## **Topical Review**

# Topics in the Routine Assessment of Newborn Puppy Viability

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*E-mail:* mege@fmvz.unesp.br (M.L.G. Lourenço) Neonatal veterinarians still observe higher mortality rates among their patients than those observed among humans. Establishment of a neonatal assessment protocol is fundamental to the identification of the medical status of the neonate and the need for medical intervention. The neonatal Apgar score evaluation, which is commonly used in clinical practice, should be complemented by other methods of analysis. This study proposes, in addition to an Apgar score analysis, the evaluation of laboratory parameters and weight. We believe that knowledge of these reference values is essential for diagnosing at-risk neonates and for establishing suitable treatments.

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### Introduction

In veterinary medicine, the neonatal mortality rate ranges from 17%-30% in dogs and is the highest during the first 7 days of life.<sup>1-4</sup> Neonatal mortality is associated with several factors, including stillbirths, maternal neglect, and agalactia as well as congenital and acquired conditions<sup>3</sup>; however, many losses are a result of inadequate reproductive management and could be avoided. A study of 1342 neonates reported that 91% of the pups were alive until weaning, which presumably reflects adequate reproduction management.<sup>1</sup> The survival rate is related to the qualifications of the medical staff involved, the number of neonatologists, and the hospital infrastructure in humans.<sup>5</sup>

The evaluation of human neonates is performed based on the Apgar score, which aims to facilitate the clinical evaluation of the newborn at the time of delivery and to target neonatal resuscitation interventions. It is the method most employed in the immediate identification of the status of the child at birth.<sup>6,7</sup> The index is based on the evaluation of 5 vital clinical signs, including heart rate, spontaneous breathing, muscle tone, grimace, and the appearance of the mucous membranes, and each criterion is evaluated on a numerical scale from 0-2. A score of 7-10, obtained by the sum of all vital signs, is considered adequate, a score of 4 to 7

indicates that resuscitation may be required, and a score below 3 is an indication for emergency care. Low scores are associated with congenital anomalies, low birth weights, and higher mortality rates.<sup>6-9</sup>

The Apgar score in pups from eutocic births reflects an initial depression of the vital functions immediately after birth, possibly because of the transition to extrauterine life. However, adequate recovery occurs within 5 minutes and is maintained an hour after birth.<sup>10</sup> It is proposed that newborns require a short period of time to adapt to extrauterine life, during which spontaneous respiration and organic adaptation of the functions previously performed by the placenta can be established.<sup>11,12</sup> However, the mortality rate 2 hours after birth is higher in newborn canines with an Apgar score of 0-6 compared with those with a score between 7 and 10.<sup>6</sup>

The objective of the present study was to describe an evaluation protocol based on the Apgar score in association with clinical and laboratory parameters.

#### **Materials and Methods**

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine and Animal Science, State University of São Paulo.

The study included 27 adult dogs of various breeds, 1-10 years of age. Female dogs were divided into 2 groups: those who had a

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 Table 1

 The Modified Apgar Scoring System Used in This Study<sup>6</sup>

Parameter	Weak (0 Score)	Moderate (1 Score)	Normal (2 Score)
Heart rate	< 180 bpm	180-220 bpm	> 220 bpm
Respiratory rate	No crying/ < 6 mpm	Mild crying/ 6-15 mpm	Crying/ > 15 mpm
Reflex irritability	Absent	Grimace	Vigorous
Motility	Flaccid	Some flexions	Active motion
Mucus color	Cyanotic	Pale	Pink

normal birth (n = 11, 49 newborns) and those that required a cesarean delivery (n = 16, 55 newborns).

Normal birth was defined as puppies born spontaneously without any type of assistance, including obstetrical assistance. All females were monitored during labor.

Females were submitted for cesarean delivery if there was no response to drug therapy or to obstetric maneuvers to correct dystocia. Anesthetic induction was performed with propofol followed by epidural anesthesia with lidocaine and maintenance with isoflurane diluted in oxygen. During the cesarean delivery, fetuses were separated from the placenta, and the umbilical cord was cut and cross-clamped. Neonatal assistance was provided immediately after birth.

