



## The use of semi-quantitative tests at Cesarean section delivery for the differentiation of canine fetal fluids from maternal urine on the basis of biochemical characteristics

Orsolya Balogh\*, Marie Roch, Stefanie Keller, Erika Michel, Iris M. Reichler

*Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland*

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### ABSTRACT

In dogs, there is no diagnostic test to identify and differentiate fetal fluids from maternal urine in the event that a clear–yellowish vulvar discharge is observed pre-whelping. The objective of this study was to find a test that could easily and accurately identify rupture of the fetal membranes preceding parturition. Maternal urine, and amniotic fluid (AMF) and allantoic fluid (ALF) from only one fetus per bitch, were collected intraoperatively during Cesarean section. Specific gravity (SG) was analyzed with a refractometer, whereas the presence of leukocytes, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, erythrocyte/hemoglobin (Hb), and the pH were assessed using a urine dipstick (Combur-Test®). Combined calcium and magnesium (Ca/Mg) content were evaluated with the Total Hardness Test. The AmniSure test, which detects rupture of fetal membranes in women on the basis of the presence of human placental alpha microglobulin-1, was also performed on canine AMF, ALF, and urine. Data were analyzed using the Fisher's exact test, Wilcoxon signed-rank test, and Pearson's correlation. Sensitivity, specificity, and positive and negative likelihood ratios (LR) were calculated for parameters with significant difference between urine and both fetal fluids. Maternal urine had higher SG and lower leukocyte, protein, Hb, and Ca/Mg content than AMF and ALF. Glucose was more often present in AMF ( $n = 17$ ) and ALF ( $n = 12$ ) than in urine ( $n = 1$ ), whereas ketone bodies were rarely detected in ALF compared with urine. Bilirubin content was higher in urine and ALF than in AMF. AMF pH was less variable and higher than the pH of ALF or urine. The AmniSure was negative in all samples tested. Sensitivity and specificity for SG and for the detection of leukocytes, protein, glucose, Hb, Ca/Mg, and glucose without ketones in urine and fetal fluids were between 42% to 100% and 65% to 100%, respectively. Best positive LR was achieved for the detection of glucose without ketones and best negative LR for SG of 1.022 or less. In conclusion, the AmniSure test, which is used in humans with high diagnostic accuracy, cannot identify AMF and ALF in dogs. On the basis of our results in 26 dogs undergoing Cesarean section, the presence or absence of fetal fluids could be best determined by a positive glucose test without ketone bodies or by SG higher than 1.022, respectively. These tests may serve as additional tools to

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\* Corresponding author. Tel.: +41 44 63 58 267.

E-mail address: [obalogh@vetclinics.uzh.ch](mailto:obalogh@vetclinics.uzh.ch) (O. Balogh).

recognize parturition if clear–yellowish vulvar discharge is present in a term pregnant bitch, but their accuracy and practicability in the clinical setting need to be confirmed.

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## 1. Introduction

When a pregnant bitch is presented for leakage of variable amounts of watery, clear or yellowish fluid from the vulva, there is no quick diagnostic test to help determine whether it is composed of fetal allantoic fluid (ALF) and/or amniotic fluid (AMF) or it is maternal urine. Urination may occur more frequently in bitches near the end of gestation because of the increased size of the uterus pressing on the bladder and may sometimes be mistaken with leakage of fetal fluids [1]. Rupture of the allantochorionic membrane (outer fetal membrane) as the fetus enters the birth canal and leakage of the transparent, yellowish ALF is normal during the second stage of labor [1,2]. However, when passage of ALF/AMF occurs before the birth of the first pup without other signs of second stage labor (i.e., visible abdominal contractions), it is a sign of dystocia. This may happen in cases of primary uterine inertia, i.e., when uterine contractions are completely lacking or too weak for fetal expulsion [1]. In the bitch, uterine inertia accounted for 14% to 49% of dystocia cases [3,4] or even up to 60% in predisposed breeds like the Boxer [5]. Fast recognition and appropriate therapy are required to avoid neonatal loss. To date, there is no tool for diagnosing rupture of the fetal membranes in dogs, whereas a rapid test exists for women. The AmniSure test (AmniSure GmbH, Wetzlar, Germany) is based on the detection of placental alpha microglobulin-1 (PAMG-1), as this protein is present in higher concentrations in AMF compared with other biological fluids [6,7] and is used as a biomarker [8,9]. Even small amounts of AMF can accurately be detected in vaginal fluids with the AmniSure test, and it appears that artifacts from blood contamination do not significantly interfere with the test's performance [10]. No AMF or ALF-specific proteins have been identified for dogs, and the AmniSure test has not been evaluated on canine fetal fluids.

