

B-mode Ultrasound and Doppler Mode for Early-stage Pregnancy Diagnosis in Shi-Tzu Bitches

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ABSTRACT

Background: The very first sign to confirm pregnancy in bitches is the gestational sac detection. In this primary moment, it is very small with only few millimeters. The average time for gestational sacs visualization is approximately 20 days after mating (2 mm) surrounded by a thin hyperechoic wall (trophoblasts). The Doppler is a new method for pregnancy diagnosis in bitches. The measurement of velocity peak and vascular resistance index of the *corpus luteum* are used for such purpose. The precocity on detection pregnancy in bitches is an important tool for differential diagnosis between physiologic uterine alterations and uterine diseases. The aim of the current study was to assess the efficiency of the B-mode and Triplex Doppler ultrasound for early-stage pregnancy diagnosis in Shi-Tzu bitches, using ecobiometry of the gestational sac and *corpus luteum* vascular index.

Materials, Methods & Results: Ten healthy Shi-Tzu bitches were evaluated. Pregnancy diagnosis was carried out using the B-mode. The ecobiometry of the gestational sac were characterized by the variables the outer (OLL) and inner (ILL) latero-lateral diameters, and the outer (ODV) and inner (IDV) dorso-ventral diameters. The *corpus luteum* vascularization was studied by the systolic velocity peak (PVS), end-diastolic velocity (EDV), vascular resistance (RI) and pulsativity indexes (PI) using the Triplex Doppler. The ultrasound pregnancy diagnosis was carried out on days 14.2 ± 2.04 post mating. The earliest pregnancy diagnosis occurred on day 12. Positive correlation on linear regression was observed between ILL and IDV and the date of the pregnancy diagnosis ($r^2 = 0.7$ and $P = 0.0027$; $r^2 = 0.7$ e $P = 0.0025$, respectively). On *corpus luteum* Triplex Doppler assessment, PVS was 11.67 ± 2.53 cm/s, EDV was 5.52 ± 1.6 cm/s, PI was 1.04 ± 0.45 and RI was 0.61 ± 0.1 .

Discussion: Early diagnosis observed in the present study for canine pregnancy is an important differential in veterinary obstetrics. Gestational sac was detected in the uterine horn on the 20th day post LH peak, 18 days following ovulation in a Beagle bitch and the ultrasound diagnosis of pregnancy was performed 18-24 days following the first and last mating, respectively, in Yorkshire dogs. Those results highlighted the difference between the dates for early-stage pregnancy diagnosis among different canine breeds. The precocity for the ultrasound pregnancy diagnosis depends on the visualization of the gestational sac, which is directly related to embryogenesis. The assessment of the ecobiometry of the gestational sacs may aid on the early-stage ultrasound pregnancy diagnosis in bitches. The values of *corpus luteum* vascular index in Shi-Tzu bitches in the current study were different from those found in other trial in bitches and in women. Such values may be justified by the differences on the *corpus luteum* structures between the canine and human species. Regarding to Triplex Doppler in pregnant bitches, the values of PI and EDV could be determined, which hadn't been reported before in veterinary medicine for the study of *corpus luteum* blood flow. In conclusion, the detection of pregnancy on early-stage using ultrasound examination is feasible in Shi-Tzu bitches, by the 12th day post conception. It was also possible to determine important ecobiometry values of the gestational sac and *corpus luteum* vascular index using Triplex Doppler.

Keywords: canine, pregnancy, ultrasound.

INTRODUCTION

The sign that confirms pregnancy in bitches is the gestational sac detection, a mature blastocyst which is responsible for the embryonic development. In this moment, the gestational sac is very small and has only few millimeters in diameter. The average time for gestational sacs visualization is approximately 20 days after mating, appearing as anechoic structures (first chorionic fluid) with 2 mm, surrounded by a thin hyperechoic wall (trophoblasts) [15].

The blood flow assessment of the *corpus luteum* and ovary using Doppler is a mean of diagnosing pregnancy and predicting fetal viability. The measurement of peak velocity and *corpus luteum* vascular resistance index are used for such purpose [1]. There are few reports of Doppler use for pregnancy monitoring and uterine arteries flow characteristics of the of pregnant and no pregnant in small animal practice [9,13].

The precocity on pregnancy detection in bitches is an important tool for differential diagnosis between physiologic uterine alterations and uterine diseases. Inside a gestation period lasting approximately 60 days [18] the diagnosis of diseases should be as fast as possible to allow interventions by the responsible clinician.

