Pesq. Vet. Bras. 33(9):1144-1150, setembro 2013

Ovarian and uterine periovulatory Doppler ultrasonography in bitches[1]

Claudia C. Barbosa[2], Mírley B. Souza[2], Sarah R.R.A. Scalercio[3], Ticiana F.P. Silva[2], Sheyla F.S. Domingues[3] and Lúcia D.M. Silva[2]*

ABSTRACT.- Barbosa C.C., Souza M.B., Scalercio S.R.R.A., Silva T.F.P., Domingues S.F.S. & Silva L.D.M. 2013. **Ovarian and uterine periovulatory Doppler ultrasonography in bitches.** *Pesquisa Veterinária Brasileira 33(9):1144-1150.* Laboratório de Reprodução de Carnívoros, Universidade Estadual do Ceará, Campus do Itaperi, Av. Paranjana 1700, Itaperi, Fortaleza, CE 60740-903, Brazil. E-mail: lucia.daniel.machado@hotmail.com

This paper aims to describe the uterine and ovarian ultrasonographic characteristics and Doppler velocimetric features of their arteries in bitches during the periovulatory period. Fifteen estrous cycles in 10 animals were evaluated. The ultrasonographic characteristics, resistance indices (RI) and pulsatility indices (PI) of the uterus and ovaries in each animal were recorded 5 days before and after ovulation (D0). The data were statistically analyzed, and the results were expressed as the mean ± standard error of mean (P<0.05). In results the ultrasonographic features of the uterus were the same on all of the cycles and evaluated days. The uterus had an average diameter of 0.85±0.02cm. An increase in the volume of the ovaries and the diameter of the ovarian follicles were measured. Ovaries had a volume of 0.64±0.06cm^3, and the follicles cavities had a diameter of 0.46 ± 0.01 cm on the day of ovulation. After ovulation, it was observed that some follicles not collapse in some cycles. Two days prior to ovulation, the uterine blood perfusion decreased. This decrease remained unchanged until ovulation. Following ovulation, we measured a gradual increase in the uterine perfusion and in the ovarian artery. This artery directed blood flow to the ovaries and increased the intra-ovarian perfusion on the day after ovulation. In conclusion, specific features are observed in the uterus and ovarian ultrasound image and Doppler values of their arteries presented on the periovulatory days and when associated allow to estimate more accurately the date of ovulation.

INDEX TERMS: Ultrasonography Doppler, ovulation, dogs.

RESUMO.- [**Ultrassonografia Doppler ovariana e uterina em cadelas.**] Este trabalho teve como objetivo descrever as características ultrassonográficas uterinas e ovarianas, e dopplervelocimétricas das suas artérias nos dias periovulatórios em cadelas. Quinze ciclos estrais em 10 animais foram avaliados. As características ultrassonográficas, índices de resistência (IR) e índices de pulsatilidade (IP) do útero e dos ovários em cada animal foram registrados 5 dias antes e depois da ovulação (D0). Os dados foram analisados estatisticamente e os resultados foram expressos em média ± erro padrão da média (P<0,05). Como resultados as características ultrassonográficas uterinas foram semelhantes em todos os ciclos e dias avaliados, tendo o corpo uterino um diâmetro médio de 0,85±0,02cm. Foi observado um aumento no volume dos ovários e nos diâmetros dos folículos, tendo os ovários um volume de 0,64±0,06cm^3, e a cavidade dos folículos um diâmetro de 0,46±0,01cm no dia da ovulação. Após a ovulação, foi observado colapso dos folículos somente em alguns ciclos. A perfusão sanguínea uterina diminuiu dois dias antes da ovulação e permaneceu inalterada até a ovulação. Após a ovulação, houve um aumento gradual na perfusão das artérias uterinas e ovarianas, direcionando o fluxo de sangue para os ovários e

[1] Received on February 7, 2013.
Accepted for publication on July 26, 2013.

[2] Laboratório de Reprodução de Carnívoros, Universidade Estadual do Ceará (UECE), Campus do Itaperi, Av. Paranjana 1700, Itaperi, Fortaleza, CE 60740-903, Brazil. *Corresponding author: lucia.daniel.machado@hotmail.com

[3] Laboratório de Biologia e Medicina de Mamíferos Silvestres da Amazônia, Universidade Federal do Pará, Av. Universitária s/n, Jaderlândia, Castanhal, PA 68745-001, Brazil.

aumentando a perfusão da artéria intra-ovariana um dia após a ovulação. Conclui-se que características específicas são observadas na imagem ultrassonográfica uterina e ovariana, e dopplervelocimétricas de suas artérias nos dias periovulatórios, e quando associadas permitem estimar com mais precisão o dia da ovulação.