AMF and ALF are produced by various mechanisms, i.e., secretions from the fetus, transudation through the fetal skin before keratinization, or placental, transmembranous, and intramembranous pathways [11–18]. The biochemical characteristics and pregnancy stage-related changes of fetal fluids were already described in women and in several domestic animal species. In cats, detailed information on fetal fluid composition (e.g., electrolytes, proteins, glucose, enzymes of the liver, kidney, and pancreas) and its time-dependent evolution was compared with maternal blood but not to maternal urine [19]. In that study, several parameters changed throughout gestation corresponding to the actual degree of fetal development; moreover, changes in fetal fluid composition diverged from those in maternal serum, which underscores the active contribution of the fetus to AMF and ALF production. In dogs, such detailed analysis of fetal fluid composition is missing. Aralla et al. [20] found decreased sodium levels in AMF in late-compared with mid-pregnant bitches, whereas AMF osmolality, urea, total protein, and potassium content were

similar between the two time points. An immunoprotective role of AMF and ALF was hypothesized after finding lysozyme and immunoglobulin G in canine fetal fluids [21]. Another recent study described the presence of insulin-like growth factor 1 (IGF1) and nonesterified fatty acids (NEFA) in the fetal fluids of term pregnant bitches, and found that both NEFA and IGF1 concentrations were dependent on the size of the dam [22]. In contrast, AMF glucose, lactate, and cortisol levels were not related to breed size, but differences in the values of these parameters were found between puppies of bitches undergoing vaginal parturition or Cesarean section (C-section), and also varied in cases of stillbirth [23]. We hypothesize that the composition of fetal fluids in dogs differs not only from maternal serum but also from urine. The objective of our study was to distinguish fetal AMF and ALF from maternal urine on the basis of biochemical characteristics using semi-quantitative tests at the time of C-section delivery.

## 2. Material and methods

### 2.1. Animals

Twenty-six bitches undergoing elective or emergency C-section at the small animal clinic of the Vetsuisse Faculty, University of Zurich, were included in the study. Informed consent was obtained from the owners, and the study was approved by the Cantonal Veterinary Authority of Zurich (permit no. 04/2013).

Elective C-sections were carried out in term pregnant dogs with high risk for dystocia. Determination of term gestation was on the basis of a combination of several diagnostic methods. Ovulation was determined by serum progesterone (P4) levels during estrus ( $P4 \geq 5$  ng/mL at ovulation [24]). Additionally, and especially if the date of ovulation was unknown, the prepartum P4 profile was followed to estimate the expected time of parturition [25]. Ultrasonography was carried out in all cases to determine gestational age by measuring fetal biparietal diameter, to visualize intestinal peristalsis as an indicator of fetal maturity, and to evaluate fetal well-being (heart rate, movements) [26]. C-section was performed only when the bitch was near term (at least 61 days from ovulation or <2 days before the expected time of parturition) on the basis of the combined results of the methods earlier.

### 2.2. Collection and testing of fetal fluids and maternal urine

During C-section, ALF and AMF (1–5 mL) were collected separately, from the fetus nearest to the site of hysterotomy. The non-villous allantochorionic membrane was visualized, and a small hole was made on it to allow placing a sterile syringe tip into the cavity. The ALF was then aspirated under visual control. Afterwards, the allantochorionic membrane was torn, the fetus in the intact amniotic membrane was visualized, and the AMF was aspirated.

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