The evaluation of neonatal viability was similar in the 2 groups. We evaluated the modified Apgar score proposed by Kustritz<sup>13</sup> and Veronesi et al.<sup>6</sup> The evaluation of heart rate was performed using a vascular Doppler ultrasound. The respiratory frequency and breathing patterns of the neonate were noted, and reflex irritability was checked by a painful stimulus. Muscle tone was determined with the neonate in a supine position by observing active movements and responses to passive movements of the limbs. The appearance of the mucous membranes was assessed by

visualization of the oral mucosa. Each parameter was scored on a scale from 0-2, and the total score was the sum of these (Table 1).

Neonatal reflexes (suckle, rooting, and righting reflexes) were also assessed at birth and 60 minutes later (Fig). The suckle reflex was elicited by inserting the clean tip of the smallest digit of the examiner into the mouth of the neonate and assessing the suckling force; the righting reflex of the neonate was assessed by placing it on its back on a soft surface and verifying that it returned to the right recumbence. The rooting reflex was assessed by approaching the nose of the neonate with a hand shaped into a circle with the forefinger and thumb and checking whether the neonate inserted its nose into the circle. The reflexes were scored on a scale from 0-2, and the total score was their sum. Joint analysis of all reflexes was used because the presence of one reflex alone does not ensure neonatal survival. The interpretation of the score was as follows: 0-2, weak viability; 3 and 4, moderate viability; and 5 and 6, normal viability (Table 2).

The rectal temperature was measured with a digital thermometer, and the neonates were weighed using scales (in grams) both at birth and 60 minutes later.

After determination of the Apgar score and neonatal reflexes, a blood sample (0.1 mL) was drawn by jugular puncture with a 1-mL syringe and a 26-G needle. All laboratory assessments were performed at the site of the birth, and the results were used to determine whether the newborns required emergency treatment.

The evaluations were performed with an i-STAT Portable Clinical Analyzer (ABBOTT) with an EG7 cartridge for blood gas analysis (ABBOTT). The following parameters were assessed: blood pH, partial pressure of oxygen and carbon dioxide (pO<sub>2</sub> and pCO<sub>2</sub>, mmHg), sodium (Na, mEq/L), potassium (K, mEq/L), ionized calcium (iCa, mmol/L), hematocrit (Hto), hemoglobin (Hgl, g/dL), base excess (BE, mmol/L), sodium bicarbonate (NaHCO<sub>3</sub>, mmol/L), total carbon dioxide (TCO<sub>2</sub>, mmol/L), and oxygen saturation (SO<sub>2</sub>). A determination of lactate (mmol/L) and glucose (mg/dL) levels

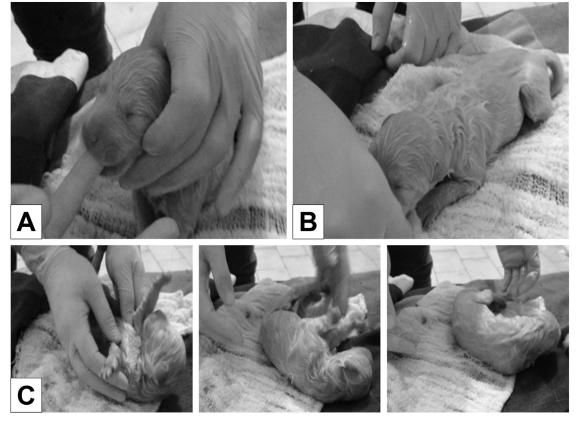


Fig. Demonstration of suckling (A), rooting (B), and righting reflexes (C) in a neonate.

Parameter	Weak (0 Score)	Moderate (1 Score)	Normal (2 Score)
Suckle	Absent	Weak ( > 3 suckles/min) <sup>27</sup>	Strong (5 suckles/min) <sup>27</sup>
Rooting	Absent	Slow muzzle fitting inside the circle	Immediate fitting muzzle within the circle
Righting reflexes	Absent (continues in initial position)	Slow body repositioning	Fast body repositioning

was performed with a portable Accutrend analyzer (for determining lactate, glucose, cholesterol, and triglyceride levels, Roche and Johnson).

