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Evaluation of feline uterine and umbilical arteries blood flow in a pharmacologically induced abnormal gestation model

P.G. Blanco^{a,b,c,*}, R. Vercellini^{c,d}, A. Rube^e, R. Rodríguez^d, D.O. Arias^a,
C. Gobello^{b,c}

^a Cardiology Service, Faculty of Veterinary Sciences, National University of La Plata (FVS-NULP), La Plata, Argentina

^b Laboratory of Reproductive Physiology, FVS-NULP, La Plata, Argentina

^c National Research Council (CONICET), La Plata, Argentina

^d Radiology Service, FVS-NULP, La Plata, Argentina

^e Ultrasonography Service, FVS-NULP, La Plata, Argentina

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ABSTRACT

The aim of this study was to describe resistance index (RI) and systolic/diastolic ratio (S/D ratio) of uterine and umbilical arteries in an experimental model of abnormal pregnancy in felids. On days 30 to 35 (32 ± 2.9) after mating, 20 domestic short-hair pregnant queens were randomly assigned to one of the following treatment groups: a treated group (TG; $n = 8$), which received 10 mg/kg of aglepristone subcutaneously twice, 24 hours apart, and a control nontreated group (CG; $n = 12$). M-mode and Doppler ultrasonographic evaluations were performed at the initiation of the treatment (Day 0) and then every other day during 8 days. In both groups, uterine and umbilical arteries were evaluated by Doppler ultrasound, whereas fetal heart rate was assessed by M-mode ultrasound. Resistance index of uterine artery augmented in TG from Day 2 onward, conversely it decreased in CG ($P < 0.01$). On Day 8, RI values were 0.64 ± 0.05 vs 0.37 ± 0.01 for TG and CG, respectively. Additionally, S/D ratio of the same artery presented an increase in TG, whereas this ratio diminished in CG ($P < 0.01$). On Day 8, this parameter showed values of 2.98 ± 0.4 vs 1.62 ± 0.06 for TG and CG, respectively. Resistance index of umbilical artery remained almost unchanged in TG from Day 6 onward, whereas it progressively decreased in CG throughout the course of the study ($P < 0.05$). On Day 8, RI were 0.89 ± 0.04 and 0.82 ± 0.01 , for TG and CG, respectively. Furthermore, on Day 8, S/D ratio of umbilical artery progressively diminished in CG but not in TG ($P < 0.01$), being 14.7 ± 9.1 vs 5.9 ± 0.3 for TG and CG, respectively. Fetal heart rate was higher in TG than in CG ($P < 0.05$). Group differences in Doppler parameters appeared on Day 2, when the other clinical or ultrasonographic signs were still absent. It is concluded that blood flow of the uterine and umbilical arteries differed between these normal and abnormal gestations predicting an adverse obstetric outcome.

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1. Introduction

Doppler ultrasound has been used in the assessment of maternal and fetal health during pregnancy in several

* Corresponding author. Tel.: +54 221 423 6663; fax: +54 221 427 7378.

E-mail address: pgblanco@fcv.unlp.edu.ar (P.G. Blanco).

species [1–3]. This noninvasive technique is useful for the evaluation of blood flow velocity, resistance index (RI), and systolic/diastolic ratio (S/D ratio) of uterine and fetal vessels.

In pregnant women, Doppler has become a routine technique for identifying fetal compromise associated with abnormal uteroplacental or fetal circulation. Increased RI of uterine or umbilical arteries indicates a high risk of fetal or

perinatal death [4,5]. Furthermore, in this species, the increase in placental resistance is generally associated with intrauterine fetal growth retardation and fetal hypoxia [6,7]. Infants with abnormal Doppler indices also have an augmented risk of neonatal complication [8]. Some reports have suggested that abnormal velocity waveforms at the level of the umbilical artery may even precede decelerations of fetal heart rate [9]. Additionally, other report stated that fetal heart rate does not seem to have any advantage over Doppler velocimetry [10].

In dogs, Doppler measurements of uterine and umbilical blood flow throughout pathologic pregnancies have been reported [11]. In that study, it has been shown that uterine artery RI increased in bitches that spontaneously aborted or in those that presented a high percentage of neonatal death. In the same animals, RI of umbilical artery did not diminish at the rate that has been described for normal canine pregnancy. Furthermore, these abnormalities occurred before heart rate decreased, suggesting that Doppler could predict compromised pregnancies and obstetrical diseases.

In cats, RI of uterine and umbilical arteries progressively decreases throughout normal gestation, guaranteeing an appropriate perfusion of the placenta and fetal viscera [12,13]. The use of Doppler ultrasound to assess feline pathologic gestations has not been reported so far. In this species, fetal stress has only been evaluated by ultrasound heart rate measurement [14], and the utility of other ultrasonographic diagnostic methods remains obscure.

