and lactate of neonates based on the induction anesthetic agent (mean  $\pm$  standard deviation).

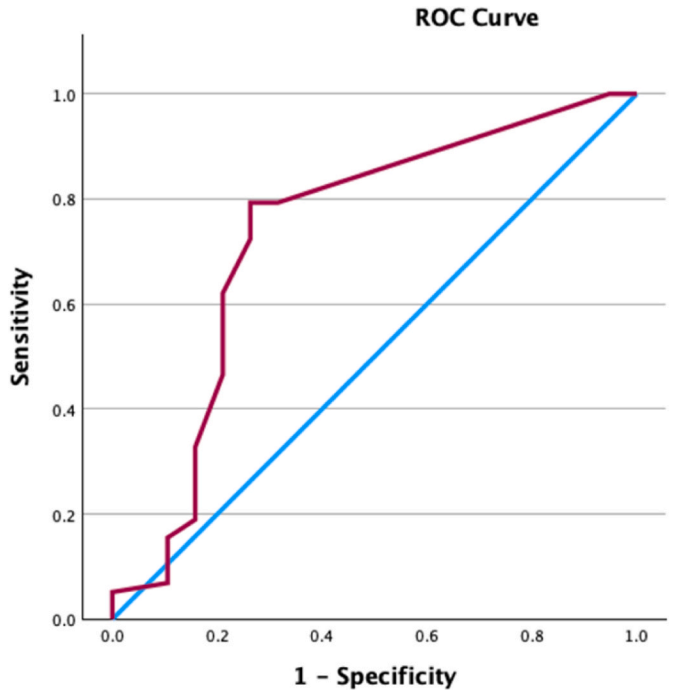
	Induction anesthetic agent		Total	Range
	Propofol	Alfaxane		
Lactate (mmol/l)	8,71 $\pm$ 0,39	8,47 $\pm$ 0,467	8,59 $\pm$ 0,43	3,20–12
Glucose (mg/dl)	126,20 $\pm$ 9,28	117,88 $\pm$ 5,8	122,04 $\pm$ 7,82	40–417
Temperature ( $^{\circ}\text{C}$ )	32,63 $\pm$ 0,08	32,73 $\pm$ 0,09	32,68 $\pm$ 0,09	31,20–34,50
Apgar score (0–10)	7,12 $\pm$ 0,31 <sup>a</sup>	8,32 $\pm$ 0,29 <sup>b</sup>	7,72 $\pm$ 0,31	3–10
Weight (Kg)	0,40 $\pm$ 0,03	0,35 $\pm$ 0,02	0,37 $\pm$ 0,02	70–900

<sup>ab</sup>: different letters in the same row and category denote significant differences ( $p < 0,05$ ).

**Table 5**  
Average lactate levels (mmol/l) based on litter size and mother size.

		Mean	Std Err	Range
Dam's weight	<10 kg	8,47	0,44	3,42–12
	>10 kg	8,80	0,43	3,17–12
Litter size	1-2 neonates	9,85	0,84	6,09–12
	3-5 neonates	7,90	0,45	3,17–12
	>5 neonates	9,10	0,45	4,33–12

<sup>ab</sup>: different letters in the same row and category denote significant differences ( $p < 0.05$ ).



**Fig. 1.** Receiver operating characteristic (ROC) curve for neonatal blood lactate as a predictor of viability.

**Table 6**  
Correlation coefficients between plasmatic neonatal lactate and neonatal parameters (Apgar score, neonatal weight, neonatal temperature and neonatal glucose level).

Neonatal parameters	Correlation coefficient
Neonatal lactate-Apgar score	−0.471***
Neonatal lactate-Neonatal glucose	−0.531***
Neonatal lactate-Neonatal weight	0.074 <sup>ns</sup>
Neonatal lactate-Neonatal temperature	−0.401***

\*\*\*Correlation was significant at 0,5 % ( $p < 0.005$ ).

<sup>ns</sup> Correlation was not significant.

**Table 7**  
Correlation coefficient of plasma neonatal lactate and maternal parameters (lactate plasma levels, glucose plasma levels, age).

Neonatal parameters	Correlation coefficient
Neonatal lactate-Mother's lactate	0.147 <sup>ns</sup>
Neonatal lactate- Mother's glucose	−0.300**
Neonatal lactate-Bitch weight	0.020 <sup>ns</sup>

\*\* Correlation is significant at 1 % level ( $p < 0.01$ ).

<sup>ns</sup> Correlation is not significant.

concentrations (Spearman's  $\rho = 0.15$ ,  $p = 0.027$ ). In contrast, a



significant moderate negative correlation was detected between maternal glucose levels and neonatal lactate concentrations (Spearman's  $\rho = -0.30$ ,  $p = 0.0016$ ). Regarding maternal age, no significant correlation with neonatal lactate levels was identified ( $p = 0.081$ ).