To be evaluated for neonatal survival, the newborns were divided into following groups: those that died within 2 hours, those that survived from 2-24 hours, those that were alive after 24 hours,<sup>6</sup> and those that were alive at 30 days. Early neonatal mortality was defined as newborns that died before 7 days, and late mortality as neonates that died between 7 and 30 days.

Newborns with Apgar scores of 0-6 at birth underwent emergency treatment to increase their chances of survival, as described by Traas.<sup>14</sup> The treatment consisted of clearing the airways with a suction bulb (size 1), drying, respiratory stimulation (cardiopulmonary resuscitation), oxygenation, ventilation by mask or tracheal tube, and, when necessary, administration of fluid therapy (NaCl 0.9% solution with or without the addition of glucose based on the blood glucose levels) and intravenous therapeutics (doxapram, aminophylline, and epinephrine). After performing the tests described and stimulating defecation and urination reflexes, the clinician inspected the newborns for congenital anomalies, performed antisepsis of the umbilical cord, and placed the neonate under a radiant heat source.

The reference values used in this study were those described in the literature by a number of different authors.

The results were expressed as the mean  $\pm$  standard error. Differences between the time periods were determined from the paired *t* test for the ionizable calcium (iCa) variable. For all other variables, differences were compared using the Mann-Whitney rank sum test with a significance level less than or equal to 5%.

## Results

A total of 49 eutocic neonates and 55 cesarean neonates, with 4 and 3 puppies being the median number of pups per litter, respectively, were evaluated, and no differences were noted between the labors. The study evaluated females of 7 different breeds: 6 Pinschers, 3 Shih Tzus, 2 Welsh corgis, 1 Boxer, 1 Samoyed and Teckel crossbreed, and 10 mongrels. The duration of labor before intervention was determined by the fetal heart rate, as determined by a Doppler ultrasound examination, and ranged from 2-24 hours.

Regarding the sex of the evaluated newborns, there were 49 females and 42 males, with 24 females and 19 males from eutocic labor and 25 females and 23 males from dystocic labor. Maternal and neonatal body weights differed between the types of delivery (Table 3).

#### Table 3

Comparison of Maternal and Neonatal Weight in Different Groups (Eutocia and Cesarean Delivery)

	Eutocia	Cesarean Delivery	P Value
Body weight Maternal body weight (kg) Neonatal body weight (g)	$\begin{array}{r} 7.19 \ \pm \ 12.88 \\ 206.4 \ \pm \ 13.3 \end{array}$	$\begin{array}{r} 8.24 \ \pm \ 4.5 \\ 277.2 \ \pm \ 14.2 \end{array}$	0.008 < 0.001

Recovery of the Apgar score was observed after 60 minutes on average. The same recovery was noted for the neonatal reflexes (suckle, rooting, and righting reflexes). Rectal temperature was higher in newborns from cesarean deliveries (Table 4).

Laboratory parameters were influenced by the type of birth and the evaluation time point (Tables 5 and 6).

Correlations between clinical and laboratory parameters were evaluated for newborns from eutocic births and cesarean deliveries (Table 7).

Regarding neonatal survival, 11.54% (12) of the 104 newborns were stillborn. Early mortality was 0% (0) up to 2 hours, 1.92% (2) between 2 and 24 hours, and 2.88% (3) between 24 hours and 7 days. Late mortality was 4.80% (5) between 7 and 15 days and 1.92% (2) between 15 and 30 days.

## Discussion

Early identification of neonates at risk is essential to minimize losses to neonatal diseases. The development of a protocol for neonatal assessment is, therefore, fundamental to this identification as well as to a greater knowledge of neonatal physiology.

The Apgar score proposed by Veronesi et al<sup>6</sup> determines the clinical condition of the neonate<sup>15</sup>; for this reason, we decided to use it in this study. The proposition of a score of neonatal viability reflexes (NVR) aimed at assessing the degree of neonatal depression based on suction reflexes, demand, and straightening has already been described by Kustritz.<sup>13</sup> The aforementioned reflexes are present early after birth to ensure breastfeeding and, hence, are indispensable to neonate survival. The low score would be observed only in situations of fetal hypoxia or neonatal triad.

