



## Reference range of gestational uterine artery resistance index in small canine breeds

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### ABSTRACT

The objectives of this study were to evaluate the uterine artery blood flow during the second half of normal pregnancy in small breed dogs and to determine the RI reference values of this artery in these bitches. Fifty-two, 1.5–6.5 kg, purebred healthy pregnant bitches were included in this study. Doppler ultrasonographic examinations of the uterus were performed every 10 days from Day 30 of the estrous cycle to parturition. Peak systolic velocity (PSV) and end diastolic velocity (EDV) of the uterine artery were measured while RI [(PSV–EDV)/PSV] was automatically calculated. Values of PSV, EDV and RI were analyzed by repeated measures ANOVA followed by Tukey test using age, body weight, litter size and parity as covariates. Resistance index reference ranges were constructed by regressing this index on gestational age. Equation of the polynomial regression curves was used to calculate the mean, 95th and 5th centiles for each gestational age (GA). A gradual increase of PSV ( $P < 0.01$ ) and EDV ( $P < 0.01$ ) and a decrease in RI ( $P < 0.01$ ) were found throughout the study period. PSV and EDV were influenced by litter size ( $P < 0.05$ ), while RI was not. None of the Doppler parameters were influenced by age, body weight or parity. A second-degree polynomial described the relationship between RI of uterine artery and GA in the different time points:  $[RI = 0.993 + 8.4 \times 10^{-5} \times GA^2 - 0.0117 \times GA]$ . It is concluded that uterine artery blood flow progressively increased throughout normal pregnancy in small breed bitches. In addition, reference ranges of uterine artery RI were 0.64–0.79, 0.57 to 0.75, 0.53 to 0.71 and 0.52 to 0.66 on Days 30, 40, 50 and 60 of gestation, respectively.

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### 1. Introduction

Doppler ultrasound has been used to assess maternal and fetal blood flow during pregnancy in many species, including dogs [1–3]. This non-invasive technique evaluates the flow velocity and resistance index (RI) within vessels. In dogs, as in many other species, it has been postulated that uterine artery blood flow progressively increases from mid to the end of normal gestation, guaranteeing an appropriate perfusion of the conceptuses [4,5]. Doppler ultrasonographic changes of uterine arteries during normal pregnancy have been described in Beagles [6], large breeds [4] and different breeds [5]. To our knowledge, these changes have not been reported in small breeds. The wide range of peak systolic

velocity (PSV), end diastolic velocity (EDV) and RI absolute values in the existing studies limits the practical application of these values. Assuming that this variability could be due, among other aspects, to maternal body weight, reliable Doppler values in a homogeneous group of toy bitches are needed.

In addition, while Nautrup (1998) [6], Di salvo et al. (2006) [4], Miranda & Domingues (2010) [7] and Blanco et al. (2011) [5] found an increase in uterine blood flow up to parturition, Rossi Feliciano et al. (2014) [8] reported that uteroplacental arteries RI remained unchanged from the 6th week of gestation onwards. Additionally, some authors reported a gestational increase of PSV and EDV in uteroplacental [6] and uterine arteries [9]. According to these authors, both velocities might triplicate their initial values during the second half of gestation. On the other hand, other studies found no significant differences in these velocities during the same period [4,8].

In human and canine pregnancy, it has been demonstrated that the absence of a decreasing tendency in uterine artery RI is

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associated with obstetrical disorders [1,9,10]. Additionally, this abnormal uterine blood flow pattern seems to precede the appearance of other ultrasonographic alterations in the same species [9]. For this reason, in pregnant women, Doppler has become a routine technique for identifying fetal compromise associated with an abnormal uteroplacental circulation [6,11]. In this species, uterine artery RI higher than 0.58 on week 24th of pregnancy may be indicative of preeclampsia, intrauterine growth restriction and intrauterine death [12,13]. The early detection of these disorders permits an appropriate intervention [14,15].

Despite the proved ability of Doppler ultrasound to predict gestational disorders, reference values of canine uterine artery RI during gestation are not available. The objectives of this study were to evaluate the uterine artery blood flow during the second half of normal pregnancy in small breed dogs and to determine the RI reference values of this artery in these bitches.

