# EVALUATION OF NEWBORN PUPS VITALITY BY MEANS OF LACTATE MEASUREMENT, APGAR SCORE AND UTERINE TOCOMETRY.

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**Abstract**

Newborn vitality evaluation and early detection of fetal distress could contribute to reduce mortality at birth in canine species. High neonatal mortality rate in dog is reported subsequent to complicated or uncomplicated whelping. Actually, the lactate level is considered an indicator of fetal distress and a valid predictor of babies' survival. Fetal acidosis recognition by umbilical lactate (UL) measurement, Apgar score classification and uterine activity monitoring during labour, can represent an advanced system in the management of the canine newborn patient. Mainly purpose of this study was to correlate UL levels with canine neonatal morbidity and death within 48 hours of birth. We evaluated relationship among some neonatal parameters at birth (mucous membrane color, heart and respiratory rate, reflex irritability, motility, suckling and vocalization, UL, weight and temperature) with labour characteristics (uterine contractions recorded by the tocometric Whelpwise® system, delivering time and pup presentation), in view to predict pup vitality. We considered also vaginal parturition *versus* elective and emergency caesarean section, and uterotonic drugs influence on delivery. UL concentration proved to be useful to predict canine neonatal mortality within 48 hours of birth ($P<0.05$). We identified in 5 mmol/L of umbilical lactate concentration the cut off value allowing distinguishing between healthy and distressed pups. Higher values of UL were related with pup suffering, whereas lower values characterized vigorous pups.

Lactate concentrations lower than 5 mmol/L and Apgar scores higher than 9, related to mean delivery time of 105 minutes with effective uterine contractions (10 mm of Hg of width or more, frequency from 4 to 12 contractions for hour, each ones of 2-5 minutes duration), should be considered good prognostic factors in canine labour and neonatology.

[1] Abbreviations

UL = umbilical lactate; C-section = caesarean section

*Keywords:* Lactate; Apgar; Tocometry; Dog; Newborn; Vitality;

## 1. Introduction

*Intra-partum* uterine and fetal monitoring has been the standard of care in human medicine since the early 1970's, consistently improving both fetal and maternal outcomes [1]. At present, canine parturition monitoring relies on temperature changes, progesterone declining and individual whelping symptoms which can only contribute a subjective and rough determination of labour. These high variable parameters frustrate early recognition of labour and possible dystocia, leading to fetal distress.

Labour is the physiologic process by which a fetus is expelled from the uterus to the outside world and is defined as a regular uterine contractions accompanied by cervical effacement and dilatation [2]. The greatest impediment to understanding normal labour is recognizing its onset [3]. To quantitatively evaluate the uterine activity, a tocometric method has been developed based on recording of uterine contractions from the abdominal surface [2]. Tocometer is an external pressure measurement device, which is used to detect changes in abdominal contour as an indirect indication of uterine contractions. This system does not require an invasive probe, allowing to be used for most pregnancies without risk to the fetus or the mother [2]. External tocometer monitoring devices are used in over 90% of all human hospital births in the United States [2]. At present a tocometer suitable in dog has been realized by Whelpwise™ Veterinary Perinatal Specialties®, allowing to objectively (quantitatively and qualitatively) record uterine activity for labour recognition [4].

Lactate plays a central role in human neonatology and obstetric clinic as a marker of fetal and neonatal distress and index of secondary pathologies due to hypoxic events occurred at parturition [5,6]. When the oxygen supply to the fetus is significantly disrupted, tissue oxygen deprivation