TERMOS DE INDEXAÇÃO: Ultrassonografia Doppler, ovulação, caninos.

INTRODUCTION

Determining ovulation timing in bitches has been a subject of study by several authors. This topic is important because it aids in identifying a more precise date for successful artificial insemination, especially with frozen/thawed semen. The ovulation date has been estimated mainly using vaginal cytology, serum hormone concentrations and ovarian ultrasound. Vaginal cytology is a low-cost technique but is not accurate for establishing the exact date of ovulation. The assessment of luteinizing hormone (LH) or progesterone levels is the most suitable method for this purpose. However, measuring LH requires taking at least two samples of blood plasma per day. Furthermore, there are no commercial kits available to measure LH and progesterone concentrations in many countries, which delays data results (Lévy & Fontbonne 2007, Uchoa et al. 2012). Ovarian ultrasonography in B-mode is an alternative method that can measure the growth of the ovarian follicles from the antral to the preovulatory stage as well as detect the newly formed corpora lutea to estimate the likely day of ovulation (Domingues et al. 2007). B-mode ultrasound is a practical, non-invasive and relatively low-cost technique (Eker & Salmanoglu 2006).

The evaluation of the ovarian arteries has shown that good perfusion in ovarian follicles is critical for producing good-quality oocytes and embryos (Nargund et al. 1996). Moreover, a well-perfused uterus is required for normal future pregnancies (Ferreira et al. 2007). In medicine, B-mode ultrasound with Doppler has been used to study uterine and ovarian hemodynamics during the menstrual cycle in fertile (Steer et al. 1990) and infertile women (Steer et al. 1994). It has also been applied to monitor the ovarian and uterus vasculature in *in vitro* fertilization (IVF) and embryo transference (ET) (Coulam et al. 1994, Yang et al. 1999, Pan et al. 2004).

In bitches, Alvarez-Clau and Liste (2005) describe the uterine arteries only on metestrous and anoestrus, and England et al. (2012) compared uterine perfusion among bitches with endometrial hyperplasia and normal uterus, before and after mating. There have also been two studies evaluating the intraovarian vessels during the follicular and luteal phases in nonpregnant bitches (Köster et al. 2001, Bicudo et al. 2010), one comparing intraovarian vessels during diestrus in pregnant and nonpregnant bitches (Polisca et al. 2013) and one report using color Doppler ultrasonography for detecting periovulatory events in ovarian artery (Bergeron 2013). There are no studies evaluating these two arteries, in the same bitch during the periovulatory period.

Assessment of uterine and ovarian perfusion characteristics in the periovulatory period is extremely important. Thus, this paper aims to describe the uterine and ovarian ultrasonographic characteristics and Doppler velocimetric features of their arteries in bitches during the periovulatory period.

MATERIALS AND METHODS

Setting and experimental animals

This study was performed at the Laboratory of Carnivore Reproduction and the experimental protocol was approved by the Animal Ethics Committee of the State University of Ceara (protocol number 08517453-0), in accordance with the guidelines of care and use of laboratory animals established by Brazilian College of Animal Experimentation.

Fifteen estrous cycles of 10 bitches comprising 1 Schnauzer, 4 French Bulldogs and 5 Brazilian Terriers, that were property of commercial kennels and were fed with commercial rations and water *ad libitum*. Age ranged from 8 months to 3 years and weight between 6 and 10 Kg. All bitches had good overall clinical and hematological status and known reproductive history.

Ultrasonography assessment

The ultrasonographic evaluations were performed on a daily basis in a room at approximately 25°C. The assessments began on the first day of proestrus and continued until the first day of cytological diestrus. The data were collected 5 days before (D-5) and after (D+5) ovulation (D0). The B-mode and Doppler velocimetry analyses were performed using a SonoAce PICO (Medison Co., Ltda, Daechi-Dong, Kangnam-ku, Cuseoul, Korea) model ultrasonography device. A 5 to 9 MHz frequency linear transducer and ultrasonography-specific gel were used in all of the examinations. Before the assessment, the bitches fasted for a minimum of 12 hours. Their abdomens were shaved, and they were placed on dorsal recumbency on a foam cushion.