We understand that an assessment performed by a single evaluator was a limiting factor of our study; however, we believed that a single assessment would reduce the bias for subjectivity in clinical examination, which would occur with multiple evaluators. The newborns were evaluated in the same order so that the interval between examinations was 60 minutes and, therefore, constant regardless of the size of the litter.

#### Table 4

Clinical Parameters Expressed as the Mean  $\pm\,$  Standard Error, Followed by the Statistical Significance of the Difference Between the Time Points

Parameters	Labor	Moment	
		At Birth	After 60 Min
Apgar	Cesarean delivery Eutocia	$\begin{array}{rrr} 4.3 \ \pm \ 0.3^{aA} \\ 7.6 \ \pm \ 0.3^{aB} \end{array}$	$\begin{array}{r} 8.8\ \pm\ 0.3^{b}\\ 8.6\ \pm\ 0.3^{b}\end{array}$
Reflexes	Cesarean delivery Eutocia	$\begin{array}{rrrr} 1.7 \ \pm \ 0.2^{aA} \\ 4.6 \ \pm \ 0.2^{B} \end{array}$	$\begin{array}{rrrr} 4.1 \ \pm \ 0.2^{bA} \\ 5.0 \ \pm \ 0.2^{B} \end{array}$
Temperature	Cesarean delivery Eutocia	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

<sup>a, b</sup> Different superscript uppercase letters indicate significant differences between evaluation periods within the same group (P < 0.05).

<sup>A, B</sup> Different superscript lowercase letters indicate significant differences between groups in the same evaluation period (P < 0.05).

Table 5 Laboratory Parameters Expressed as the Mean  $\pm$  Standard Error According to the Evaluation Time Point

Parameter	Value at Birth	Value 60 Min After Birth	P Value
$HCO_3$ $PO_2$ $SO_2$ $TCO_2$ Lactate	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	< 0.001 0.02 0.02 < 0.001 0.001

The value of eutocia was apparent from the Apgar scores for pups at both time points. In cesarean deliveries, moderate viability was observed at birth, which tells us that newborns initially presented with depression, as has also been reported by Crissiuma et al<sup>16</sup> and Silva et al.<sup>17</sup> This depression is associated with the fetal distress arising from the dystocia, with the fetus spending more time in the uterus or vaginal canal and being affected by anesthetic agents. At 60 minutes, however, we observed recovery in the Apgar score for pups from cesarean deliveries, which was equal to the scores of the eutocic pups.<sup>6,10</sup>

The score reflects a temporary condition of the neonate and, therefore, is useful in verifying the effectiveness of interventions. An elevation of this score, depending on the type of delivery, demonstrates the ability of the neonate to adjust to an extrauterine environment, even under adverse conditions. The scores obtained in this study were higher than those of Groppetti et al<sup>8</sup> for both types of birth, and of Vivan,<sup>18</sup> in cesarean deliveries; such a difference probably reflects the anesthetic protocols used in the different studies.

We believe that the weight of the neonates reflected the weight of mothers because the average weight of females in cesarean deliveries was lower than that of females in eutocia. This result likely occurred because smaller females have

#### Table 6

Laboratory Parameters Expressed as the Mean  $\pm\,$  Standard Error, Followed by the Statistical Significance of the Difference Between the Time Points

