Analyzing Blood Gasometry at 3 Time Points of the Fetal-Neonatal Transitional Period of Dogs Born by Elective Caesarean Parturition

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ABSTRACT

Blood gasometry (pH, $PvCO_2$, PvO_2, and HCO_3) was performed in 44 puppies of 9 gestating females at term. Venous blood samples were obtained at 3 consecutive time points: moment 0' (sample obtained from one of the umbilical arteries of each fetus); moment 30', and moment 90' (samples obtained from the jugular vein of each newborn). No significant variation was observed in the average venous blood pH of any of the puppies between moments 0' (7.17 ± 0.08) and 30' (7.18 ± 0.10). Notwithstanding, a significant increase of the venous blood pH was observed in moment 90' (7.31 ± 0.06). The $PvCO_2$ and PvO_2 varied significantly during the 3 time points assessed: while the average $PvCO_2$ decreased over time (moment 0' = 59.59 ± 9.80 mmHg, moment 30' = 55.27 ± 14.40 mmHg, and moment 90' = 45.75 ± 7.90 mmHg), the average PvO_2 increased (moment 0' = 17.91 ± 6.40 mmHg, moment 30' = 22.23 ± 6.44 mmHg, and moment 90' = 25.64 ± 6.19 mmHg). The average HCO_3 values only varied between moments 30' (21.70 ± 2.72 mmol/L) and 90' (23.84 ± 2.79 mmol/L). It was concluded that the physiological changes taking place in the first 90 minutes after birth can be reflected by the blood gasometry of the fetal-neonatal period.

INTRODUCTION

Fetal physiology depends on the mother's circulation to carry gases and nutrients.[1–3] Right after birth, due to the separation of the placental circulation and development of asphyxia,[4,5] it is fundamental that the newborn respiratory and circulatory systems are capacitated to maintain their homeostasis in the aerial environment.[2,5,6] The increase in the partial pressure of carbon dioxide inside the umbilical vessels ($PvCO_2$) as well as the

body's loss of temperature stimulate the first inspiratory reflex.[4,5] Because the acid-base equilibrium regulation is one of the main factors for maintaining organ equilibrium, any problem in this process during the perinatal period may result in irreversible consequences to the newborn.[4]

Clinical aspects related to cardiac and respiratory frequencies, mucosa coloration, temperature, degree of hydration, and neurological responses presented at birth are routinely used to determine neonate vitality.[7–9] However, most parameters observed are subjective and incapable of accurately predicting the degree of depression or individual organ function.[10] Therefore, in aiming to improve the evaluation of neonatal vitality, the objective of this work was to analyze fetal and neonatal blood gasometry during this transitional period of dogs born by elective caesarean parturition.

EXPERIMENTAL PROCEDURE

Animals

Forty-four dogs in the fetal-neonatal transitional period were included in this study (with owners' consent). All animals were mixed bred, weighing from 0.8 to 1.2 lbs at birth, born by caesarean section from females accompanied for ≥32 days before parturition. The puppies were identified and their data recorded.

EXPERIMENTAL DESIGN

Prenatal Care

Nine healthy pregnant females were dewormed with fenbendazole (50 mg/kg/day orally; Panacur®, Hoechst Roussel Vet, Germany) for 3 consecutive days starting on the 40th day of gestation.[11,12] In order to avoid stressful situations, all females were kept by their owners and remained in their homes until it was time for the elective caesarean section. Prenatal care, including clinical, radiographic, and ultrasonographic exams, was provided on a weekly basis. The scheduling of the caesarean section was based on the clinical data obtained throughout the weekly assistance. The clinical examination consisted of abdominal and pelvic muscle tone inspection, behavioral changes (such as search for isolation, quietness, loss of appetite), beginning of uterine contractions, and abrupt decrease in body temperature.[13] Using the ultrasonographic examination, the fetal renal visualization, decrease in heart rate, biparietal diameter of the majority of fetuses, and intestinal motility were considered, given that they are only visible through ultrasound between 58 and 63 days into gestation.[14–16] Once the intervention date was set, the dams were submitted to 12 hours of food deprivation and 3 hours of water deprivation[17] and taken into the surgical center.