71 develops, acids begin to accumulate, and acidemia ensues [6]. Lactate is a major component of
72 metabolic acidosis. Sampling of blood from the fetus's scalp or umbilical cord, during labour and
73 parturition, to analyze lactate, is regarded as the ideal method of identifying *intra-partum* fetal
74 hypoxia in human [7,8]. In human and horse, the evaluation of the newborn's condition at delivery
75 is performed by Apgar score method and umbilical cord blood gas analysis, which provide
76 important information about the state of the neonate [9]. There are limited data concerning blood
77 gas parameters in neonatal dogs [10,11]. Aim of this study was to evaluate the meaning of lactate in
78 newborn puppies developing distress at delivery, compared with healthy neonates. We correlate
79 umbilical cord blood lactate levels with neonatal morbidity and death within 48 hours of birth.
80 Newborn puppy's vitality was evaluated by Apgar scoring adapted for dog. We adopted seven
81 behavioural parameters to assess neonatal conditions at delivery, that is mucous colour, heart and
82 respiratory rate, reflex of irritability, motility, suckling and vocalization. Neonatal measurements
83 (Apgar score, lactate, weight and temperature) were related with characteristics of delivery (uterine
84 contractions analyzed by tocometry, interval time elapsed between the expulsions of two
85 consecutive pups, pup presentation), in view of predicting puppies vitality. We considered also
86 vaginal parturition *versus* elective and emergency caesarean section, and uterotonic drug influence
87 on delivery.
88
89 **2. Materials and methods**
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91 *2.1. Animals*
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93 The present study was conducted with approval of the Ethic Committee of the Faculty of Veterinary
94 Medicine, Università degli Studi di Milano, Italy.

For this study 133 pups from 23 litter of 21 bitches, delivered by vaginal parturition (n=14), elective caesarean section (n=6) and emergency caesarean surgery (n=3), were evaluated. Bitches of different breeds weighted from 5 to 50 Kg and were 4 to 8 year old.

Two out of 21 bitches (9.5 %) belonged to small size breeds (< 10 kg body weight), 8 out of 21 (38.1 %) to medium size breeds (10 to 34 kg body weight) and 11 out of 21 bitches (52.4 %) to large-giant size breeds (>34 kg body weight). 13 bitches were *primipara*.

### *2.2. Uterine tocometry*

From the last week of pregnancy until delivery bitches were monitored by tocometry to assess uterine activity and by ecodoppler to evaluate fetal heart rate, using the Whelpwise™ Veterinary Perinatal Specialties® method. This system consists of a tocodynamometer (uterine sensor), a recorder, a modem, and a hand-held ecodoppler unit. The uterine sensor was placed and kept pressed under the lightly clipped caudo-lateral abdomen area, with the bitch in lateral recumbency. During uterine monitoring bitches were kept quiet, avoiding any movements throughout 20 to 30 minutes each session. Uterine records were performed at 12 hour interval at the beginning, and then more frequently as the parturition approached. We assumed a width of 2 to 8 mm Hg and frequency of 0 to 3 contractions per hour, each ones of 2 to 5 minutes duration, as indicators of pre-labour. Labour was considered when uterine contractions were 10 mm Hg of width or more and frequency ranged from 4 to 12 contractions per hour, each ones of 2 to 5 minutes duration. The staff of Whelpwise™ Veterinary Perinatal Specialties®, available 24 hours a day, received and interpreted uterine contractile records and subsequently communicated such findings to the Authors.

Obstetrical assistance, under tocometric monitoring, consisted of 10% calcium gluconate sc injection at 0.2 mL/kg dose (Calcio pH, Fatro, Ozzano dell'Emilia, BO, Italy), given 15 minutes before oxytocin (Izossitocina, IZO spa, Brescia, Italia) at 0.2-0.5 IU sc micro-doses, in case of

uterine inertia, or at 1-2 IU im standard doses, in case of fetus engaged in the birth canal, associated with fetal gentle traction if needed.

### *2.3. Surgical procedures*

In bitches subjected to caesarean section (C-section) anesthesia was inducted by 1% propofol iv injection at 6.5 mg/kg dose (Propovet, Esteve spa, Milano, Italy) and, after tracheal intubation, maintained with isoflurane at 1.5-2 % (Isoba, Schering-Plough Corporation, Kenilworth, NJ, USA) in oxygen. Surgical approach was carried out on ventral midline to expose the uterine body and horns and puppies were extracted as rapidly as possible. Uterine incision was sutured by absorbable 2-0 USP Monocryl (Ethicon Inc, Johnson & Johnson Company, Plaza New Brunswick New Jersey) on a taper point needle in a single continuous oblique seroserous layer. Abdomen was closed routinely using a 1 or 2 USP simple interrupted Vicryl (Ethicon inc, Johnson & Johnson company, Plaza New Brunswick New Jersey) suture then an subcutaneous continuous pattern with 0 or 1 USP Assucrom (Assut Europe spa, Magliano dei Marsi, AQ, Italy) suture. Skin suture was performed by using 3-0 Daclon (SMI, Hünningen, Belgium) suture. Post-surgical pain was controlled by im injection of 4 mg/kg tramadol chlorhydrate (Altadol, Formevet spa, Milano, Italy).