Therefore, the aim of the current study was to assess the efficiency of the B-mode and Doppler ultrasound examination for early-stage gestational diagnosis in Shi-Tzu bitches and determination of ecobiometric parameters of the gestational sacs and *corpus luteum* vascular index.

MATERIALS AND METHODS

Ten no pregnant multiparous Shi-Tzu bitches weighting 4-7 kg, aged 4-6 years old, from a private kennel were evaluated. The animals were previously submitted to physical and obstetric examination and only patients considered healthy were admitted in the current study.

The detection of proestro phase beginning was performed by the kennel owner, who was advice to contact the research team in order to confirm the phase of the estral cycle. The estrus signs (i.e., acceptance of matting) and vaginal cytology carried out the confirmation of the optimal fertility period. Following confirmation, the female were put along with a male for three days or artificial insemination was performed.

The early-stage gestational diagnosis was performed two weeks following the first mating or artificial insemination, analogically to studies performed

on human obstetrics [5], according to methodology previously described [11].

The B-mode ultrasound and Doppler were performed using the MyLabTM30 VET machine¹ and a 7-12MHz linear transducer. A single examiner carried out the ultrasound.

Prior to the procedures, the abdomen of the bitches were widely clipped. The animals were put in dorsal recumbency, which was changed to right or left lateral recumbency as required for optimal ultrasound scanning.

The presence of gestational sac was determined using the B-mode ultrasound examination. The ecobiometry of the gestational sac was determined using the measurement of the outer (OLL) and inner (ILL) latero-lateral diameters, and outer (ODV) and inner (IDV) dorso-ventral diameters.

The *corpus luteum* vascularization, assessed by pulsed Doppler, was used to determine the peak systolic velocity (PSV), end-diastolic velocity (EDV), resistance index ($RI = [PSV - EDV] / PSV$) and pulsatility index ($PI = [PSV - EDV] / \text{mean velocity}$) [6]. Three evaluation per animal were performed and a mean value was determined for each index.

Pulse Doppler was employed to determine sample volume, using the uniform insonation method. The cursor was positioned in the area of a vessel within the ovarian parenchyma to measure the trace of spectral curve flow and vascular index, which were obtained automatically following software identification of the ultrasonic scanner for each waveform [7].

The power Doppler function was employed to increase the sensitivity of the blood flow measurements and to transform the examination from angle-independent to insonation or incident angle [4].

Statistical analysis

The current study followed a randomized design. The data were previously tested for normality and homogeneity of the variance (F-test). The PROC MEANS-SAS® and GraphPad Prisma 4 were used. The significance level adopted in the current study was 5%.

The values for the date of early-stage gestational diagnosis and gestational sac ecobiometry were correlate using linear regression, and the coefficient of determination and the linear regression equation were determined. The equation was $\hat{Y} = a + bxi$; “ $\hat{Y}$ ” means the date of gestational diagnosis; “a” means the linear coefficient of the line; “b” means the regression coefficient; “xi” means the diameter of the gestational sac.

RESULTS

The mean pregnancy period in 10 Shi-Tzu bitches was 58 days and the number of puppies per pregnancy, on average, was four. The ultrasound pregnancy diagnosis was accomplished at 14.2 ± 2.04 days following mating, requiring only a single ultrasound examination to detect pregnancy. The earliest pregnancy was detected on the 12th day post mating (Figure 1) and the mean number of gestational vesicles detected per animal was four.

The mean values of the ecobiometry of the gestational sac (EGS) found in the current study and

the correlation between the date of pregnancy diagnosis (DPD) in Shi-Tzu bitches are shown in Table 1 and Figure 2.

Regarding the Triplex Doppler mode, peak systolic velocity was 11.67 ± 2.53 cm/s, end-diastolic velocity 5.52 ± 1.6 cm/s, pulsativity index was 1.04 ± 0.45 and vascular resistance index was 0.61 ± 0.1 for the left ovary. Statistical values were not possible to be measured on the right ovary due to its anatomic positioning, to the presence of artifacts and to reduced sampling. However, it was possible to detect the presence of *corpus luteum* on those structures.