Caesarean Anesthesia

At the surgical center, each female had a cephalic vein punctured using an intravenous catheter (Jelco 20G, Johnson & Johnson Medical Vascular Access, Texas, USA) fixed with impermeable sticking tape (Cremer, Brazil), connected to an infusion set (Medicinska Plastika A.D. Tetovo, Russia) containing a 0.9% physiological solution (sodium chloride 0.9%; JP Pharmaceuticals S.A. [Japanese Pharmacopoeia]), and maintained with a continuous infusion (30 mL/h). Anesthesia induction was performed using the following protocol: an injectable 6.0-mg/kg dose of propofol (Propofol emulsion I.V., 10 mg/mL, Abbott Laboratórios do Brasil Ltda.)[18] followed by epidural blockade with an injectable 5.0-mg/kg dose of lidocaine chloride 2% (without vasoconstrictor; Hipolabor, Brazil).[9] After confirming blockade by observing anal sphincter, tail relaxation, and loss of pelvic muscle tone,[19] the dogs were placed on dorsal recumbency to prepare for the surgical procedure. Additional propofol in bolus at a 2.0-mg/kg dose[18] was administered as needed throughout the surgical procedure.

Surgical Procedure and Sample Collection

After the lidocaine latency period,[20] the surgical procedure was initialized with a ventral midline incision from umbilicus to pubis. The uterine horns were exteriorized, and an incision was made at the uterine body. Fetuses were removed, first from the right uterine horn, then from the left one.[21] The females were monitored, using a Vital Signs Monitor (Life Window 6000, Digicare Biomedical Technology, Florida, USA), throughout the whole surgical procedure.

As each fetus was exteriorized from maternal uterus with its placenta, the amniotic sac was ruptured and its fluid removed.[21] Two hemostatic tweezers were placed on the umbilical cord. Cordocentesis was immediately performed between the tweezers, using a plastic 1.0-mL syringe and needle (sterile disposable syringe; BD Plastipak 13 × 3.8 27.5 G ½, Becton, Dickinson, and Company, Brazil) previously rinsed with heparin.[22] A venous blood sample (0.3 mL) was obtained from one of the umbilical arteries of each conceptus.[23,24] The blood samples were collected in anaerobiosis and immediately sealed with cork to avoid blood gas tension alteration.[22,25] The syringe was rolled between the palms of the hands after the blood sample was obtained so that it would mix with the anticoagulant.[22] This procedure was done before each neonate took its first breath. An incision was made between the tweezers, and the neonates were then handed over to an assistant.

The 9 females delivered a total of 70 puppies from which only 44 were included in the analysis in order to keep the original number of puppies per female. Therefore, only the first 5 puppies of each female were included.

Each neonate's respiratory tract was immediately cleared using sterile surgical gauzes while the head was inclined downwards, with the posterior limbs elevated, allowing for all the mucus and fluid from the respiratory tract to be drained. Respiration was stimulated by friction made by the gauze onto the thoracic region.[26] The following physiological and vigor signs were observed and recorded for each neonate: temperature, heart beat, spontaneous breathing, movement, and vocalization. The umbilical cord was then tied at a 2.0-cm distance from the body using a monofilament nonabsorbable suture (Ethilon Nylon Suture 3-0, Ethicon, New Jersey, USA). The neonates were then identified and placed on a thermal cushion.

Other venous blood samples were obtained from the jugular vein of all neonates 30 and 90 minutes after birth, so that new blood gas analysis evaluation could be made at 2 other time points. All blood samples were identified and stored at 4°C until the laboratory analysis could be made within 30 minutes of its collection.[22]

Laboratory Analysis

Samples were analyzed individually by the Blood Gas and pH Analyzer (Radiometer Copenhagen model ABL™-5, Radiometer Copenhagen, Denmark). For the fetal blood samples, the apparatus was calibrated to venous blood, 37°C temperature (average temperature of most females at the moment of caesarian parturition), 12 mg/dL hemoglobin (average hemoglobin level of most females on day of surgery), and 21% oxygen concentration fraction of inspirated gas. As for the neonate samples, the same parameters were used, except for the temperature, which was reduced to 35°C.[9,27]

RESULTS

Repeated measures analysis of variance showed that there is a significant variation in pH (Table 1, Figure 1) with time ($P = 0.0001$) in the group of puppies where the increase in pH was observed, particularly at 90 minutes after birth. Repeated measures analysis of variance was performed in order to evaluate the pH evolution with time. Table 2 provides the results of the repeated measures analysis of variance for pH and the times that differed significantly from one

Table 1. Descriptive Analysis of pH, $PvCO_2$, PvO_2, and HCO_3, With Time, for the 44 Puppies Born by Elective Caesarean Parturition.