### *2.4. Lactate measurement*

To analyze newborn venous lactate concentration by Accutrend Lactate® (Roche, Germany), pup umbilical cord was doubly clamped at the time of delivery to isolate blood vessels from the placenta and blood samples of 25 µL were collected as soon as possible and never later than 5 minutes after birth, as described by Huch [12]. The Accutrend Lactate® technology is based on enzymatic

144 determination and reflectance photometry (wave length 660 nm) of lactate in sample of whole blood
145 [13]. The measuring range is from 0.8 mmol/L to 22 mmol/L.
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147 *2.5. Apgar score*
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149 An Apgar scoring scheme, derived from the one proposed for the horse [14] was adapted to puppy
150 patterns according to the canine neonatal physiology and was used to objectively classify newborn
151 healthy and vitality immediately after birth. The parameters evaluated in the Apgar score included
152 mucous membrane color, heart and respiratory rate, reflex irritability, motility, suckling and
153 vocalization, as described in Table 1.
154 Each parameter was assigned a value 0, 1 or 2. The resulting Apgar score, summing up each value,
155 ranges from 0 to 14. The test was done within 10 minutes and repeated 24 hours after birth. Pups
156 totalizing 0 to 4 Apgar score were considered as severe hypo-vital, 5 to 9 score as moderate hypo-
157 vital and 10 to 14 as healthy.
158 Newborn heart rate was recorded by fetal ecodoppler (Whelpwise™ Veterinary Perinatal
159 Specialties®). All others neonatal parameters were clinically evaluated. Birth body weight and
160 rectal temperature were measured within 15 minutes after delivery.
161 After the required procedures to assess neonatal vitality were performed, pups were entrusted to
162 maternal cares. In case of critical conditions, emergency nursing such as removal of fluid from
163 airways and stomach, oxygen therapy (after caesarean parturition), vigorous massage, warming and
164 stimulation of *Ren Zhong* acupoint (GV 26), were provided to neonatal patients.
165 Statistics
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167 *2.6. Statistical analysis*
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Records were enclosed in a data base and statistically analyzed using SPSS statistics software package [15] for a descriptive and inferential evaluation. Frequency and proportion of categorical variables as well as median, mean and standard deviation of scale variables and Apgar scores were calculated. Pearson Chi-Square was applied to analyze the differences in Apgar scores (categorized as low when 0 to 4, medium when 5 to 9 and high when 10 to 14) in relation to the type of whelping, pup presentation, number of pups delivered for litter and dead pups within 48 hours of birth. Pearson Chi-Square was also used to analyze the relation between number of pups for litter and dead pups within 48 hours. The U Mann-Withney non parametric test was used to assess UL concentration in relation to pup mortality and pup delivering time respect to pharmacological assistance at parturition and theirs mortality after 48 hours. The Spearman's correlation coefficient was used to measure the association between umbilical lactate concentration (UL) values and elapsing time between two consecutive pup expulsions, between UL and heart and respiratory rate frequency, UL and body temperature at birth and between pup delivering time and body temperature. Non parametric analysis of variance (ANOVA) was used to test the effect of UL on Apgar score, on number of pups for litter, on body weight at birth, and on uterotonic drug administration. ANOVA was also applied to evaluate the relation between Apgar score of pups at birth and delivering time and between Apgar score and body weight at birth. $P<0.05$ value was considered statistically significant, $P<0.001$ highly significant.

## 3. Results

A total of 23 parturitions from 17 bitches were considered (6 bitches delivered twice), delivering in *toto* 133 pups.

Out of a total of 133 pups, 95 were born by vaginal parturition, 23 by elective caesarean section and 15 by emergency surgery.

For statistical purposes, only 94 puppies resulting from 17 parturitions were analyzed. Number of pups delivered in relation to the type of parturition is shown in Table 2.

The records from the remaining pups were excluded from statistical analysis due to incomplete observations of neonatal vitality or uterine activity, or unfeasibility to collect umbilical cord blood samples. Blood collection from neonatal umbilical cord was not possible in case of placenta and fetal membranes rupture during delivery or because umbilical bleeding. These incomplete records, represented by 6 litters, were used to standardize and acquire practice.