#### 4. Discussion

The primary objective of this study was to evaluate neonatal blood lactate concentration at birth in puppies delivered via emergency cesarean section and assess its potential as a biomarker of neonatal viability. Since lactate is a product of anaerobic metabolism and reflects both tissue oxygenation and metabolic stress, its measurement immediately after birth could provide valuable insights into neonatal adaptation and survival. While previous studies have investigated lactate concentration in amniotic fluid [5,26], data on direct neonatal blood lactate measurement and its relationship with viability parameters remain limited.

In this study, neonatal lactate levels showed a significant correlation with well-established viability indicators, such as Apgar scores, neonatal temperature, and glucose concentration, reinforcing its potential as a prognostic biomarker [1]. These findings align with previous research suggesting that elevated lactate levels are associated with increased neonatal stress and perinatal hypoxia [18]. The observed neonatal mortality rate was 15.25 %, with 11.86 % of neonates stillborn and 3.39 % dying within the first few hours postpartum. These results are consistent with previous reports, where perinatal mortality rates range from 11.3 % within the first 48 h [2] to as high as 25 % in some studies [5]. In other species, such as foals, lactate has been recognized as a valuable health indicator, with elevated concentrations being associated with bacterial infections and systemic inflammatory response syndrome (SIRS) [27,28].

Previous studies have reported an association between elevated lactate concentrations (>12.2 mmol/L at 48 h) and increased neonatal mortality risk [1]. However, the predictive value of lactate may vary depending on perinatal conditions, resuscitation efforts, and postnatal management [29]. In the present study, neonates delivered via dystocia cesarean section had an average lactate concentration of 8.59 mmol/L. While this value is above the previously reported survival threshold (6.55 mmol/L), it remains below the critical level associated with increased mortality risk (12.2 mmol/L). Notably, all neonates survived the initial 4 h post-birth during hospitalization. Given that dystocia is associated with fetal distress and perinatal hypoxia, the elevated lactate levels observed in this study may reflect the metabolic response to birth-related stress [21]. Other authors have reported biochemical alterations in calves and lambs, including changes in lactate levels. Their findings indicate that lactate concentrations are significantly higher in non-surviving neonates (16 mmol/L) compared to those that survive (5.6 mmol/L) [27].

Several studies have proposed different lactate cut-off values for neonatal prognosis. Some authors suggest that concentrations around 5 mmol/L may be indicative of favorable outcomes [2], while others report that values exceeding 8 mmol/L could be associated with increased neonatal risk. However, variations in study populations, resuscitation protocols, and measuring devices must be considered when interpreting these thresholds [1]. However, considerable variability exists among reports regarding these threshold values, highlighting the need for further research to establish a clear consensus. In the present study, although the mean lactate concentration surpassed the 8 mmol/L threshold, no neonatal deaths were observed within the first 4 h of life. A lactate threshold of 9.96 mmol/L was established as a predictor of neonatal viability, as it was significantly associated with Apgar scores above seven at one and 5 min postpartum. This discrepancy may be attributed to the increased fetal stress and subsequent tissue hypoxia experienced by neonates born from emergency dystocic cesarean sections. While this threshold provides a valuable reference for clinical decision-making, it should be interpreted with caution until validated in

larger, multicentric studies and further research is needed to confirm its applicability across different populations, breeds, and clinical settings. Although our study did not include lactate measurements in neonates born via elective cesarean section or natural birth, previous studies have reported no significant differences between delivery methods. However, higher lactate concentrations have been observed in neonates delivered by emergency cesarean section, whereas the lowest levels are typically found in those born via elective cesarean section [2,17].

Although the main objective of this study was to assess how lactate levels can serve as an indicator of neonatal viability immediately after birth, other authors have explored its utility during the first days of life. Several studies have reported that lactate concentrations are highest immediately after birth and gradually decrease as the neonate develops [17,30]. Additionally, lactate was not evaluated as a long-term marker due to the inability to control neonatal management at home, which could introduce other causes of mortality unrelated to lactate concentrations. Additionally, previous studies have reported that Apgar scores may vary depending on neonatal weight, which could influence the overall results. For instance, significant differences have been observed between large and small neonates, potentially affecting their scores [11]. However, further research is needed to better understand the influence of neonatal size and breed on Apgar scoring, as only a limited number of studies have addressed this aspect. Refining these parameters could help establish more precise cut-off values for neonatal viability assessment at birth.

Recent studies have reported variability in Apgar scores depending on the breed. For instance, in Chihuahuas, the survival cut-off after cesarean section has been established at 4 points [31], whereas in our study, we considered a minimum score of 7 points for good viability. Additionally, other studies analyzing multiple breeds have suggested a cut-off of 6 points to identify adequate neonatal viability [32]. Given that this is a preliminary study aiming to establish a cut-off for neonates born via emergency cesarean section, as well as to compare it with other parameters that have not yet been fully explored, further research is needed to assess potential differences between breeds and delivery methods.