Variable	Moment of Collection of Blood Sample (minutes)	Mean ± SD	Variance (minimum; maximum)
pH	0	7.17 ± 0.08	(6.98; 7.31)a
	30	7.18 ± 0.10	(6.95; 7.31)a
	90	7.31 ± 0.06	(7.17; 7.43)b
$PvCO_2$ (mmHg)	0	59.59 ± 9.80	(45; 92)a
	30	55.27 ± 14.40	(37; 99)b
	90	45.75 ± 7.90	(30; 63)c
PvO_2 (mmHg)	0	17.91 ± 6.40	(9; 52)a
	30	22.23 ± 6.44	(14; 39)b
	90	25.64 ± 6.19	(17; 44)c
HCO_3 (mmol/L)	0	22.80 ± 3.23	(14; 28)
	30	21.70 ± 2.72	(17; 27)a
	90	23.84 ± 2.79	(13; 28)b

pH = hydrogenionic potential of venous blood; $PvCO_2$ = partial pressure of carbon dioxide in venous blood; PvO_2 = partial pressure of oxygen in venous blood; HCO_3 = bicarbonate content in venous blood; SD = standard deviation; moment 0 = time point corresponding to the blood sample collected from the double-clamped umbilical cord, before the neonate took its first breath; moment 30 = time point corresponding to the blood sample collected from the left jugular vein of each neonate 30 minutes after birth; moment 90 = time point corresponding to the blood sample collected from the right jugular vein of each neonate 90 minutes after birth.
Different letters in the same column differ statistically.

Figure 1: pH variation with time in the group of total puppies born from elective caesareans.

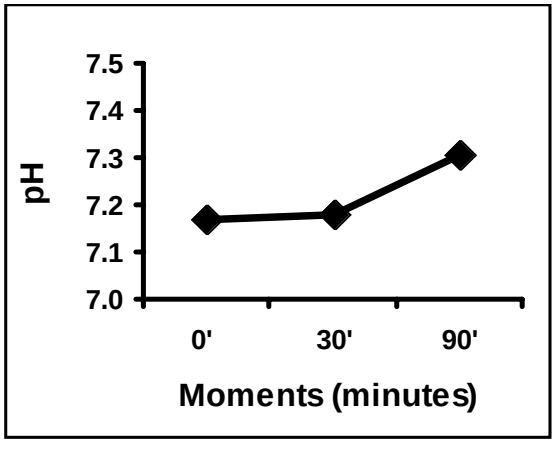
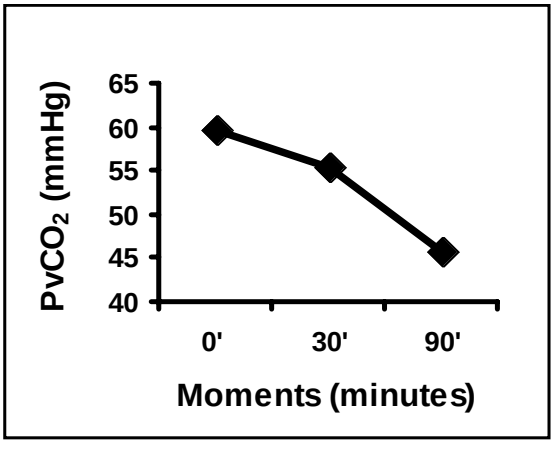


Figure 2: $PvCO_2$ variation with time in the group of puppies born by elective caesareans.



another according to the Bonferroni test. While the repeated measures analysis of variance showed that there was a significant decrease in the values of $PvCO_2$ throughout the 3 evaluated time points (P = 0.0001), the values of PvO_2 increased in the group of total puppies (Table 1, Figures 2 and 3). It was verified that at moment 0', $PvCO_2$ was significantly greater than at moment 30' and moment 90', whereas PvO_2 was significantly smaller. On the other hand, at moment 30', $PvCO_2$ was significantly greater than at moment 90', whereas PvO_2 was significantly smaller. Repeated measures analysis of variance was performed in order to evaluate the evolution of $PvCO_2$ and PvO_2 with time. Table 2 provides the results of this analysis as well as the times that differ significantly according to the Bonferroni test. The repeated measures analysis of variance showed

Figure 3: PvO_2 variation throughout the 3 time points in the group of total puppies born by elective caesareans.