Umbilical cord blood samples were successfully collected in 70 out of 94 pups.

The measurement values of UL in pups ranged from 1.9 mmol/L to over 22 mmol/L (upper detection limit of Accutrend Lactate®, Roche). UL levels of newborn pups were not statistically comparable with the type of parturition. Nevertheless pups delivered by emergency C-section showed the highest mean UL values (8.3 ± 5.9 mmol/L) and pups born by elective C-section the lowest one (3.75 ± 1.2 mmol/L).

**Commentato [u1]:** che cosa vuol dire?

**Commentato [u2]:** nella tab. 3 c'è scritto 3.5

For a quick and easy clinical management of neonates, depending on newborn vitality on the basis of the Apgar scoring, pups were further subdivided as follows: healthy pups those totalizing 10 to 14 Apgar score (high); moderate hypo-vital, those pups with 5 to 9 Apgar score (medium) and severe hypo-vital the pups with 0 to 4 Apgar score (low). On account of this classification 45.3% of newborn pups showed low Apgar score at birth, 33.7% had medium Apgar score and 21% appeared healthy. Apgar scoring of pups at birth in relation to type of whelping is described in Table 3. ~~Low~~ Apgar scores were significantly associated to the type of whelping ~~and emergency C-section showed~~ (P<0.001). Actually all pups born by emergency surgery were severe hypo-vital.

UL concentrations were related to Apgar scoring ~~with~~ (P<0.05). High lactate values were associated with low Apgar scores. The mean umbilical blood lactate concentration in relation to Apgar score in pups at birth is described in Table 4.

The onset of labour, objectively detected by Whelpwise® tocometric measurement of uterine activity, showed a beginning trend from 2 P.M. to 10 P.M...

Vaginal deliveries were completed in 3.5 to 10 hours from the onset of the second stage of labour, with a mean of 6 hours and 24 minutes and an elapsing time between two consecutive pup births of 111.5 minutes.

We documented 0 to 3 light (2 to 8 mm of Hg) contractions per 30 minutes, each 12 hours, in pre-labour bitches. When frequency increased to 4 or more light contraction, the uterine monitoring was repeated 2 hours later. We recorded 3 to 7 strength (> 10 mm of Hg) contractions per 30 minutes in bitches delivering puppies within 30 minutes and 3 hours. Intra-partum detection of 0 to 3 light contractions per 30 minutes without parturition constituted recommendation for uterine inertia therapy. Bitches showing 3 strong intra-partum contractions not followed by parturition within 3 hours were submitted to emergency C-section as occurred dystocia.

Pups died within 48 hours of birth required a median value of 71 ~~156.6 ± 201.5~~ minutes to be delivered, compared with 43 ~~110.4 ± 176.6~~ minutes of healthy pups. Delivering time and mortality of pups within 48 hours of birth ~~showed~~ were significantly correlated (P<0.05), with prolonged parturition time negatively associated to neonatal survival., ~~although this result have to be considered with caution because of very scattered data.~~ The UL concentration evaluated in newborn pups at birth was significantly correlated with ~~in relation with~~ the time of expulsion ~~showed~~ (P<0.05). The longer was the time elapsing between two consecutive pup births, the higher was the UL value. For an average expulsion time of 1 hour and 45 minutes, the newborn UL concentration was lower than 5 mmol/L. No significant correlation was observed between the expulsion time and Apgar score, nevertheless Apgar scoring tended to decrease with the increasing of the expulsion time.

The 35.4% of vaginal delivered pups showed cranial presentation, while the 24% showed caudal presentation. Remaining data have not been detected. UL values in newborn pups were not

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statistically evaluable in respect to the type of pup presentation. However pups with cranial presentation showed a lower average UL of 6.95 ± 4.4 mmol/L, compared with average UL of 8.27 ± 4.3 mmol/L in pups with caudal presentation. Instead, Apgar score ~~in relation with~~was significantly related to pup presentation ~~displayed (~~P≤0.05): lower Apgar values were actually associated with caudal presentation.

