



# Biochemical composition of fetal fluids in at term, normal developed, healthy, viable dogs and preliminary data from pathologic littermates

M.C. Veronesi <sup>a</sup>, B. Bolis <sup>a,\*</sup>, M. Faustini <sup>a</sup>, A. Rota <sup>b</sup>, A. Mollo <sup>c</sup>

<sup>a</sup> Department of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy

<sup>b</sup> Ambulatorio Associato Pellegrini e Rota, Almenno San Bartolomeo, Bergamo, Italy

<sup>c</sup> Department of Animal Medicine, Productions and Health, Università di Padova, Padua, Italy

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## ABSTRACT

A proper canine neonatal assistance, required to reduce the high perinatal loss rate, imply a full knowledge about the fetal-to-neonatal physiology. Because fetal fluids play an important role throughout mammals pregnancy, influencing fetal growth and development, fetal well being, and contributing to guarantee the most suitable environment for the fetus, the knowledge about fetal fluids biochemical composition is of major importance. At first, the biochemical composition of fetal fluids collected by normal developed, healthy and viable newborns, is necessary to depict the normal features, and represent the first step for the further detection of abnormalities associated to fetal/neonatal distress and useful for the early identification of newborns needing special attention, immediately after birth. The present study was aimed to define the biochemical composition of amniotic and allantoic fluids collected from fetus delivered by caesarean section at term of pregnancy. To reduce the possible confounding effect of maternal labor or troubles at parturition, fetal fluids were collected only from puppies born by elective caesarean section, at term of normal pregnancies. Fetal fluids from 76 puppies, 70 normal and six pathologic newborns, born by elective caesarean section were collected and analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, lactate dehydrogenase (LDH), creatine-kinase (CK), alkaline phosphatase (ALP), creatinine, urea, amylase, lipase, gamma-glutamyl transferase ( $\gamma$ -GT), triglycerides, cholesterol, total proteins, albumin, globulins, glucose, magnesium, potassium, chloride, sodium, calcium, phosphorus and osmolarity. No significant differences were found between biochemical composition of amniotic or allantoic fluid in normal and pathologic newborns, maybe due to the small number of the pathologic puppies. Although some correlations between the two fluids were found (albumin, phosphorus, glucose and triglycerides), the results showed significant differences between the amniotic and allantoic biochemical composition (for all the parameters, except of alanine aminotransferase, triglycerides, cholesterol, albumin, amylase and glucose), suggesting that diverse sources could concur to the final composition of each fluid. A wide variability within and among litters was found for both amniotic and allantoic biochemical composition, and for some parameters an influence of breed body size (amniotic amylase, cholesterol, and allantoic calcium and glucose), maternal parity (amniotic and allantoic CK, glucose, LDH, chloride) and newborn gender (allantoic phosphorus) was found. Further investigations are needed for addressing the origin of each fetal fluid biochemical composition in the dog and also to indeep possible differences in fetal fluids biochemical composition between normal and pathologic puppies, providing potential markers for the quick identification of newborns that need special surveillance and cares immediately after birth.

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## 1. Introduction

In the last years canine perinatology received an increasing interest, mainly aimed to a better management of newborn puppies and to reduce the perinatal loss rate, currently too high. In the dog species, that give birth to multiple neonates, the process of

\* Corresponding author. Department of Veterinary Medicine, Università degli Studi di Milano, Via Celoria, 10, 20133, Milan, Italy.

E-mail address: [barbara.bolis@unimi.it](mailto:barbara.bolis@unimi.it) (B. Bolis).

parturition can last several hours, affecting the newborns survival rate [1]. This is in contrast to the need of the as high as possible puppies survival rate, due to the economic value of breeding dogs, but also to the emotional impact of the birth process in pet dogs. In this species, the perinatal mortality refers to the sum of fresh born dead puppies plus puppies dying during the early neonatal period, considered as the first week of life [2]. The canine perinatal mortality rate is reported to be very variable in the different studies, ranging between 8% and 26% [2–4], with a considerable impact for the breeders.

Canine perinatal mortality could be limited by a correct management of whelping coupled to a proper neonatal assistance [5]. The proper canine neonatal assistance requires, in turn, the full knowledge about fetal-to-neonatal physiology to allow, on one hand, the correct management of the normal newborns, but also for the soon recognition of those puppies needing special surveillance or cares after birth. Fetal fluids, in example, recognized to play an important role throughout mammals pregnancy, influencing fetal growth and development, fetal well being, and contributing to guarantee the most suitable environment for the fetus, are scantily studied in dogs.

In humans, the origin and composition of amniotic fluid are fully known, and markers related to some maternal, fetal or placental diseases were identified, with relevant clinical impact.