The relationship between lactate concentration and the Apgar score has been previously investigated, with some studies reporting no significant correlation within the first 10 min of life and even suggesting the absence of correlation during the first 8 h postpartum [1,32]. Contrary to these findings, our study identified a moderate yet statistically significant negative correlation between Apgar scores and lactate levels, demonstrating that lower Apgar scores were associated with higher lactate concentrations. Similar results have been described in previous studies, suggesting that elevated lactate concentrations may reflect increased tissue hypoxia and reduced neonatal viability [18]. Given the moderate strength of this correlation, it is essential to consider additional parameters when assessing neonatal viability rather than relying exclusively on lactate and Apgar scores. However, since the association is statistically significant, lactate may still serve as a potential predictive model for neonatal condition. Therefore, it could be a useful tool for determining the level of postnatal care required, helping to identify neonates who may need closer monitoring and medical intervention.

Neonatal glucose measurement is a commonly used parameter at birth, providing insight into the energy reserves of neonates. Due to hepatic immaturity, neonates have limited glycogen stores, making them susceptible to hypoglycemia [33,34]. Several factors, including birth weight and maternal condition, have also been implicated in neonatal glucose fluctuations [1]. While some studies have reported an association between low glucose levels, poorer Apgar scores, and increased mortality risk [18], others have indicated that excessively high glucose concentrations may also correlate with increased neonatal mortality [35]. However, glucose alone has been deemed an unreliable predictor of neonatal mortality [36,37]. In the present study, a moderate yet statistically significant positive correlation was observed between neonatal glucose and lactate concentrations at birth. This relationship

could be attributed to perinatal stress, which activates fetal and neonatal adaptive responses, particularly in cases of prolonged or complicated deliveries such as dystocic cesarean sections. Neonatal hypoxia, a common consequence of dystocia, promotes anaerobic metabolism, leading to increased lactate production. Simultaneously, stress-induced catecholamine release may elevate neonatal glucose levels, potentially explaining the observed correlation [5]. Few studies have explored the direct correlation between blood glucose and neonatal lactate levels, although an association between amniotic fluid glucose and blood lactate has been described [1]. Previous studies have reported that neonates born from dystocic deliveries exhibit higher blood glucose levels compared to those delivered eutocically [5]. These findings align with our results, further supporting the hypothesis that neonatal metabolic adaptations to birth stress influence both glucose and lactate dynamics. Future investigations should focus on refining the predictive value of combined glucose and lactate measurements in neonatal viability assessment, particularly in high-risk deliveries. In other species, such as pigs, a potential relationship between glucose and lactate has been described, linked to metabolic cycles. Authors have reported that gluconeogenesis occurs simultaneously with other energy-demanding processes, leading to lactate release from muscle cells and, consequently, lactic acidosis. These metabolic pathways, which remain poorly studied in neonates, could also explain the positive correlation observed between glucose and lactate in our study. Further research is needed to elucidate the physiological mechanisms underlying this association and to determine its clinical relevance in neonatal viability assessment [38].

Birth weight has been widely recognized as a risk factor for neonatal survival, as low-birth-weight neonates are more susceptible to oxygen restrictions and the secondary effects of hypoxia [39,40]. Several studies have reported a significant correlation between low birth weight and increased mortality within the first 2–4 days of life [41–43]. However, in the present study, a weak and statistically insignificant positive correlation was observed between lactate levels and birth weight.

Neonatal temperature is a critical viability parameter, as hypothermia has been associated with reduced neonatal responsiveness and lower success rates in resuscitation efforts [1,18,31]. Moreover, hypoxia in neonates has been linked to bradycardia, respiratory depression, and a diminished suckling reflex, ultimately contributing to lower Apgar scores [1]. For the first time, this study identified a statistically significant moderate negative correlation between neonatal temperature and lactate concentration at birth. This finding suggests that neonates with lower body temperatures tend to exhibit lower heart and respiratory rates, leading to an accumulation of lactate due to insufficient oxygen delivery to tissues [44]. Neonates with lower Apgar scores often struggle with respiration and circulation, linking hypothermia to higher lactate levels and increased metabolic distress. This highlights the need for immediate thermoregulation to reduce hypoxia and acidosis. Further research is needed to assess temperature monitoring in predicting viability and improving early interventions.

One of the objectives of this study was to assess whether maternal weight and litter size influence neonatal lactate concentrations at birth. Previous research has examined these parameters, reporting a non-significant negative correlation between maternal weight and neonatal lactate, as well as a non-significant positive correlation between maternal age and lactate levels [44]. In the present study, no significant correlation was observed between neonatal lactate concentration and maternal weight. A plausible explanation for this finding is that lactate is primarily a metabolite associated with tissue hypoxia and neonatal stress [1], both of which are influenced by perinatal conditions rather than maternal body size.