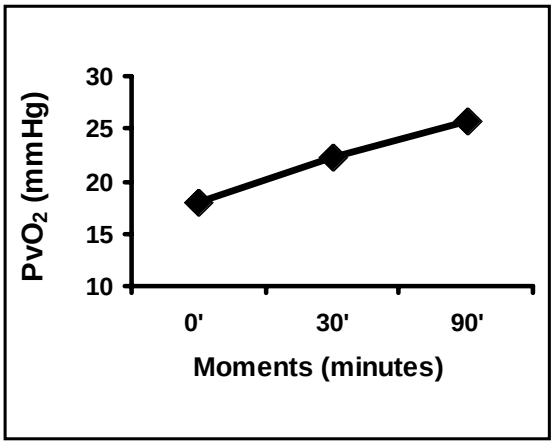


Figure 4: HCO_3 variation throughout the 3 time points in the group of total puppies born by elective caesareans.

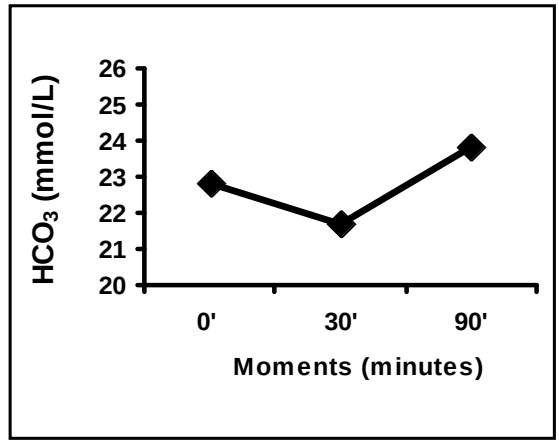


Table 2. Results of the Repeated Measures Analysis of Variance for pH, $PvCO_2$, PvO_2, and HCO_3 for the 44 Puppies Born by Elective Caesarean Parturition.

Variable	*P*-Value	Significant Differences Between Times
pH	0.0001	$FS \neq NS_2$
		$NS_1 \neq NS_2$
$PvCO_2$	0.0001	$FS \neq NS_1$
		$FS \neq NS_2$
		$NS_1 \neq NS_2$
PvO_2	0.0001	$FS \neq NS_1$
		$FS \neq NS_2$
		$NS_1 \neq NS_2$
HCO_3	0.0002	$NS_1 \neq NS_2$

pH = hydrogenionic potential of venous blood; $PvCO_2$ = partial pressure of carbon dioxide in venous blood; PvO_2 = partial pressure of oxygen in venous blood; HCO_3 = bicarbonate content in venous blood; FS = fetal sample; NSy = neonatal sample (if y = 1, first neonatal collection at 30 minutes; if y = 2, second neonatal collection at 90 minutes).

that the values of HCO_3 (Table 1, Figure 4) increased significantly between moment 30' and moment 90' (P = 0.0002) in the group of total puppies. In order to evaluate the HCO_3 evolution with time, independent of group, a repeated measure analysis of variance was performed. Table 2 provides the results for this test as well as the times that differ significantly according to the Bonferroni test.

DISCUSSION

For all 44 puppies, the increase in pH was insufficient to promote significant hemogasometric changes between moments 0' and 30'. However, there was a significant pH evolution between moments 30' and 90'. If acidemic fetuses in dogs are considered to be those with blood pH below 7.25 as in human fetuses,[28,29] it can be suggested that dogs are born in acidemia and are not able to reverse this situation until the first 30 minutes after birth. From this moment, the pH increases and is normalized around 90 minutes after birth, contradicting the Casey et al[30] proposal that affirms that human fetuses are born acidemic and that the pH should worsen in the neonatal period. However, since no other studies with dogs were found in veterinary literature that could have its results confronted with those obtained in this work, care should be taken in characterizing the fetuses' and neonates' acid-base state as acidemia at moments 0' and 30'. Also, care should be taken in affirming that this situation is normalized around moment 90', since the values obtained as results for pH, $PvCO_2$, PvO_2, and HCO_3 in each moment may simply be reflecting the physiological hemogasometric pattern for this fetal-neonatal transitional period in dogs.

