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# Power and pulsed Doppler evaluation of ovarian hemodynamic changes during diestrus in pregnant and nonpregnant bitches

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ABSTRACT

The aim of the study was to further characterize the relationship between hemodynamic changes in the ovary and luteal function in pregnant and nonpregnant bitches. Fourteen German Shepherd bitches were monitored three times a week from the first day of cytological diestrus (D1) until parturition or the end of diestrus (progesterone <2 ng/mL) by color Doppler, pulsed wave spectral Doppler, and power Doppler (PD) ultrasonography. By means of PD the total number of color pixels were calculated. The Doppler parameters evaluated were: peak systolic velocity (PSV), end diastolic velocity (EDV), and both resistive and pulsatility indices. Blood samples were collected three times a week throughout the experiment to determine progesterone (P4) concentrations. The length of diestrus in pregnant versus nonpregnant group was significantly shorter ($P < 0.01$; $57 \pm 1$ vs. $63 \pm 1$, respectively). By means of pulsed wave spectral Doppler the waveform showed a typical pattern of a low–resistive vessel characterized by a rapid systolic peak followed by a slow telediastolic decrease with a relatively high end-diastolic velocity. Blood flow parameters did not differ between left and right ovary. In both groups PSV and EDV showed a gradual decrease with the progress of diestrus; however, the values of PSV and EDV were significantly higher ($P < 0.05$) in the pregnant group versus nonpregnant group from D31 to D61 and from D49 to D58 respectively. Moreover, a significantly decrease ($P < 0.05$) of PSV and EDV in the pregnant group was observed from D46 to D58 and from D49 to D55, respectively. The resistive and pulsatility indices showed an increase during diestrus and the values were significantly lower ($P < 0.05$) in the pregnant group from D49 to D61. By means of PD, the pixel number was significantly higher ($P < 0.05$) in the pregnant versus nonpregnant group from D40 to D61. In particular, a significant decrease ($P < 0.05$) in the pixel number in the pregnant group was observed from D46 to D61. The comparison of the P4 values with the ovarian pixel number in the pregnant and nonpregnant group showed a direct correlation ($r = 0.792$, $N = 59$ and $r = 0.774$, $N = 59$, respectively). In particular, the P4 values were higher ($P < 0.05$) in the pregnant than in the nonpregnant group from D37 to D52. In conclusion, significant physiological differences between pregnant and nonpregnant bitches in terms of P4 and ovarian blood supply are reported. In addition it was possible to define that blood flow pattern during diestrus in pregnant bitches is not always closely related with P4 production.



## 1. Introduction

The development of a corpus luteum (CL) and its endocrine function depends on active angiogenesis

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characterized by endothelial cell proliferation principally sustained by vascular endothelial growth factor [1–4]. This process makes the ovary, and particularly the CL, one of the most perfused structures in the body [5–7]. The hemodynamic changes during the luteal phases have been investigated in different mammalian species, including humans, by immunocytochemical or ultrastructural study [8–17].

The recent availability of color Doppler (CD), pulsed wave spectral Doppler (PW), and PD ultrasonography techniques has provided a major advance in the study of reproduction and represents a reproducible, noninvasive method for monitoring local ovarian blood flow changes during follicular development, ovulation, and CL formation [18–22]. A relationship between luteal blood flow and luteal function in women [23,24], cow [25,26], mare [21,22], ewe [27], and bitch [20] has been reported. However, the physiological ovarian and uterine blood flow in domestic animals was assessed using the CD and PW [18–21], and only one study employed the PD to study luteal blood flow [22]. Power Doppler is three to five times more sensitive to detect slow blood flow than CD [28], and this is independent of the angle of insonation [29]. This study was carried out with an aim to further characterize the hemodynamic changes in the ovaries of pregnant and nonpregnant bitches during the luteal phase using PD and PW. Luteal function, assessed by peripheral serum progesterone (P4) concentrations was also defined, and correlation with Doppler data were established.