Mean number of pups for each litter was 5.7 ± 3.4. We defined as small sized litter those with a number of 3 or less pups, middle sized litter with 4 to 7 pups and large sized litter with 8 or more pups. Based on this criterion we observed 7 small, 10 middle and 6 large sized litters. We recorded the lowest UL levels in middle sized litters (6.7 ± 3.6 mmol/L) and the highest one in large litters (7.9 ± 5.3 mmol/L), with lowest Apgar score in small sized litters, still without significant differences.

The male to female ratio was 62/70; sexual recognition was unfeasible in one further pup because of a case of fetal monster. 15 out of 133 pups died within 48 hours of birth. This percentage does not include 4 pups euthanized after whelping due to severe congenital disorders (cleft palate, frontal meningocele, onphalocele, *atresia ani*).

14 out of 94 statistical analyzed pups died 48 hours after delivery. 10 pups were born by vaginal delivery, 2 by elective C-section and the remaining 2 pups by emergency surgery. Mean UL value of pups surviving later than 48 hours was 6.55 ± 3.3 mmol/L. Dead pups within 48 hours of birth showed a mean lactate value of 12.2 ± 6.7 mmol/L. The difference in UL concentration between alive and dead pups was significant with $P<0.05$. No statistical differences were detected between alive pups and those dead within 48 hours in relation to Apgar score assigned at birth.

Depending on type of whelping, the mean UL concentration and Apgar score varied in newborn pups alive compared with dead pups within 48 hours of birth, as shown in Table 5. However, this difference between alive and dead pups was not significant.

Immediately after birth pups were clinically evaluated to settle their vitality. Mean heart rate at delivery was 134 ± 44.7 BPM in surviving and 108 ± 79.8 BPM in pups dead within 48 hours of birth. Considering all pups the average heart rate increased from 129 ± 50 BPM at delivery to 210 ± 20.5 BPM after 24 hours of birth. UL concentration ~~and~~ was proven to be significantly related to heart rate of pups at birth ~~showed (~~ P<0.01~~)~~. Statistical analyses indicated a decreasing of UL related to the raise of heart rate in newborn pups. Median ~~R~~respiratory rate ~~average~~ was ~~22.5 ± 14.9~~ ~~bpm~~ 20 in surviving and ~~12.2 ± 15.7 bpm~~7 in pups dead after 48 hours of birth. The average respiratory rate recorded in all pups progressive increased from 21 ± 15 bpm at delivery to 39 ± 12 bpm 48 hours after birth. No statistical correlations have been observed between respiratory rate and UL of pups at birth.

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Both heart and respiratory rate are parameters of Apgar score therefore value of this scoring follows the same tendency and increased 24h after birth.

Mean rectal temperature in pups immediately after birth was 37.4 ± 1.6 °C. We recorded a decrease of body temperature at 36.3 ± 1.3 °C level 24 hours after birth, then it rose again at 37.1 ± 0.5 °C the day after. UL values were correlate with body temperature of pups at delivery with P<0.001. Pups with higher rectal temperature at birth showed higher UL concentration. Instead temperature values detected 24 and 48 hours after parturition did not present any statistical relation with UL concentrations at birth. Apgar scoring at birth was not associated to body temperature of pups at delivery or 24 and 48 hours later.

The birth weights of pups at birth ranged from 155 to 572 g. Pups surviving later than 48 hours showed a mean weight of 421.7 ± 92.8 g, while dead pups weighed 340.8 ± 77.2 g. Body weight at birth was not statistically related to UL concentration or Apgar score.

The parturition of 12 pups required medical assistance, that is admistration of 10% calcium gluconate with oxytocin, because of uterine inertia. Medical augmentation of labour was performed in bitches characterized by 0 to 3 light contractions per 30 minutes. In these cases ~~mean~~ median

elapsing time between two consecutive pup births was ~~291 ± 257~~304 minutes, that is about 5 hours. Delivery time was related to drug administration with $P<0.05$, showing increase of expulsion time in pups born with medical assistance. Mean UL concentration detected in these pups was 9.93 ± 4.61 mmol/L and Apgar score average at birth was 5.1 ± 3.8. . No statistical evidences were detected about UL concentration and Apgar score in pups delivered after medical therapy in respect to untreated parturitions.