Similarly to humans and sheep, also in dogs, it may be assumed that the amniotic fluid is composed by several secretions from the urinary system, the respiratory tract, the gastrointestinal system and the skin before keratinization [6,7], while the allantoic fluid, less investigated in humans, is supposed to be mainly provided by the fetal urines, due to the final urethral canalization in the fetus.

To the authors knowledge, despite the wide scientific literature about human amniotic fluid composition, only few studies focused on the canine fetal fluid composition, although, fetal fluids can be collectible at the time of caesarean section, as previously reported by Ref. [8], without interfering with neonatal assistance. The availability of normal data about the biochemical fetal fluids composition will not only add some lacking information about dogs perinatology, but will also represent the first step for a further detection of differences between normal and pathologic puppies, providing potential markers for the quick identification of newborns at risk, that need special surveillance and cares, immediately after birth.

For all the above mentioned reasons, the aim of the present study was to report the biochemical composition of fetal fluids collected from at term, normal developed, healthy, viable puppies and the preliminary data from pathologic littermates. To reduce the possible confounding effect of maternal labor or troubles at parturition, fetal fluids were collected only from fetuses delivered by elective caesarean section, at term of normal pregnancies. The possible effect played by some maternal and fetal parameters on the normal biochemical fetal fluids composition was also assessed.

## 2. Materials and methods

### 2.1. Clinical data

The study was performed on 24 purebred bitches, belonging to several breeds. According to breed body weight, the bitches were classified, according to Federation Cynologique Internationale, as belonging to small sized breeds (body weight < 10 kg, n = 13), or large breeds (body weight > 20 kg, n = 11). No bitches belonging to medium sized (body weight 10–20 kg) breeds were enrolled.

All the bitches, were regularly vaccinated and dewormed, and healthy from the time of mating, throughout pregnancy, until parturition.

All the enrolled bitches belonged to breeds at high risk of dystocia or had previous history of troubles at whelping. Therefore, in all cases, an elective caesarean section (CS) at term of pregnancy was planned, to ensure the health of mothers and puppies.

The date of elective CS was estimated based upon the measurement of progesterone plasma concentration at mating, coupled to the prediction of parturition date by the ultrasonographic measurement of the inner chorionic cavity and biparietal diameter of the fetal skull, as reported by Meloni et al. [8]. However, CS was performed only on the base of progesterone plasma concentrations  $\leq 2$  ng/ml [8].

The same anesthesia and surgical protocol was performed in all bitches, and mainly aimed to minimize the negative impact on puppy newborns, as reported by Meloni et al. [8]. Briefly, after premedication with metoclopramide, ceftazolin and oxygen mask, induction was obtained by propofol infusion and lidocaine used for infiltration on the site of surgical incision, followed by anesthesia maintenance using isoflurane in oxygen. The caesarean section was performed by ventral midline laparotomy and, only after all the fetuses were removed, tramadol and oxytocin were injected to the dam [8].

Newborn viability was assessed within 5 min after birth as reported by Veronesi et al. [1], and three levels of newborn distress identified: no distress for Apgar score between 10 and 7, moderate distress for score between 6 and 4, and severe distress for score between 3 and 0.

Each puppy was additionally evaluated for gender, absence of gross physical defects and weighed before nursing. Puppies born alive and classified as viable (absence of neonatal distress as evidenced by an Apgar score  $\geq 7$ ), normal weighed and without malformations were considered as “normal”, while puppies born dead, less viable, underweighted or malformed were considered as “pathologic”. Birth body weight was compared to the reference range reported for each breed by the Italian Kennel Club (ENCI).

Newborns survival was checked at 24 h of age and at 7 days after birth.

### 2.2. Fetal fluids collection

The study was performed during the year 2015, when the national regulation did not require an institutional approval for the use of waste biological materials, obtained during routine clinical management of patients, such as bitches requiring elective CS.

Before surgery the owners signed an informed consent, to specifically allow the collection and use of fetal fluids for research purposes.

In order to avoid every possible interference of fetal fluids collection on the neonatal first cares and reanimation procedure, fetal fluids collection and puppies cares and reanimation were always performed by two different veterinarians.

For each puppy, the amniotic and the allantoic fluids were separately collected as previously reported [8], and without any disturbance for the newborns. In some cases, the allantoic fluid was lost during the fetal bags opening procedures. Immediately after collection, fetal fluids were centrifuged at room temperature at  $1000 \times g$  for 10 min, and the separated supernatant frozen at  $-20^\circ\text{C}$  until analysis, performed within 3 months from fluids collection.

### 2.3. Biochemical analysis

Samples were thawed and centrifuged at  $1750 \times g$  for 10 min at room temperature, and the supernatant was immediately analyzed on BT1500 (Biotechnica, Rome, Italy) automated chemistry analyzer. On board were used Gesan's reagents (Italy) for all biochemical

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