## 2. Materials and methods

### 2.1. Animals

Fifty-two,  $3.5 \pm 0.2$  (1–6) years old,  $3.7 \pm 0.1$  (1.5–6.5) kg, purebred (27 Miniature Poodle, 10 Yorkshire Terrier, 5 Malteses, 4 Miniature Dachshund, 2 Chihuahuas, 2 Miniature Schnauzer and 2 Bichón Frisé) healthy pregnant bitches with a history of  $3.1 \pm 0.27$  (1–5) uneventful pregnancies and parturitions were included in this study. The protocol was reviewed and approved by the Animal Care and Use Committee of the Veterinary School of the National University of La Plata, Argentina. Consent forms were signed by all of the owners.

### 2.2. Ultrasonographic follow up

The female dogs were evaluated every 10 days from Day 30 of the estrous cycle to parturition. Day 0 of the estrous cycle was defined as the first day of typical estrus vaginal cytology [16]. Pregnancy was confirmed using ultrasound examination [17].

Bidimensional and Doppler ultrasonographic evaluations were carried out with a 7.5–8 MHz linear transducer (Toshiba Core Vision Pro, Japan). The dogs were positioned in lateral recumbency, acoustic gel was applied to the transducer and coupled directly to the clipped skin. Uterine body was observed by bidimensional ultrasound in a transversal axis [15]. Color Doppler was used to localize uterine arteries at both sides of the uterine body and pulsed-wave Doppler was performed to obtain the waveforms [18]. PSV (m/s) and EDV (m/s) were measured while RI [(PSV–EDV)/PSV] was automatically calculated [1]. To minimize variability, a single trained operator (PRB) recorded three uniform consecutive waveforms which were averaged.

### 2.3. Statistical analysis

Normal distribution of the data was assessed and corroborated by Kolmogorov – Smirnov test. Peak systolic velocity, EDV and RI of the left and right uterine arteries were compared using Student's t-tests. Values of PSV, EDV and RI were analyzed by repeated measures ANOVA followed by Tukey test using age, body weight, litter size and parity as covariates. Resistance index reference ranges were constructed by regressing this index on gestational age (GA). Polynomial regression model was fitted to Mean. The information about standard deviation of the measurement was contained in the residuals around the fitted curve. Equation of the polynomial regression curves was used to calculate the mean, 95th and 5th centiles for each gestational age [19,20].  $P < 0.05$  was considered significant (SPSS 19.0, SPSS Inc. Chicago, IL, USA).

## 3. Results

All the females whelped normally  $3.4 \pm 0.2$  (2–6) healthy puppies. No differences were found between right and left uterine arteries ( $P > 0.1$ ), therefore values of PSV, EDV and RI were averaged. A gradual increase of PSV ( $P < 0.01$ ) and EDV ( $P < 0.01$ ; Fig. 1) and a decrease in RI ( $P < 0.01$ ; Fig. 2) were found throughout the study period in all the cases. Although PSV and EDV were influenced by litter size ( $P < 0.05$ ), RI was not ( $P > 0.1$ ). Both velocities were greater in larger litters. None of the Doppler parameters were influenced by age, body weight or parity ( $P > 0.1$ ). A second-degree polynomial described the relationship between RI of uterine artery and GA in the different time points:

$$[RI = 0.993 + 8.4 \times 10^{-5} \times GA^2 - 0.0117 \times GA].$$

The reference values estimated for the mean, 95th and 5th centiles for Days 30, 40, 50 and 60 were presented in Table 1.

## 4. Discussion

In these bitches, uterine blood flow progressively increased during normal gestation. Uterine artery PSV and EDV increased throughout the experiment. This finding is in line with previous articles [5,6] and opposite to other reports in this species [4,8]. In the present study, uterine artery RI showed a pronounced decrease. This result is consistent with previous reports in dogs [4,5] and in disagreement with another canine study in which RI showed a constant value of 0.5 throughout the second half of gestation [8].

The decrease in vascular resistance may be explained by the additive effect of different physiological mechanisms. In pregnant women, this decrease has been attributed to concurrent invasion of the trophoblast into the endometrium during placentation and the loss of the musculoelastic coat of the arteries [20]. Additionally, the increased availability of a potent vasodilator i.e. nitric oxide, a decrease of blood viscosity and a gradual reduction of the peripheral vascular resistance in the maternal compartment are also present factors that contribute [21,22,23]. A similar process might be present in dogs, although it remains to be described [5,7].