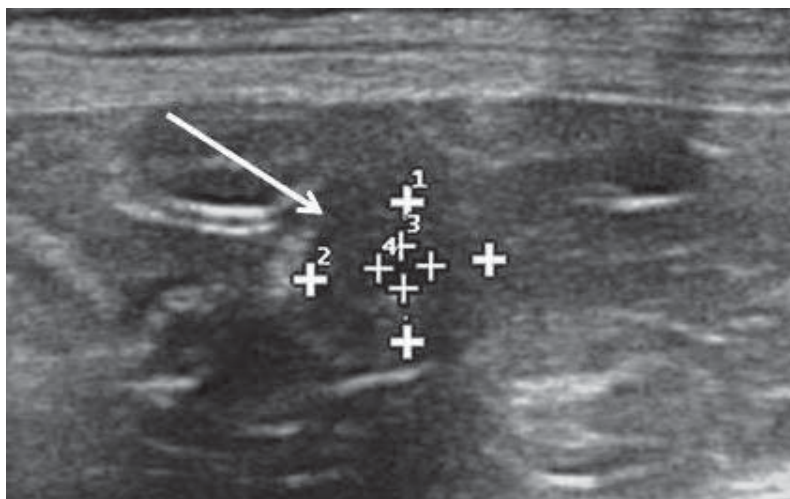


Figure 1. Ultrasound imaging of the early-stage and ecobiometry of the gestational sac in Shi-Tzu bitches.

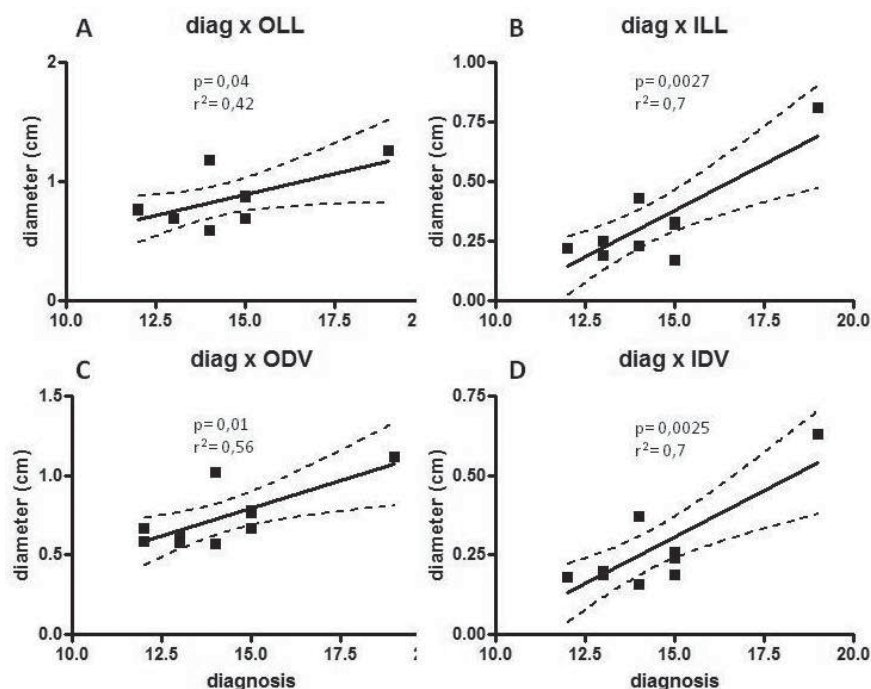


Figure 2. (A), (B), (C) e (D) Linear regression between the values of date of pregnancy diagnosis and ecobiometry of the gestational sac in Shi-Tzu bitches.

Table 1. Mean values of OLL, ILL, ODV and IDV of the gestational sacs and linear regression between the date of pregnancy diagnosis and the ecobiometry of the gestational sacs in Shi-Tzu bitches.

	OLL	ILL	ODV	IDV
Mean values	0.83 ± 0.22	0.31 ± 0.18	0.73 ± 0.19	0.26 ± 0.14
Regression equation	Y = 0.069x - 0.15	Y = 0.077x - 0.78	Y = 0.069x - 0.25	Y = 0.058x - 0.57
r ²	0.42	0.7	0.56	0.7
Slope	0.069 ± 0.028	0.077 ± 0.018	0.069 ± 0.021	0.058 ± 0.013
P (5%)	0.0426*	0.0027*	0.0128*	0.0025*

*P < 0.005. Outer latero-lateral diameter (OLL); inner latero-lateral diameter (ILL); outer dorso-ventral diameter (ODV); inner dorso-ventral diameter (IDV); r²: coefficient of determination.