Parameters	Labor	Moments	
		At Birth	After 60 Min
рН	Cesarean delivery Eutocia	$\begin{array}{r} 7.2 \ \pm \ 0.01^{a} \\ 7.2 \ \pm \ 0.01^{a} \end{array}$	$\begin{array}{rrr} 7.3 \ \pm \ 0.01^{\rm b} \\ 7.3 \ \pm \ 0.01^{\rm b} \end{array}$
BE	Cesarean delivery Eutocia	$\begin{array}{rrrr} -7.8 \ \pm \ 0.8^a \\ -6.7 \ \pm \ 0.8^a \end{array}$	$\begin{array}{rrr} -2.9 \ \pm \ 0.8^b \\ -4.3 \ \pm \ 0.8^b \end{array}$
pCO <sub>2</sub>	Cesarean delivery Eutocia	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrr} 45.8 \ \pm \ 1.3^{\rm b} \\ 46.6 \ \pm \ 1.4 \end{array}$
Na <sup>+</sup>	Cesarean delivery Eutocia	$\begin{array}{r} 143.2\ \pm\ 0.6\\ 143.0\ \pm\ 0.6^a\end{array}$	$\begin{array}{r} 143.5 \ \pm \ 0.6 \\ 142.4 \ \pm \ 0.6^{b} \end{array}$
$K^+$	Cesarean delivery Eutocia	$\begin{array}{rrr} 4.0 \ \pm \ 0.08^{a} \\ 4.2 \ \pm \ 0.09 \end{array}$	$\begin{array}{rrr} 4.3 \ \pm \ 0.08^{\rm b} \\ 4.2 \ \pm \ 0.09 \end{array}$
iCa	Cesarean delivery Eutocia	$\begin{array}{rrr} 1.5 \ \pm \ 0.01^{a} \\ 1.6 \ \pm \ 0.01 \end{array}$	$\begin{array}{rrr} 1.4 \ \pm \ 0.01^{\text{bA}} \\ 1.5 \ \pm \ 0.01^{\text{B}} \end{array}$
Hematocrit	Cesarean delivery Eutocia	$\begin{array}{rrrr} 54.9 \ \pm \ 1.2^{aA} \\ 47.5 \ \pm \ 1.3^{B} \end{array}$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$
Hemoglobin	Cesarean delivery Eutocia	$\begin{array}{rrrr} 18.\ 7\ \pm\ 0.4^{aA} \\ 16.2\ \pm\ 0.4^{B} \end{array}$	$\begin{array}{rrr} 19.4 \ \pm \ 0.4^{bA} \\ 16.1 \ \pm \ 0.4^{B} \end{array}$
Glucose	Cesarean delivery Eutocia	$\begin{array}{rrrr} 85.6 \ \pm \ 7.6^{a} \\ 106.0 \ \pm \ 8.1^{a} \end{array}$	$\begin{array}{rrr} 56.4 \ \pm \ 7.6^{bA} \\ 123.9 \ \pm \ 8.1^{aB} \end{array}$

 $^{\rm a,b}$  Different superscript uppercase letters indicate significant differences between evaluation periods within the same group ( $P\,<\,0.05$ ).

<sup>A,B</sup> Different superscript lowercase letters indicate significant differences between groups in the same evaluation period (P < 0.05).

## Table 7

Correlation Between Clinical and Laboratory Parameters According to the Evaluation Time Point

Parameters	At Birth		Was the Correlation
	R Value	P Value	Maintained at 60 Min?
Eutocia			
pH vs glycemia	-0.642	0.001	Yes
pH vs lactate	-0.606	0.001	No
Glucose vs BE	-0.445	0.002	Yes
BE vs lactate	-0.563	< 0.001	Yes
Apgar vs lactate	-0.324	0.034	Yes
Blood glucose vs weight	0.328	0.031	No
BE vs Apgar	0.319	0.037	No
Cesarean section			
pH vs weight	0.293	0.43	Yes
pH vs Apgar	0.526	< 0.001	Yes
pH vs RVN	0.423	0.002	Yes
BE vs weight	0.65	< 0.001	Yes
BE vs Apgar	0.686	< 0.001	No
BE vs RVN	0.686	< 0.001	No
Apgar vs RVN	0.877	< 0.001	Yes
Apgar vs weight	0.581	< 0.001	Yes
pH vs lactate	-0.606	< 0.001	No
Apgar vs lactate	-0.558	< 0.001	Yes

a greater chance of developing dystocia owing to maternal-fetal disproportion.<sup>19</sup>

Variations in body temperatures of the newborns were associated with the differences to which the pups were subjected in postpartum management. Although the eutocic pups remained with their mothers, the newborns from cesarean deliveries were kept in temperature-controlled environments during the recovery of the parturient mother, thereby reducing heat loss. Body temperature decreases are known to be a consequence of a deficit of simultaneously increasing thermogenic mechanisms such as thermolysis in these animals.<sup>9,16</sup> Newborns remained hypothermic regardless of the type of delivery, whether or not subjected to artificial heating.