As a first approach to describe abnormal feline gestation by Doppler, a pharmacologically induced abortion was caused. For this purpose, aglepristone, a safe selective progesterone receptor antagonist that has shown 88.5% efficacy 33 days after mating was selected [15].

The aim of this study was to describe RI of uterine and umbilical arteries in an experimental model of abnormal feline pregnancy.

2. Materials and methods

2.1. Animals

Twenty domestic short-hair pregnant queens, 1 to 5 (2.5 ± 0.2) years of age, weighing 2.5 to 3.9 (3.1 ± 0.1) kg, born in our institutional cat colony were included in this study. The animals were exposed to a 10-hour dark, 14-hour light photoperiod, and fed with dry commercial premium cat food and water *ad libitum*. This study was reviewed and approved by the Animal Care and Use Committee of the Veterinary School of the National University of La Plata, Argentina, and all experiments were conducted under the guidelines established in The Guide for The Care and Use of Laboratory Animals, USA.

2.2. Experimental design and ultrasonographic evaluations

In all the cases, pregnancy was confirmed on days 18 to 20 after mating, using bidimensional ultrasonographic examinations (Toshiba Nemio XG, Tokyo, Japan) with a 14-MHz linear array transducer [16]. Then, the number of gestational vesicles per queen was ultrasonographically determined [17]. On days 30 to 35 (32 ± 2.9) after mating,

the animals were randomly assigned to one of the following treatment groups: a treated group (TG; $n = 8$), which received 10 mg/kg of aglepristone (Alizin; Virbac, Carros, France) subcutaneously twice, 24 hours apart [18], and a control nontreated group (CG; $n = 12$).

M-mode and Doppler ultrasonographic evaluations were performed at the initiation of the treatment (Day 0) and then every other day during 8 days. The female cats were gently restrained in lateral recumbency without sedation, and the hair of the ventral skin was clipped after 2 minutes of acclimatization. Acoustic gel was applied to the transducer and coupled directly to the skin. Bidimensional ultrasonography was used to identify the uterine body in a transversal axis. Uterine arteries were localized at both sides of the uterine body with color flow mapping, and pulsed-wave Doppler was used to obtain the waveforms [13]. In all the cases, Doppler evaluation of umbilical artery and fetal M-mode ultrasonography were performed in the most caudal fetus of the right uterine horn, according to previous reports [12]. To minimize variability, three uniform consecutive waveforms were recorded by the same trained operator (PGB). Peak systolic velocity (PSV) and end-diastolic velocity (EDV) were measured. Resistance index $[(PSV - EDV)/PSV]$ and S/D ratio (PSV/EDV) were automatically calculated in all the vessels [19]. Fetal heart rate was obtained by M-mode ultrasonography as previously described [13]. In all the queens, ultrasonographic evaluations were performed in periods no longer than 15 minutes.

2.3. Statistical analysis

To verify the comparability of the groups, comparisons with regard to weight, age, parity, and litter size were performed by Student's *t* tests. Resistance index and S/D ratio of the left and right uterine arteries were compared using the same test. Repeated-measures ANOVA followed by Tukey test was carried out to analyze RI and S/D ratio of uterine and umbilical arteries and FHR (SPSS 18.0; SPSS, Chicago, IL, USA). The level of significance was set on 0.05.

3. Results

Both groups of queens resulted similar, considering weight, age, parity, and litter size ($P > 0.1$). No differences were found between left and right uterine arteries ($P > 0.1$); therefore, values of RI and S/D ratio were averaged. At the beginning of the study, RI and S/D ratio of uterine and umbilical arteries and FHR were not different between groups ($P > 0.1$). All the queens in CG delivered healthy kittens at term. Conversely, all the animals in TG interrupted their pregnancy 6.8 ± 4 days after treatment. At the time of abortion, most of the queens presented hemorrhagic vulvar discharge. Ultrasonographic examinations performed at the time point of abortion revealed both live and dead fetuses. Resistance index of uterine artery augmented in TG from Day 2 onward, whereas it decreased in CG ($P < 0.01$; Fig. 1). On Day 8, RI values were 0.64 ± 0.05 vs 0.37 ± 0.01 for TG and CG, respectively. Additionally, S/D ratio of the same artery increased in TG, whereas this ratio diminished in CG ($P < 0.01$; Fig. 2). On Day 8, this parameter