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## 4. Discussion

In the dog, the rate of stillbirth and neonatal mortality, that is between birth and the age of 2 to 3 weeks [16], is highly variable and related to many factors, including the quality of labour, congenital abnormalities and acquired disorders [17]. For the neonate, the first minutes after birth represent the most critical phase. In agreement with bibliographic reports, neonatal mortality rate recorded within 48 hours of birth in our study was 11.3% [17,18]. Among the 14 dead pups whose records were submitted to statistical analysis, 28.6% of them were delivered by caesarean section, both elective and emergency, and showed Apgar score lower than 3.

UL concentration proved to be useful to predict canine neonatal mortality within 48 hours of birth and statistically related. We identified in 5 mmol/L of umbilical lactate concentration the cut off value allowing to distinguish between healthy and distressed pups. Higher values of lactate were related with pups suffering, whereas lower values characterized vigorous pups.

Severe low vitality and poor Apgar scores at birth were observed in 100% of pups born from emergency C-section ($P<0.001$), in 92% born from elective C-section and in 30.3% delivered by vaginal parturition. However Apgar score at delivery was not related to pup survival within 48 hours of birth and 21.3% of pups with Apgar score lower than 2 developed satisfactory healthy conditions after parturition.

317 As regard to lactate concentration in relation to type of parturition, our results agree with the
318 findings of Borruto et al. in the human species [19]. As in the case of babies, the lowest values of
319 lactate were detected in healthy pups born from elective caesarean section, that is performed before
320 the labour onset, compared with lactate concentration in case of emergency caesarean surgery,
321 carried out during labour and the highest lactate levels observed after vaginal delivery [19]. The
322 most critical events in babies and pups seem to occur during emergency C-section. In this condition
323 fetal distress can be consequence of dystocia or hypoxic-ischemic effect of the uterine contractions
324 on placental vessels. In this regard, there is physiological evidence for fetal acidosis at birth induced
325 by uterine activity. Fetal acidosis at birth is mainly due to hyper-lactemia, induced by hypo-
326 perfusion of peripheral tissues and associated with increased uterine activity during the first and
327 second stage of labour [20]. When contractions occur in women and they exceed 30 mm Hg, the
328 maternal spiral arteries are compressed and placental perfusion is strangulated. Elevated uterine
329 activity during the first and second stage of labour increases the risk of adverse fetal outcome [20].
330 Indeed after oxytocin and calcium administration we detected high values of UL and low Apgar
331 scores in newborn pups and elapsing time between two consecutive pup births was significantly
332 longer in parturition requiring obstetrical assistance. Oxytocin induces a prolonged, often
333 permanent, depolarization of cellular membranes which increases duration and frequency of action
334 potential and decreases width and speed of spread [21]. Calcium administration increase strength of
335 myometrial activity [22]. Arbitrarily giving uterotonic drugs can be catastrophic if labour is either
336 not present, or has not gone long enough to make the cervix soft enough to facilitate delivery [1].
337 Pharmacological assistance during labour should be performed under cardiotocographic support to avoid not
338 necessary therapy administration and subsequent possibility of fetal distress.
339 Early recognizing of fetal suffering and dystocia are crucial to the successful management of pregnancy and
340 optimal neonatal health. Monitor of uterine activity allows to know the real onset of parturition and the stay

of the fetus in the birth canal. External uterine monitoring supplies an objective tool to identify labour, to predict parturition and to manage uterine problems verifying as well therapy efficacy [1].

The concept of fetal suffering, previously inappropriately referred to a generic distress, has been revised on the basis of clinical deterioration of cardiotocographic layout [7]. Cardiotocography has revolutionized the method of fetal monitoring in human, allowing a continuous record of fetal heart rate and its variation compared to uterine contractile activity during parturition and at delivery. This technology has been recently applied and settled for veterinary practice furnishing an advanced system to evaluate fetal vitality and to reduce the number of unnecessary obstetric interventions.