Uterine B-mode and Doppler assessment

Using the B-mode, the uterine body was located in the pelvis entrance area and assessed for ultrasound echogenicity. The uterine body diameter was measured immediately cranial to the cervix, in the longitudinal plane, using electronic calipers. The uterine artery was evaluated parallel to the uterine body and was located using longitudinal B-mode and color Doppler scans (Alvarez-Clau & Liste 2005). The calipers for the pulsed Doppler evaluation were placed on the uterine artery lumen image with insonation angle < 60° and a graphic representation of the artery flow was performed with visualization of consecutive waves of equal velocity and amplitude. At least three waves were measured on each side of the uterine artery for analysis. The resistance index (RI) and pulsatility index (PI) were recorded and automatically calculated by the ultrasound machine:

RI = (PSV - EDV) / PSV;
PI = PSV - EDV / M;
(PSV = peak systolic velocity; EDV = end diastolic peak; M = mean PSV and ECV) (Szatmári et al. 2001).

Ovarian B-mode and Doppler assessment

To evaluate the ovaries with B-mode, scans were performed in the caudal region and surrounding the kidneys in the sagittal and transverse planes on the flank. The ovaries ultrasonographic characteristics were evaluated. The volume was also calculated automatically by the device using two measurements on the longitudinal plane (the length and width) and one in the transverse plane (the thickness). Next, the ovaries were evaluated in all planes to monitor follicular development. The diameters of the two largest follicular cavities in each ovary were measured using the electronic calipers.

The ovarian arteries were examined bilaterally and evaluated in two locations: peripherally to the ovary (ovarian arteries) and internally to the ovary (intraovarian arteries). The methods for Doppler velocimetric analysis of the ovarian arteries were similar to those described to assess the uterine arteries. The color Doppler method was applied to check for blood flow in the arteries. The pulsed Doppler method was used to evaluate the RI and PI.

Vaginal cytology and hormone concentration assessment

The females were observed daily until a hemorrhagic vulvar discharge was noted. This date was considered to be the first day of proestrus. From this day, vaginal swabs were collected every days for vaginal smears. The percent keratinization of the vaginal epithelial cells was measured under light microscopy (x100-400; Opticus Co. Ltd., Olympus, Tokyo, Japan) (Uchoa et al. 2012).

The ovulation day, D0, was defined as the day that serum progesterone concentration reached approximately 5 ng/mL (Bouchard et al. 1991). Daily collections of blood samples (4 mL) were obtained through jugular venipuncture during the periovulatory period. The blood was collected in dry tubes. These samples were centrifuged (1000 g/15 min), and the serum samples were stored at –20°C until the progesterone (ng/dL) and estrogen (pg/mL) assays could be performed. The tests were performed using an automated Elecsys 2010 analyzer (Roche Diagnostics®). Electrochemiluminescence immunoassay reactions (EQL) were conducted according to the protocol detailed by the manufacturer (Elecsys 2010, Roche Diagnostics, Basel, Switzerland).

Statistical analyzes

A repeated-measures ANOVA was used to determine the main effect of days during the periovulatory period on the uterine body diameter, ovarian volume, presence of follicular and cavitary structures after ovulation, E2 and P4 hormonal concentrations, and the RI and PI uterine and ovarian arteries. The differences between days (from one day to the previous one) were identified using a Fisher's protected least significant difference posthoc test. A paired Student's t-test was also used to compare the left and right ovaries, as well as the uterine and ovarian arteries, within specific days. Stat View software (SAS Institute Inc., Cary, NC, USA) was used for all analyses, and all data were reported as the mean + S.E.M. for all analyses, P < 0.05 was considered to be statistically significant.

RESULTS

The ultrasonographic examinations required an average duration of 45 to 60 minutes. The animals remained calm during this period. In B-mode, the uterine body was easily visualized cranial to the cervix (Fig.1A) as a tubular, hypoechoic, homogeneous structure with echogenic walls. The structure had a mean diameter of 0.85±0.02cm on all of the evaluated days (P>0.05).