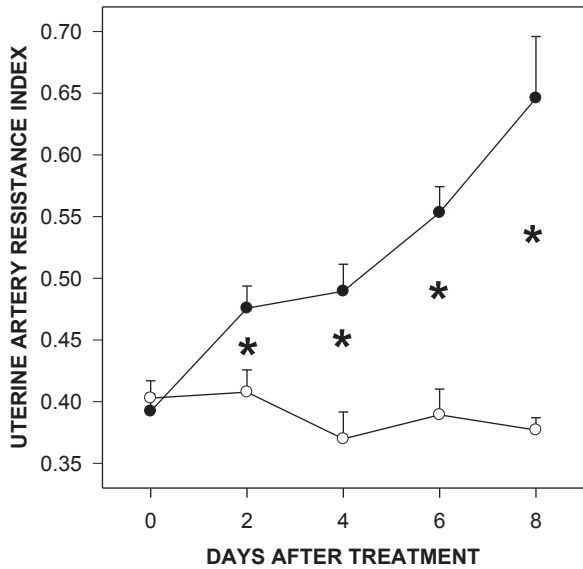


Fig. 1. Resistance index of uterine artery of 20 female cats treated with two aglepristone doses (10 mg/kg; n = 8; solid symbols) or control that did not receive any treatment (n = 12; open symbols) and followed up for 8 days. Asterisks indicate differences (P < 0.01) between groups.

showed values of 2.98 ± 0.4 vs 1.62 ± 0.06 for TG and CG, respectively.

Resistance index of umbilical artery remained almost unchanged in TG from Day 6 onward, whereas it progressively decreased in CG throughout the course of the study (P < 0.05; Fig. 3). On Day 8, RI were 0.89 ± 0.04 and 0.82 ± 0.01 , for TG and CG, respectively. During the same day, S/D ratio of umbilical artery progressively diminished in CG but not in TG (P < 0.01; Fig. 4), being 14.7 ± 9.1 vs

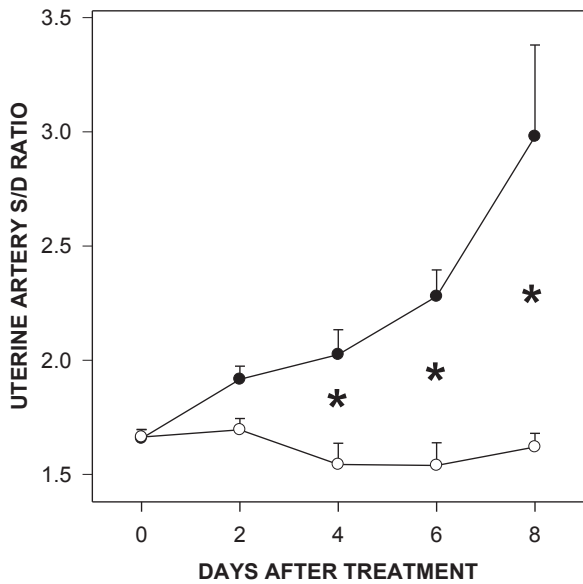


Fig. 2. Systolic/diastolic ratio of uterine artery of the same animals of Figure 1. Asterisks indicate differences (P < 0.01) between groups.

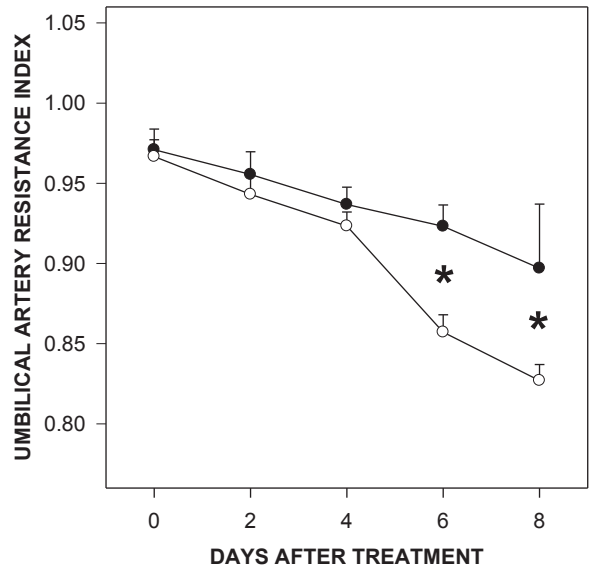


Fig. 3. Resistance index of umbilical artery of the same animals of Figure 1. Asterisks indicate differences (P < 0.05) between groups.

5.9 ± 0.3 for TG and CG, respectively. Fetal heart rate was higher in TG than in CG (P < 0.05; Table 1).