## 2. Materials and methods

### 2.1. Animals

Fourteen German Shepherd bitches, ranging in age from 5 to 9 y and weighing $34 \pm 3$ kg, were included in this study. They were judged healthy on the basis of routine clinical examination. According to the clinical history of each subject, the interestrus intervals of previous cycles were normal. From the first appearance of vulvar serosanguineous discharges, indicating the onset of proestrus, vaginal smears were taken daily from each bitch and promptly examined using hematoxylin-eosin staining until the first day of cytological diestrus (D1). The females were assigned in two groups: pregnant group ($N = 7$) and nonpregnant group ($N = 7$). The bitches in the pregnant group were mated twice every 48 h from ovulation and pregnancy was confirmed by ultrasound at D18. All procedures were carried out with respect to the Italian legislation on animal care (DL n.116, 27/01/1992).

### 2.2. Ultrasound scanning

For ultrasonographic examinations, the ultrasound system My Lab 30 Gold (Esaote, Genova, Italy) equipped with a 5.5 to 7.5 MHz microconvex probe for B-Mode as well for CD, PW, and PD measurements was used. Ultrasonography was performed from the left and right shaved flank and the ovaries were detected behind the caudal pole of the ipsilateral kidney. Intraovarian vascularization was examined, starting at D1 until D67, every 3 days by means of CD (Duplex mode), PW (Triplex mode), and PD.

The PD mode was used for color flow mapping of the ovary in various transverse sections. In order to minimize variation, standardized presets (depth 3–5 cm, PD gain 58%–85%, and pulse repetition frequency 1.0–1.7 kHz) were used throughout the examinations. The PD sample box was set to include the entire section of the ovary. Optimal still images displaying the maximum number of color pixels in the ovary without flash artifact were recorded. Three different images in each ovary were taken for each examination. The total number of color pixels was calculated by a computer-assisted image analysis system using open source software ImageJ (http://rsbweb.nih.gov/ij/).

The peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI) were determined using PW Doppler spectral tracing. The measurements of three different waveforms in both ovaries were taken. The values of blood flow parameters were automatically calculated for each waveform. The values obtained on three sweeps were averaged to obtain a single mean value for each measure at each location. The size of the sample volume, which determines Doppler information, was keep constant at 1 mm and, when the insonation angle was $\leq 60^{\circ}$, angular correction was applied.

### 2.3. Blood sampling

All subjects, every 3 days, from D1 to D67, were subjected to blood collection (2 mL) from the cephalic vein. Serum was separated from blood collected in glass tubes without anticoagulant by centrifugation ($3000\times$ *g* for 15 min), and stored at $-20\ ^{\circ}C$ until assayed. In order to detect the ovulation day, blood samples (2 mL) were collected daily from late cytological proestrus until P4 reached a value $>5$ ng/mL [30].

### 2.4. Hormone assays

Serum progesterone concentrations were determined by a commercial RIA kit (DLS, Webster, TX, USA). The intra- and interassay coefficients of variation averaged 6.9% and 12.9%, respectively. Sensitivity was 0.12 ng/mL. For estimating the ovulation day, P4 was assayed daily by enzyme linked fluorescent assay (Minividas Biomerieux, Marcy l'Etoile, France).

### 2.5. Statistical analysis

For the purpose of this work, functional luteolysis was defined as a 50% decrease in the serum progesterone concentrations from the mean values observed between days D1 to D25 [31], and complete luteolysis was defined as the failure of CL to secrete progesterone with a consequent drop in blood levels to below 2.0 ng/mL, a level found in anestrous bitches [30]. Data relative to P4 production were analyzed by Student *t* test.

For the analysis of correlation between luteal function and ovarian blood flow, as assessed by P4 and PD pixel numbers respectively, the values were expressed as relative percentages of the corresponding values that were averaged up to day D25 and set to 100 [31]. These data, and the other hemodynamic parameters were analyzed by