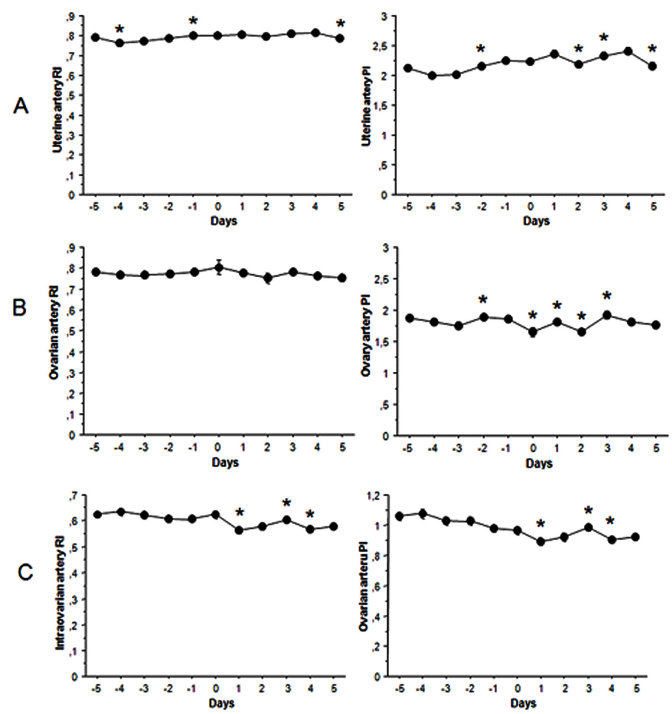

Fig.2. (**A**) Mean ±SEM of RI and PI on uterine, (**B**) ovarian and (**C**) intraovarian artery during the periovulatory period in bitches (n=15). *Indicate the differences in relation of the previous day (P<0.05).

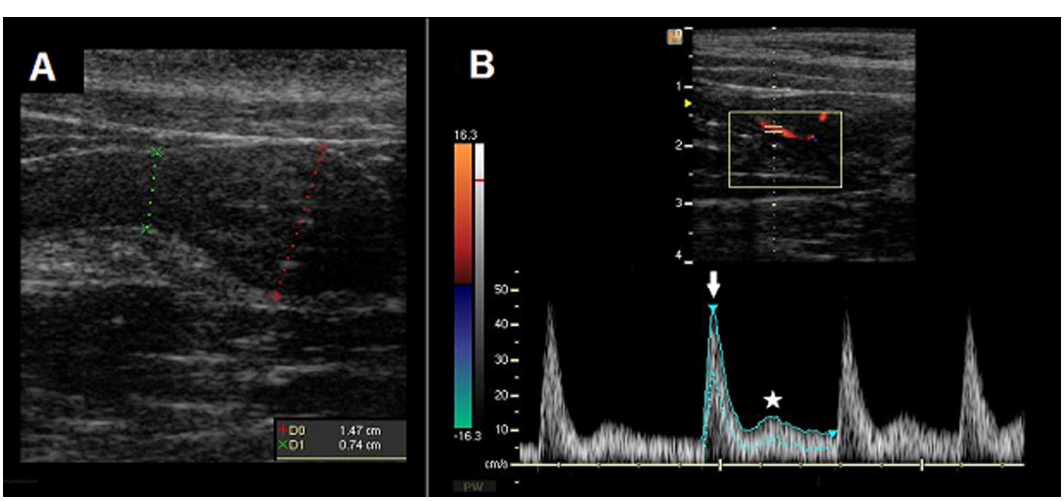

Fig.1. (**A**) Diameter of the uterine body (dotted line green) and cervix (dotted line red). (**B**) Spectral Doppler of the uterine artery during the periovulatory period in bitches (n=15). Biphasic waveform with an evident systolic peak (arrow) followed by a small diastolic peak (*).

In spectral Doppler mode, the uterine artery produced a biphasic wave with a high systolic peak and a small diastolic peak (Fig.1B). No difference was observed between the right and left uterine arteries in each day (P>0.05). On the day of ovulation, the RI of the uterine artery was 0.80±0.01. The PI was 2.23±0.04. Variations were observed in both the RI and the PI on the days that the evaluations were performed. Before ovulation, there was an increase in the RI between D-3 and D-2. The PI also increased between D-2 and D-1. After this, the PI decreased between D1 and D2 and increased between D2 and D3. Finally, both the RI and PI were reduced between D4 and D5 (P<0.05) (Fig.2A).

The ovaries were visualized in the caudal region of the kidney. They were oval to rounded in shape and had hypoechoic parenchyma compared to the adjacent tissue. The ovarian volume was similar on the right and left sides (P>0.05) on the evaluated days. On the day of ovulation, the average volume was 0.64±0.06cm^3. Over the evaluated days, the ovarian size increased, and the maximum size was measured on D3, D4 and D5 (P<0.05) (Fig.3).