In both types of pups at birth, mixed acidosis as described by Lúcio et al<sup>10</sup> and Vivian<sup>18</sup> was observed, with a drop in the pH, HCO<sub>3</sub>, and BE and an increase in blood lactate. The pH was greater than that noted by Crissiuma<sup>20</sup> (7.17), with an increase in this value at 60 minutes. This fact can be explained by compensatory alkalosis, which developed in accord with the mixed acidosis that resulted from the hypoxia. The observed values for HCO<sub>3</sub> were quite similar to those mentioned by Crissiuma et al.<sup>11</sup>

Hypercapnia occurred along with hypoxia, especially in cesarean delivery neonates, similar to those described by Gabas et al,<sup>21</sup> Crissiuma et al,<sup>20</sup> and Vivan.<sup>18</sup> The observed changes in blood gases reflect the perinatal asphyxia established during childbirth and represent neonate adaptation to the extrauterine environment. Standardization of these values was observed at 60 minutes, indicating that the hypoxia was transient, regardless of the type of delivery.

The partial  $CO_2$  value in cesarean delivery pups was greater than in the eutocic pups owing to the higher respiratory distress<sup>18,21</sup> experienced by the cesarean delivery pups; once the obstructed labor intensified the respiratory depression, a delay in pulmonary fluid reabsorption caused aspiration of the fluid.<sup>16</sup> The variable, however, stabilized at 60 minutes, also indicating recovery of the neonate.

The partial  $O_2$  at birth approached the values noted by Crissiuma et al.<sup>20</sup> At 60 minutes, however, there was an unexpected decline in this value, resulting in divergence from the reference. This decline is associated with the amendment of the dissociation curve of hemoglobin, which favors an increase in

oxygen extraction by the tissue because newborns were more vigorous at 60 minutes and exhibited an elevation in the Apgar score and reflexes, contrary to the alleged hypoxia detected.

The lower calcium concentration in dystocic neonates was considered to be a causal factor, and not a consequence of the type of delivery, because the presence of this ion is essential for uterine contractions.<sup>22</sup>

In cesarean deliveries, environmental conditions and the period that newborns must wait to breastfeed favored hemoconcentration, leading to increased packed cell volume and hemoglobin.

The length of time to initial feeding was also responsible for higher values of glycemia in eutocic pups at both time points. The decrease in glycemia observed at 60 minutes in the cesarean delivery group was associated with fasting during the anesthetic recovery of the mother in the period following parturition.

Lactate values were also greater than that found in references  $(5 \text{ mmol/L})^8$  because there was neonatal hypoxia in both groups, with the development of lactic acidosis.<sup>10,11,16,17,23,24</sup> However, a decrease in lactate level indicates the time frame for recovery from the acidosis detected at birth, which was corroborated with a clinical examination and an analysis of laboratory variables.

In eutocic labor, newborns with low birth weights presented with lower glycemic indices, indicating that these newborns may be more debilitated, with less glycogen in reserve. The increase in the lactate was related to the decline in pH and BE at birth, stressing the newborns and causing them to present with metabolic acidosis. As lactate is an indicator of tissue hypoxia, its elevation also explains the decrease in the Apgar score because it is an indicator of the condition of the animal. For this same reason, a correlation is demonstrated between the Apgar score and BE.

The positive correlations between pH and BE and Apgar scores and RVN are explained by the elevation of the pH and BE, which indicate recovery of the clinical picture of the animal, as do the clinical evaluation scores of the newborns. The concomitant elevation of Apgar and RVN is also logical because both scores evaluate the viability of the neonate.

The laboratory tests are important because they detect early changes in the acidemia. Although the clinic is sovereign concerning the establishment of therapeutic maneuvers, early identification of these changes allows us to implement prophylactic measures for the neonates. In addition, the study of these variables helps in understanding the neonatal adaptation mechanisms.