If considering, however, that acidemia occurs at moments 0' and 30' and is reversed

at moment 90', the increased values of $PvCO_2$ at moments 0' and 30' characterize the acidosis for these 2 time points as respiratory, since the puppies presented hypercapnia.[28,29] Still, care should be taken in affirming that hypercapnia also occurs in dogs, since the values obtained may simply be reflecting the physiological hemogasometric state at this moment of life. However, independent of this classification, moment 0' was the one where the puppies presented the highest value of $PvCO_2$. These high levels of carbon dioxide in the fetal blood were fundamental at the time of birth since the asphyxia development in the neonate is one of the factors responsible for stimulating the first inspiratory reflex.[4,5]

Once breathing has been initiated in the neonate, $PvCO_2$ started to reduce, although at moment 30' this reduction, though statistically significant, did not abolish the supposed neonate's hypercapnia condition. Consequently, a still high $PvCO_2$ would have contributed to the maintenance of a low pH at this moment. This phenomenon can be explained by the physiological alterations that take place in the fetal-neonatal transitional period, particularly in the respiratory and cardiovascular patterns,[2,5] which must adapt to extra-uterine life in order to remove oxygen from the air and eliminate carbon dioxide from the body. The strong pulmonary expansion at birth facilitates lung inflation through liberation of stocked surfactant,[4,31] but, naturally, requires a certain time so that a sufficient number of alveoli are inflated and gaseous exchange can take place.[32] At moment 90', the values of $PvCO_2$ measured decreased, indicating a more satisfactory respiratory function than before and, if this is considered, at moment 90' the puppies no longer presented with hypercapnia. Besides this, it is known that, in humans, the functional alterations in respiratory and cardiovascular patterns, which mark the transition of the fetal life to the neonatal one, are only concluded on the first post-natal day,[2,5] indicating that even after the 90 minutes after birth, the physiological efforts to adapt to extra-uterine life must continue, which is probably what also occurs in dogs.

Human fetuses presenting PvO_2 smaller than 20 mmHg are considered hypoxemic.[28,29] Considering similar values to characterize hypoxemia in canine fetuses, the results obtained in this work indicate that dogs are born under hypoxemia and that this condition is reversed through the first 30 minutes after birth. This suggests that the beginning of pulmonary ventilation, though still insufficient, is capable of reverting the initial state of hypoxemia that tends to decrease with time, as demonstrated by the higher PvO_2 values recorded at moment 90'.

The kind of parturition that resulted in birth is another factor that should be considered when evaluating the results obtained in this study. It is known that, in humans, the presence of several degrees of hypoxia and the transitional interference in the maternal-fetal respiratory exchanges are common in all types of delivery.[28] In natural delivery, repeated uterine contractions, with reduction of uterine blood flow, cause a certain degree of fetal hypoxia. Nevertheless, neonates born by elective caesareans may present transitory respiratory stress, since they do not reabsorb pulmonary fluid as fast as those born by natural delivery.[2] In the present study, the initially lower PvO_2 values may also not be assumed as representative of hypoxemia, but as physiological to this transitional period. For this reason, assuming the evolution of the results of the hemogasometric values at the 3 time points studied (0', 30', and 90') as physiological, care should be taken in observing that these are normal values for elective caesarean deliveries in dogs.

As to the variation in bicarbonate with time, the absence of statistically significant variation between moments 0' and 30' in this work can be justified by the primary intention of respiratory adaptation to the phenomenon of birth. The statistically significant variation that occurred in bicarbonate values between moments 30' and 90',

however, may reflect the compensatory metabolic effort associated with respiratory compensation to achieve pH equilibrium in this last moment.

In synthesis, 2 interpretations may be considered for the results obtained in this work. The first is that the blood samples collected from the umbilical cords indicate that the fetuses, moments before birth, present respiratory acidosis indicated by the low pH, high $PvCO_2$, low PvO_2, and normal bicarbonate. At 30 minutes after birth, the puppies persist in respiratory acidosis, though there are signs of the attempt to reverse the acidemic state, represented by the decrease in $PvCO_2$ and increase in PvO_2. At 90 minutes after birth, $PvCO_2$ continues to decrease while PvO_2 increases. The retention of bicarbonate increases and the pH is normalized, indicating that in this time interval after birth, the neonate's physiological adaptation to extra-uterine life, particularly from respiratory origin, is already capable of equilibrating blood pH. The second interpretation that may be considered for the results is that the values obtained in this study for pH, $PvCO_2$, PvO_2, and HCO_3 at the 3 time points do not characterize posteriorly compensated acidemia, but represent the normal, or physiological, values for each evaluated moment in elective caesarean birth.

The results obtained in this work suggest that other studies be made with the purpose of better understanding the physiological events that involve the birth of dogs as well as interpreting the influence of different anaesthetic protocols used in caesarean parturition, in representative patterns of neonatal vitality, to facilitate the creation of therapeutic measures necessary to increase survival after birth.

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