Five days before ovulation, rounded structures with anechoic contents in both the left and right ovaries were observed. The structures were an average of 0.29±00cm diameter, compatible with the characteristics of follicles (Fig.4A). Over the evaluated days, the follicular diameter increased. The largest follicular diameter was observed on D1 (0.46±0.01cm) (Fig.5A). On the day of ovulation, the ultrasonographic characteristics of the follicles appeared differently in the cycles. In four cycles, all of the follicles collapsed on the day of ovulation. In five cycles, all of the follicles collapsed on D1,however, in one of them it was not possible to define a cavity in the *corpus luteum* (cl) on the remaining days (Fig.5B); and in the other four cycles, cavities in the cls were observed on D3 and D4 (Fig.5D). In seven cycles, the follicles were found to be partially collapsed, and cavitary structures were visualized on the remaining days. The maximum diameter of these structures was measured on D3 and remained constant until D5 (Fig.4B and 5E). Moderate to large quantities of anechoic free fluid were observed in the ovarian bursa in all cycles until D3 (Fig.5C).

On color Doppler, there was an increase in the intraovarian blood flow following ovulation (Fig.6). On spectral Doppler, the ovarian and intraovarian arteries showed biphasic waves on each day. The waves of the ovarian arteries were similar to those of the uterine artery and were characterized by a high systolic peak and a small diastolic peak. In contrast, the waves of the intraovarian artery showed a small systolic peak and a flat diastolic peak (Fig.7). The arteries were similar bilaterally (P>0.05). The ovarian artery showed an increase in the PI between D3 and D-2. The PI was reduced between D1 and D2 and then increased between D2 and D3. The ovarian artery RI showed only small changes on each of the evaluated days (Fig.2B). The RI and PI of the intraovarian artery were similar. They were reduced from D0 to D1, increased from D2 to D3 and reduced on D4 (P<0.05) (Fig.2C).

On the day of ovulation, 96% of the cells were keratinized in vaginal cytology. Furthermore, 6.53±0.80ng/mL of progesterone and 39.25±6.26pg/mL of estrogen (Fig.8) were observed in the serum. There was an increase in the progesterone concentration and a decrease in the estrogen concentration on the days analyzed (P<0.05).

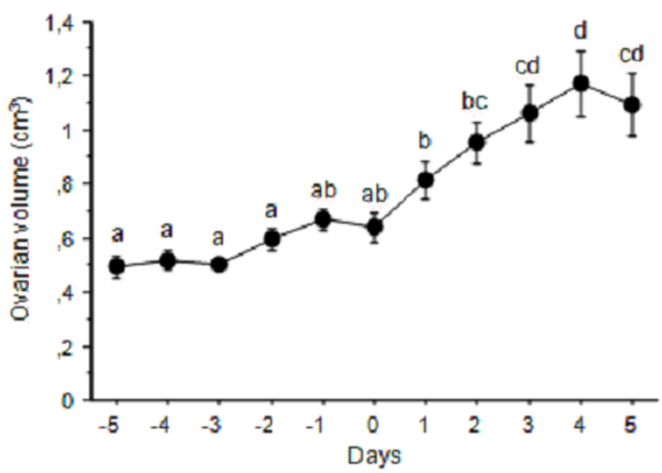

Fig.3. Mean ±SEM of the ovarian volume (cm^3) obtained using ultrasound during the periovulatory period in bitches (n=15). [a, b] Indicate the differences between the days (P<0.05).