4. Discussion

To the authors' knowledge, this is the first report of uterine blood flow in abnormal feline pregnancy. As expected, and opposite to what has been described for normal pregnancy, RI and S/D ratio of uterine artery increased in these induced abortions. This is also in line with what was

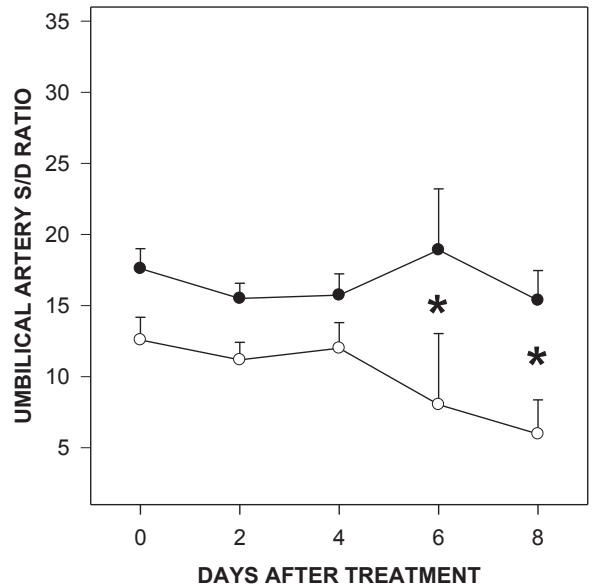


Fig. 4. Systolic/diastolic ratio of umbilical artery of the same animals of Figure 1. Asterisks indicate differences (P < 0.05) between groups.

Table 1
Fetal heart rate (mean \pm SEM) of the same animals of Figure 1.

Fetal heart rate (bpm)	Day 0	Day 2	Day 4	Day 6	Day 8
TG	238 \pm 5	246 \pm 2	253 \pm 3	246 \pm 4	248 \pm 15
CG	231 \pm 5	245 \pm 2	238 \pm 3	242 \pm 3	251 \pm 4

Abbreviations: CG, control nontreated group; TG, treated group.

experimentally described in abnormal canine gestation, where RI and S/D ratio progressively increased to the time point of abortion [20]. Furthermore, these results were later confirmed in spontaneous abortion canine cases [11]. These findings are also consistent with those of women, where an increased RI reflected a high resistance in uterine microcirculation and, therefore, augmented risk of abortion [19,21]. Importantly, in the present cat report, group differences in Doppler parameters appeared as early as 48 hours after abortion treatment.

It has been postulated that progesterone augments uterine blood flow during pregnancy in several species [22,23]. In women, the administration of oral progesterone was followed by a significant decrease in uterine artery RI and S/D ratio [19,24]. In the present feline study, increased RI and S/D ratio may have been provoked by the pharmacologically induced uterine progesterone deprivation [25]. Further studies are necessary to unveil the signaling transduction pathways by which progesterone may alter uterine artery blood flow in queens.

In felids, aglepristone mid-term abortion is assumed to be the result of damage of uterine venous blood vessels and a subsequent interstitial hemorrhage, resulting in an uteroplacental detachment [26]. Reflex vasoconstriction of the uterine artery in response to this hemorrhage could have also contributed to the decrease in blood flow observed in these queens.

The RI and S/D ratio of umbilical artery were also higher in TG than in normal pregnancies. These findings agree with a report in dogs, where umbilical artery RI did not diminish in animals with spontaneous abortion or a high percentage of neonatal death [11]. It is also consistent with studies in humans, where Doppler parameters obtained from this artery have shown to be useful indicators of fetuses that are at risk [6]. It has been suggested that an absent or reversed EDV in the umbilical arteries is associated with a higher rate of human perinatal mortality and serious morbidity among survivors [27]. In the present study, diastolic flow of umbilical artery was first detected at Day 30 of gestation, and there was not reversal or absence after this time point in any animal.

Fetal heart rate is generally decreased as a result of stress and hypoxemia in feline pregnancy [14]. The heart, as a vital organ, is affected (i.e., decelerated) after uterine or umbilical blood flow impairment [28]. Studies in humans have suggested that changes in umbilical and other vessels occur weeks before the onset of fetal heart rate deceleration [9,29]. It is worth noting that in the present study, fetal heart rate did not decelerate in the antagonist-treated gestations and that this parameter was even higher than in normal pregnancies. An explanation for this acceleration might be that maturation of vagal cardiac control depends on fetal weight and low weight results from poor fetal

perfusion [30]. In addition, plasma catecholamines concentrations increase progressively with greater hypoxia [31].

The present results suggest that Doppler could detect pregnancy abnormalities before other clinical or ultrasonographic signs are present. This emphasizes the importance of routine serial Doppler examinations during feline pregnancy. Maternal and fetal blood flow assessment has the potential to provide useful information in case of compromised feline pregnancy. Further work, including spontaneous abnormal pregnancies, is required to better describe the pathologic changes of maternal and fetal blood flow during abnormal feline gestation.

It is concluded that, in these queens, uterine and umbilical arteries blood flow differed between normal and abnormal pregnancies predicting the adverse obstetric outcome.

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Competing Interests

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

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