Total length of parturition and the time of pup expulsion are commonly considered the most important parameters involved in neonatal vitality. It has been shown in dogs that neonatal mortality is directly correlated to duration of labour [23]. One study found that if delivery was complete within 1 to 4.5 hours of the onset of second stage labour, puppy mortality was 5.8%; whereas, neonatal mortality increased to 13.7% if this stage prolonged more than 5 to 24 hours. The outcome for the bitch and the puppies is favorable when second stage of labour is lower than 12 hours [23,24]. We identified the onset of labour, by means of the tocometer Whelpwise® system, when uterine contractions were 10 mm of Hg of width or more and frequency ranged from 4 to 12 contractions per hour, each ones of 2 to 5 minutes duration. We observed the highest UL values in pups spending long time in parturition. Pups died within 48 hours of birth showed 46.2 ± 24.9 minutes of parturition longer in duration when compared with healthy pups. Moreover pups born after a prolonged vaginal whelping displayed statistically increased UL values and Apgar evaluation tended to lower scores at birth. Newborn pups with UL concentration lower than 5 mmol/L were, on average, born 1 hour and 45 minutes after the previous pup.

In women during the second stage of labour, it has been demonstrate a significant raise of maternal lactate due to skeletal muscles activity [25], and a statistical relation between the increase of fetal lactate and duration of this stage [26]. Large litters may present a high risk of uterine intertia, due to

overstretching of the uterus preventing uterine contractions [18]. We observed the highest UL values in litters of eight or more pups. Body weight at birth was not related to pup vitality, UL concentration, Apgar score or surviving time.

Apgar score and UL concentration in pups at birth showed a significant correlation, useful to evaluate neonatal vitality. High UL values were associated with low Apgar scores and were indicative of fetal suffering. Among parameters of Apgar score, heart rate frequency of pups at delivery demonstrated to be the most important factor associated to vitality and UL values with a significant relation.

Before lactate analysis was introduced as routinely practice in obstetric, acid-base balance was used as index to predict baby vitality. Actually, a great number of newborns having an acid–base balance considered unfavorable do not present complications and, furthermore, the same number of newborns with a low Apgar score do not show alterations; according to the same parameters, the examination is regarded as ambiguous and so it has lost its validity [7]. At present, the standard assessment of the fetal condition during labour and delivery in neonatal human medicine is by means of lactate measurement together with clinical observation and cardiotocography [7].

Many studies of lactate in babies have been performed from fetal scalp, umbilical arterial or venous blood samples. The most contribution to fetal lactate concentration during second stage of labour is from the fetus himself, mainly in case of prolonged labour or acidemia at birth [26]. Only a little quota of lactate cross the placenta, so lactate acid in fetal blood during labour is thought to be primarily of fetal origin, rather than maternal, and is the end product of anaerobic glycolysis [26-28]. It has been reported that umbilical cord venous lactate concentrations are not usually higher than umbilical cord arterial lactate concentrations [27]. For blood lactate measurement in newborn pups we used the umbilical venous sampling because of the large diameter making easier its identification and collection, compared to arteries, mainly in small sized pups. Hand-held analyzer Accutrend Lactate® allowed rapid lactate detection in small blood volumes (25 µL), particularly

391 adapt to canine neonatology demands. Umbilical cord blood samples have been successfully
392 collected in 70 out of 94 pups, independently of pup size. We met difficulties in blood collection in
393 case of placenta and fetal membranes rupture during vaginal delivery or because umbilical bleeding,
394 often occurring during C-section. To avoid this loss of data lactate measurement should be carry out
395 directly from pup mucous prick.
396 In conclusion, on the basis of results obtained in this study, UL demonstrated to be predictive of
397 pup mortality within 48 hours of birth, and related to the more traditional and simple but less
398 objective method of Apgar score, to evaluate newborn pup's vitality. Moreover lactate evaluation in
399 newborn pups, at birth and eventually after neonatal critical care, could provide a valid parameter to
400 verify the effectiveness of resuscitation therapy.
401 UL concentrations lower than 5 mmol/L with Apgar score higher than 9, associated to delivering
402 time of less than 1 hour and 45 minutes and effective uterine contractions (width 10 mm of Hg or
403 more, frequency 4 to 12 for hour, 2 to 5 minutes duration), should be considered favorable
404 prognostic factors of pup wellbeing. Once this procedure has been standardized and assimilate,
405 lactate measurement can be easily performed in newborn pups from umbilical vein.
406 Lactate detection with Apgar score and perinatal uterine tocometer assessment can reduce the
407 number of unnecessary obstetric and pharmacological interventions, allowing early recognition of
408 pup distress and improving management of neonatal care.