The proposal was to use neonatal reflexes to score viability aimed at assessing the degree of depression of the neonate and its ability to breastfeed. Suckling, rooting, and righting reflexes were selected as these present early after birth to ensure feeding. Clearly, feeding itself is essential for the survival of the neonate, and therefore, any assessment of reflexes alone would be inadequate. Reflex assessment was not performed, therefore, simply as a means for identifying the neurologic status of the puppy because it is known that the nervous system is not fully developed at birth.<sup>25</sup> Because a hypoglycemic state, in addition to fetal and neonatal hypoxia, is common in the perinatal period, a check of reflexes is appropriate for all newborn puppies. The average blood glucose levels of the neonates remained within the limits set by Vivan,<sup>18</sup> both at birth and 60 minutes later. However, we found that newborns with glucose levels <40 mg/dL usually had low Apgar scores and poor reflexes.

Of the 27 litters examined, the total mortality rate, including stillbirths, was 23.07%, the early neonatal mortality rate (up to 7 days) was 14.42%, and the late neonatal mortality rate (up to 30 days) was 9.6%, which is comparable to the findings reported in the literature.

Among all breeds, mortality occurred in 33.3% of the offspring, with 14.42% of deaths occurring in those up to 7 days of age (11.54% stillbirths and 2.88% in the first 24 hours). We consider the rate of stillbirths to be high. Among the 7 breeds we evaluated, neonatal mortality occurred in 40% of the mongrel litters, 50% of the Pinscher and Welsh Corgi litters, 33.3% of the Shih Tzu litters, and 100% of the Teckel litters. Perinatal mortality as described by Tonnessen et al<sup>26</sup> was comparatively minor for the Pinscher, Welsh Corgi, Shih Tzu, and Dachshund litters at 10.5%, 11.7%, 21.1%, and 20.1%, respectively. In 2 of the breeds evaluated, Boxer and Samoyeds, there was no neonatal mortality.

We observed that in neonates with early mortality, hypoglycemia and temperature at birth were 27.6  $\pm$  20.2 mg/dL and 33.9  $\pm$  1.2°C, respectively. We believe that these values show a developing trend as the puppy fades, and this fading is characterized by neonatal hypoglycemia, dehydration, and hypothermia. In this first stage of life, this process can easily lead to death; therefore, strict control of blood glucose levels and temperature in newborns is recommended for those pups that have these low values at birth.

Regarding newborns that died between 24 hours and 30 days, 2 cases of cannibalism were observed in a primiparous female, along with 1 case of worms detected by necropsy. The highest mortality rate was observed among puppies 2 weeks of age. According to Tonnessen et al,<sup>26</sup> perinatal mortality is influenced by the canine breed, number and size of litters, and maternal age, with the breed being the determining factor for perinatal mortality relative to size. The risk of premature death is greater in dogs that have had their first offspring at greater than 6 years of age and in litters with stillbirths. None of the pregnant females in this study were older than 6 years, but no association between stillbirths and early neonatal mortality was observed in our study.

Neonatal mortality in veterinary practice remains high compared with that in humans despite considerable progress in the development of biotechnologies over recent decades.

The Apgar score is an inexpensive and practical method to evaluate the condition of a newborn and to determine the effectiveness of the resuscitation actions. We would like to note that the score should not be used as a prognostic index because it is subject to sudden changes between time points, as described by Veronesi et al<sup>6</sup> and Groppetti et al.<sup>8</sup> We observed the same in relation to neonatal reflexes, which showed rapid recovery 60 minutes after the first evaluation.

Reflexes continue to be an essential part of routine examination because their absence indicates that the neonate is entering the critical fading puppy stage, which increases the chance of death. Measurements of blood glucose levels reinforce the need to identify animals that are fading and help to ensure that neonatal assistance is provided as soon as possible.

Blood gas analysis identified acidosis, even in clinically healthy animals, and thus complemented the clinical evaluation. It was with the same objective that we conducted an examination of lactate levels, which in addition to being practical, is also a less costly alternative. We reiterate that the acidosis observed was expected because it is part of the cardiorespiratory adaptation of the neonate to extrauterine life. The weight can be measured after the other parameters but must not be neglected, because newborns with a low birth weight require greater attention from both the veterinarian and the owner to ensure proper development.

#### **Author Contributions**

All authors were involved in conducting the experiment. F.G.V. and M.L.G.L. analyzed the data and drafted the paper.

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