DISCUSSION

Early diagnosis observed in the present study for canine pregnancy is an important differential in veterinary obstetrics for several reasons including: (1) confirmation of pregnancy as soon as possible for the owners anxious; (2) provide subsidies to the vet for interference in cases of accidental and unscheduled mating [14]; promote obstetric planning management for the pregnant female as well as an effective health strategy [10]; and (3) assist in the diagnosis of gestational alterations that may compromise this physiological process in bitches [21].

Even if the ultrasound is considered a non-invasive diagnostic technique [14], the stress resulting from the immobilization of the animal [12] or even atypical environment [15] may have a negative effect on the reproduction of the animals [19]. In pregnant bitches, hormonal, metabolic, and neural alterations caused by stress can promote embryonic absorption, fetal mortality and problems during parturition [3]. Therefore, in this study we adopted strategies to minimize possible stress to the animals, such as: the constant presence of the owner; the prior presentation and interaction of the animal with the sonographer; and a peaceful and quiet environment with pleasant temperatures and humidity.

Gestational sacs can be early viewed between 19th and 20th days of pregnancy in bitches but such small fluid structures can be overlapped by intestinal gas at the beginning of pregnancy. B-mode ultrasound presented precision of 94, 98 and 99% for de diagnosis of pregnancy in bitches following 24, 25 and 28 days of pregnancy, respectively [17]. Other studies reported the visualization of gestational sacs during B-mode ultrasound examination between 15 and 20 days of pregnancy [20]. Regarding the date of the gestational

sac detection reported in those studies, it is suggested that the current trial showed early-stage ultrasound pregnancy diagnosis in Shi-Tzu bitches.

A gestational sac was detected in the uterine horn on the 20th day post LH peak, 18 days following ovulation in a Beagle bitch [17]. The ultrasound diagnosis of pregnancy was performed 18-24 days following the first and last mating, respectively, in Yorkshire dogs [8]. Those results highlighted the difference between the dates for early-stage pregnancy diagnosis among different canine breeds. Such fact reveals the importance of studies on other specific canine breeds for better understanding of the early-stage ultrasound pregnancy diagnosis, as performed in the current trial.

The precocity for the ultrasound pregnancy diagnosis depends on the visualization of the gestational sac, which is directly related to embryogenesis. The morula is formed 8 days following fertilization and consequently blastulation occurs (organization of the blastocyst, blastocoele trophoblast) [16]. The ultrasound visualization of anecogenic content (which aids on the differentiation of the gestational sac from the uterine tissue in bitches), may be associated to the formation of the blastocoele. Therefore, the assessment of the ecobiometry of the gestational sacs may aid on the early-stage ultrasound pregnancy diagnosis in bitches.

The inner latero-lateral and dorso-ventral diameters may be associated to the accumulation of fluid (blastocoele) and the visualization of the gestational sac. Such proposal is in accordance with the positive correlation for the values of the linear regression between the date of pregnancy diagnosis and the ecobiometry of the gestational sac in the current study (ILL, r² = 0.7 and P = 0.0027; IDV, r² = 0.7 and P = 0.0025).

Higher values of diameter of gestational sacs were found in bitches [13] and queens [2] in comparison with the results obtained in Shi-Tzu in the current study. The lateral diameter was also found higher than the dorso-ventral diameter in other trials [2,13] as reported in our study. Such fact occurs due to the direction of the fetal development, which occurs cranio-caudally.

The values of *corpus luteum* vascular index in Shi-Tzu bitches in the current study were different from those found in other trial in bitches [11] and in women [5] which were (left ovary: PVS = 30.31 cm/s, IR = 0.52; right ovary: PVS = 20.46 cm/s, RI = 0.42) and (PVS = 27 cm/s, RI = 0.41 - 0.57), respectively. Such values may be justified by the differences on the *corpus luteum* structures between the canine and human species.

Regarding the Triplex Doppler in pregnant bitches, the values of PI and EDV could be determined, which hadn't been reported before in veterinary medicine for the study of *corpus luteum* blood flow.

CONCLUSION

In conclusion, the detection of pregnancy on early-stage using ultrasound examination is feasible in Shi-Tzu bitches, by the 12th day post conception. It was also possible to determinate important values of ecobiometry of the gestational sac and *corpus luteum* vascular index using Triplex Doppler.

SOURCE AND MANUFACTURER

¹MyLabTM30 VET - ESAOTE, Genoa, Italy.

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Ethical approval. The trial was conducted following approval by the Animal Welfare and Ethics Committee of the School of Agrarian and Veterinary Sciences of the São Paulo State University (FCAV/UNESP Jaboticabal) [process no 017314/10].

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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