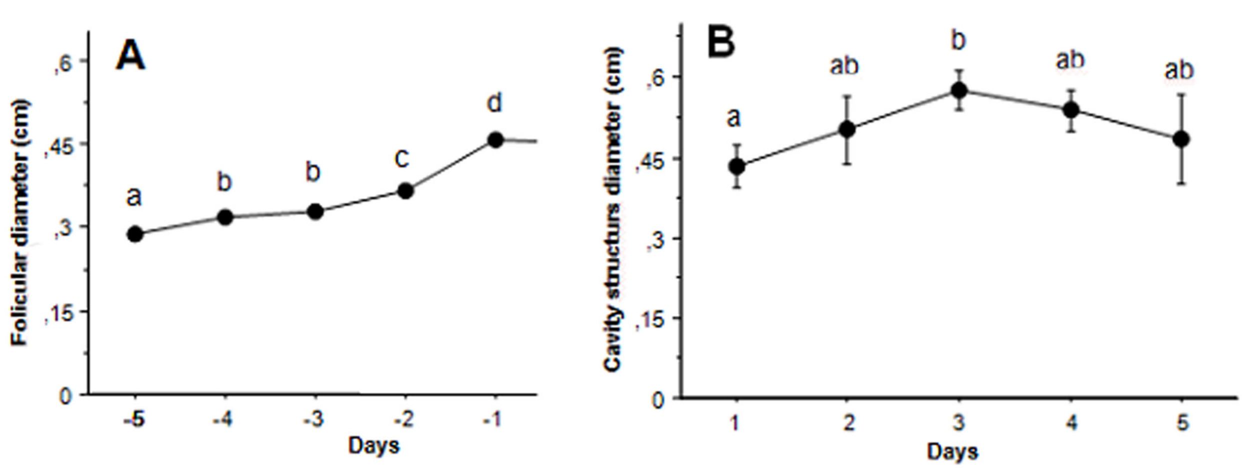

Fig.4. (**A**) Mean ±SEM of the follicular cavities diameter (cm) and (**B**) the cystic cavities after ovulation, obtained by ultrasound during the periovulatory period in bitches (n=15). [a,b] Indicate the differences between the days (P<0.05).

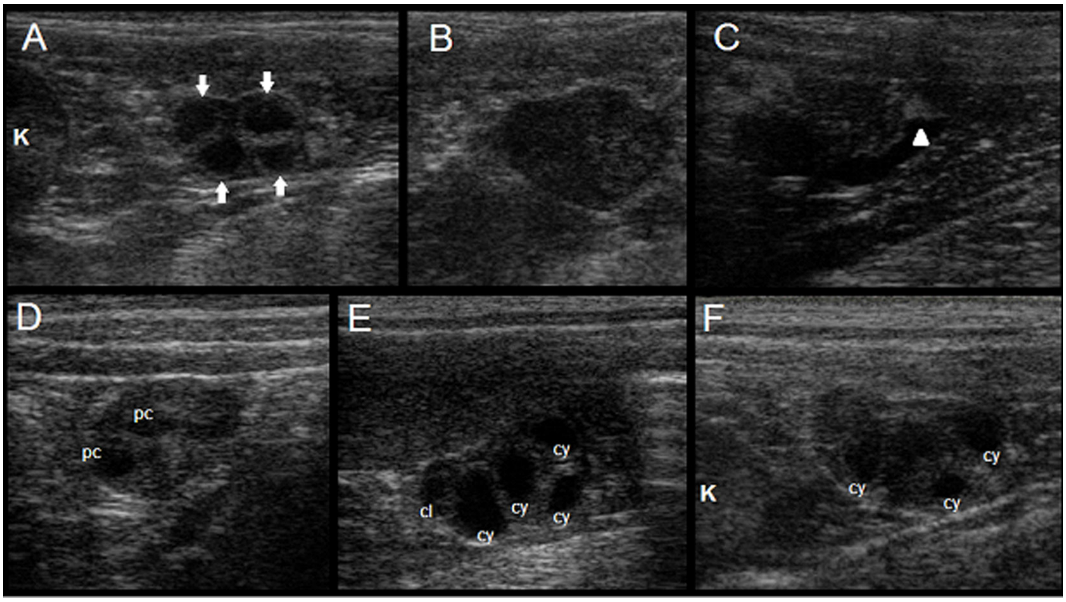

Fig.5. Longitudinal sections of the ovaries of different bitches during the periovulatory period as measured by B-mode. (**A**) Kidney (K) and images of four follicles (arrows) in D-1; the follicles were observed as round anechoic structures that were bounded by echogenic walls. (**B**) Ovary in D1, illustrating a total collapse of the follicles. (**C**) Anechoic fluid contained in the ovarian bursa (Δ). (**D**) Ovary in D0, with partially collapsed follicles characterized as anechoic structures that were round and had irregular contours (pc). (**E**) Ovary in D3, showing the corpus luteum, an echogenic wall, a small anechoic cavity (cl) and several cystic structures (cy). (**F**) Kidney (K) and ovary in D5, showing the no more cavity corpus luteum and still cystic structures (cy).