In this study, absolute values of uterine artery PSV, EDV and RI were higher than previously reported in pregnant Beagles and large breed dogs [4,6]. These results suggest that despite the general

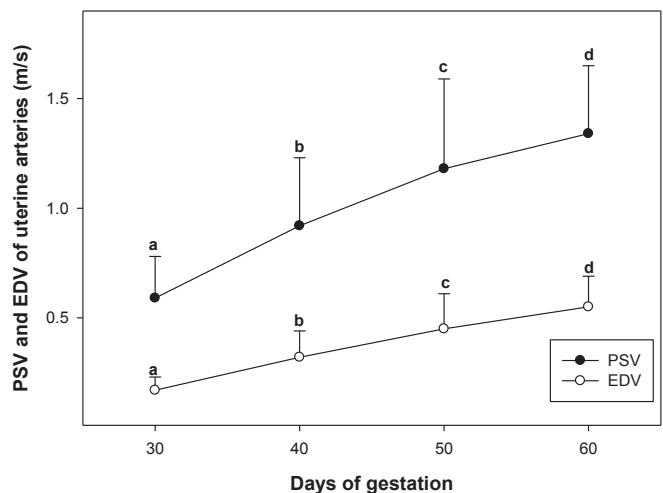
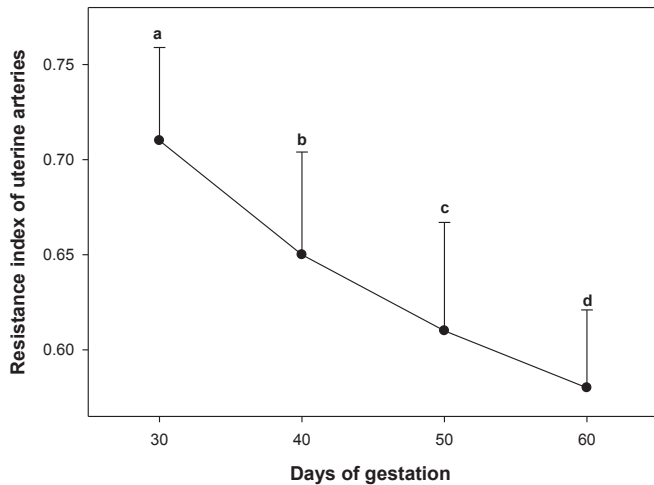


Fig. 1. Peak systolic velocity (PSV; mean  $\pm$  SD) and end diastolic velocity (EDV; mean  $\pm$  SD) the uterine arteries of the same dogs of Table 1. Different letters indicate differences ( $P < 0.05$ ) among days.



**Fig. 2.** Resistance index (RI; mean  $\pm$ SD) of the uterine arteries of the same dogs of Table 1. Different letters indicate differences ( $P < 0.01$ ) among days.

tendency for RI is to descend throughout (canine) pregnancy, there might be maternal body weight variations. To our knowledge, this is the first study carried out on a sample size  $n > 50$  bitches.

In the present study, the bitches with larger litter sizes showed higher absolute values of PSV and EDV. This may be due to the larger surface to perfuse, which is reflected in the increase in both velocities [1,24]. As the RI is calculated from PSV and EDV, the proportional increase in both velocities may explain the lack of influence of litter size on the RI [1]. Monotocous domestic species present higher RI values in the contralateral uterine artery to the conceptus [25–28]. However, as none of the bitches included in this study had a singleton fetus, this difference could not be demonstrated [29]. The influence of age and parity on canine uterine artery RI has been documented during estrus and during the early luteal phase of pregnant and non-pregnant Labrador Retriever bitches [30,31]. However, to the authors' knowledge, there were no published studies about this influence during the second half of canine gestation. In the present study, the absent influence of age and parity on RI during gestation may be associated to the homogeneity group.

The reference range for uterine artery RI for small bitches established in this article provides a useful diagnostic tool to predict adverse obstetric outcomes during gestational ultrasound evaluations. Pregnant bitches with values outside P95 and P5 should be considered at high risk of obstetrical disease and submitted to further diagnostic tests.

It is concluded that uterine artery blood flow progressively increased throughout normal pregnancy in small breed bitches. In addition, reference ranges of uterine artery RI were 0.64–0.79, 0.57 to 0.75, 0.53 to 0.71 and 0.52 to 0.66 on Days 30, 40, 50 and 60 of gestation, respectively.

**Table 1**

Mean, 95th(P95) and 5th(P5) centiles estimated for the uterine artery resistance index (RI) of 52 small breed bitches, evaluated every 10 days from Day 30–60 of gestation.

		Days of gestation			
		30	40	50	60
RI	Mean	0.72	0.66	0.62	0.59
	P95	0.79	0.75	0.71	0.66
	P5	0.64	0.57	0.53	0.52

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