No significant differences in neonatal lactate levels were observed between different litter sizes. While the impact of litter size on neonatal biochemical parameters in cesarean section deliveries has not been extensively studied, previous research has demonstrated its influence on gas exchange and other physiological parameters in natural births, particularly when comparing the first and last neonates born [40,45]. A

possible explanation for the lack of significant differences in lactate levels based on litter size in this study is the timing of delivery. Unlike natural births, where prolonged delivery increases intrauterine hypoxia, emergency cesarean sections allow for rapid fetal extraction, potentially homogenizing neonatal lactate levels regardless of litter size. While litter size may influence perinatal stress in natural births, its effect on lactate levels in cesareans appears reduced due to shorter delivery times. Further research comparing lactate dynamics across different delivery methods is needed to clarify this relationship.

Maternal parameters were evaluated in relation to neonatal lactate levels. A weak, non-significant positive correlation was observed between maternal and neonatal lactate concentrations. This finding aligns with previous literature indicating that the amount of lactate crossing the placenta during labor is minimal, with most of the neonatal lactate concentration being the product of glycolysis produced by the neonate itself [46]. A moderate negative correlation was found between maternal glucose and neonatal lactate levels, suggesting that lower maternal glucose is linked to higher neonatal lactate. This may be due to fetal reliance on maternal glucose; in hyperglycemia, increased fetal insulin promotes aerobic metabolism, reducing lactate. However, as gestation progresses, metabolic changes can impair glucose homeostasis, predisposing bitches to hypoglycemia [20]. This maternal hypoglycemia may negatively impact uterine function, potentially resulting in uterine inertia or weak contractions. Additionally, hypoglycemia can impair neuromuscular function, reducing coordination and contraction strength, which may impede the normal progression of labor [21].

Anesthesia in cesarean sections is a critical factor, particularly because some anesthetic agents can induce respiratory depression in neonates [21,23]. This study assessed the impact of the induction agent (alfaxalone vs. propofol) on neonatal lactate levels, finding no significant differences between protocols. This aligns with existing literature, which suggests that while some anesthetics cross the placenta, their effect on neonatal viability is limited [4,44,47]. In this study, no significant differences in neonatal lactate levels were found, and no neonatal mortality occurred at birth, supporting the safety of the anesthetic protocol. This aligns with previous research showing that while alfaxalone may slightly improve Apgar scores, differences from propofol are not statistically significant, with both agents considered safe for cesarean sections [24,25,48]. It is important to note that although all drugs cross the placental barrier [21], this study specifically evaluated propofol and alfaxalone. Future studies should also consider the potential influence of other drugs, such as fentanyl, to determine whether there is a correlation between their use and lactate levels in cesarean sections.

This study provides relevant insights into neonatal blood lactate as a potential biomarker of viability. Although the power analysis conducted indicates that the sample size (28 bitches and 118 neonates) was sufficient for statistical analysis, a larger cohort is required to strengthen conclusions and improve external validation. This is a preliminary study, and we aim to continue advancing research in this area to further refine lactate thresholds and explore additional neonatal viability biomarkers. Lactate measurements were performed immediately postpartum, but serial assessments over the first 24–48 h could offer a more comprehensive understanding of lactate dynamics and its role in predicting neonatal survival beyond the immediate postnatal period. Additionally, this study focused on emergency cesarean sections due to dystocia, a high-risk neonatal scenario. Further research should explore lactate thresholds in elective cesareans and natural deliveries to establish broader reference values.

## 5. Conclusion

Plasmatic neonatal lactate may serve as a valuable parameter for assessing metabolic stress and hypoxic conditions in neonates, particularly in high-risk populations such as those delivered via emergency cesarean section. However, its use as a standalone biomarker should be

approached with caution, as lactate levels can be influenced by multiple perinatal factors, including delivery conditions, neonatal thermoregulation, and maternal metabolic status. Considering these complexities, lactate assessment should be incorporated into a broader diagnostic framework that includes additional biochemical and clinical parameters. A multimodal approach would allow for a more comprehensive evaluation of neonatal viability, facilitating more accurate clinical decision-making during the perinatal period. Further studies should aim to refine neonatal lactate thresholds and evaluate its integration with other biomarkers to enhance the accuracy of viability assessments in newborn puppies.

### CRedit authorship contribution statement

**Rodríguez Raquel:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Batista Miguel:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iusupova Kseniia:** Writing – review & editing, Methodology, Formal analysis. **Alamo Desirée:** Writing – review & editing, Formal analysis, Data curation.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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