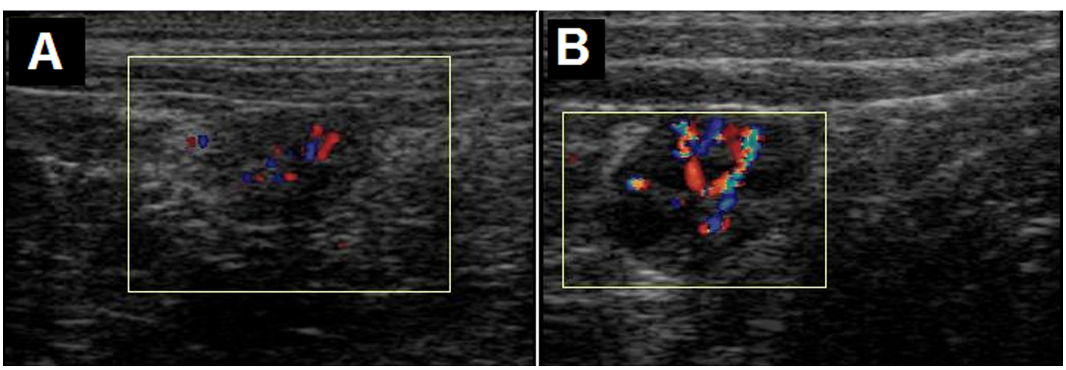

Fig.6. Doppler of intraovarian vascularization on (**A**) D2 and (**B**) D0 during the periovulatory period in bitches (n=15).

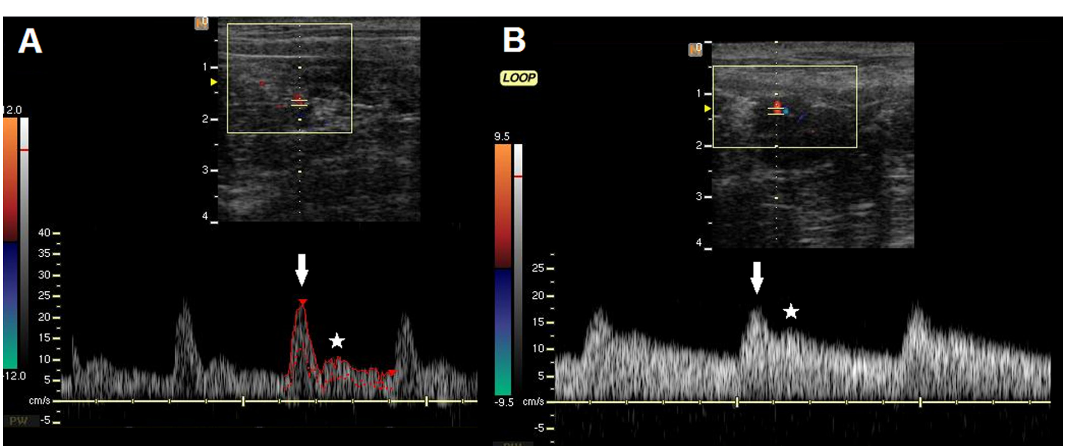

Fig.7. Spectral Doppler of the ovarian (**A**) and intraovarian arteries (**B**) in the periovulatory period in bitches (n=15). (A) Biphasic waveform with an evident systolic peak (arrow) and a small diastolic peak (*). (B) Small systolic peak and a flattened diastolic peak.

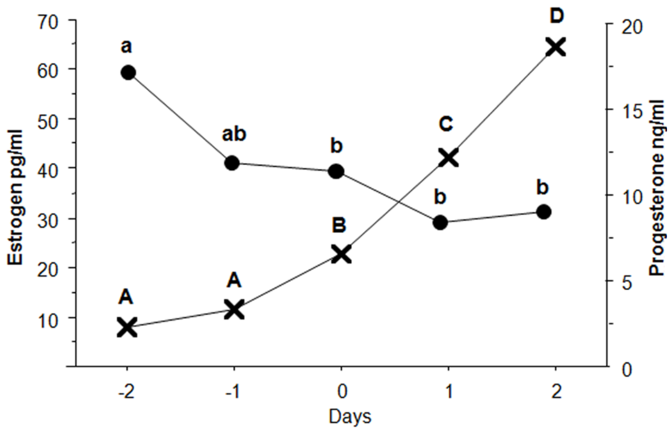

Fig.8. Serum concentrations (mean ±S.E.M.) of progesterone (ng/mL) and estrogen (pg/mL) during the periovulatory period in bitches (n=15). [a,b] Indicate the differences between the days (P<0.05).

DISCUSSION

Detailed knowledge about the reproductive physiology of normal dogs is important to support and enhance the success of canine reproduction biotechnology (Lévy & Fontbone 2007, Uchoa et al. 2012). Knowledge about the uterine and ovarian ultrasonographic features as well their arterial blood flow patterns in cyclical bitches is important. Combining Doppler and ultrasound may also allow the detection of infertility that can be related to poor uterine and ovarian perfusion, since the blood is responsible for delivering hormones and nutrition support to the organs (Ziegler et al. 1999, Acosta & Miyamoto 2004).

With the use of high-resolution ultrasound and transducers, follicles ultrasound evaluation to monitor and detect ovulation in the bitch has been increasingly successful. This technique has yielded an accuracy of 91.7% to determine ovulation and is particularly accurate in small and medium breeds and nonobese animals (Lévy & Fontbone 2007). The transducer used in the present work allowed the ultrasonographers to easily identify the evaluated structures. We identified an optimal resolution to view ultrasound images for this purpose. The evaluations were facilitated by the fact that the animals in this study were of medium size and were also docile and calm. The B-mode ultrasound characteristics, measurements of ovary and follicle preovulatory cavity, the cytological and hormonal profiles were in agreement with those reported in the literature (England et al. 2003, Eker & Salmanoglu 2005). Thus, the bitches used in the study were healthy and showed no hormonal or structural pathologies.

The ultrasound images of the ovaries around ovulation are more difficult to analyze in bitches than in other species. Previous studies have shown that the ovarian follicles just before and after ovulation appear very similar. Some follicles do not collapse at the time of ovulation. Furthermore, non-ovulated follicles often remain after ovulation (Wallace et al. 1992, Silva et al. 1996, Lévy & Fontbonne 2007). However, it is possible to identify anechoic fluid in the ovarian bursa several days after ovulation (Eker & Salmanoglu 2005). In the present paper, daily evaluations detected ovulation in 100% of the dogs. The findings are similar to those described previously (Wallace et al. 1992, Silva et al. 1996, Lévy & Fontbonne 2007).

Doppler ultrasonography can be applied to noninvasively evaluate the uterine and ovarian vasculature *in vivo*. Studies applying this method in women have concluded that the uterine hemodynamics show cyclical changes throughout the menstrual cycle. The uterine arteries of infertile women showed increased resistance during the midluteal phase compared with those in fertile controls. Pregnancy rates were reduced during cycles with elevated uterine resistance (Ziegler et al. 1999). In the present study, a decrease in uterine blood perfusion was observed two days prior to ovulation. This parameter remained unchanged until ovulation. One day after ovulation, uterine perfusion gradually increased. This pattern was similar to those described in women (Ziegler et al. 1999), cows (Bollwein et al. 2000) mares (Bollwein et al. 1998, Bollwein et al. 2002) and capuchin monkeys (Domingues et al. 2007).

These findings are justified because before ovulation, blood flows from the uterine artery to the ovarian artery. This flow increases perfusion to the ovarian and intraovarian arteries and, therefore, the ovarian follicles. During the follicular phase, the endometrium undergoes vascular neoangiogenesis. This process forms new tributaries and lengthens the circulatory bed the uterine artery supplies. During this process, blood flow through the uterine artery may be reduced as a result of the blood vessel lengthening in the endometrium. During the luteal phase, the arterioles increase in diameter during the secretory phase, thereby increasing the blood flow to the uterine artery (Ziegler et al. 1999).

The assessment of ovarian vascularization is also very important. The percentage of pregnancies per cycle is significantly higher for embryos originating from highly vascular follicles compared to those from medium to poorly vascular follicles (Borini et al. 2004). In the present study, we observed that ovarian artery perfusion increases during ovulation. This change directs blood to the ovaries and increases intraovarian artery perfusion on the day after ovulation. These results are similar to those reported in previous studies examining bitches (Bicudo et al. 2010, Köster et al. 2001, Bergeron et al. 2013), capuchin monkeys (Domingues et al. 2007), mares (Bollwein et al. 2002) and women (Tan et al. 1996, Koster et al. 2001). The increase in the ovarian vessel diameter has also been identified by laparoscopy during the periovulatory period in bitches (Silva et al. 1996).

Occasionally, the uterine, ovarian and intraovarian arteries showed an oscillating blood perfusion pattern during that period, as reported by Bicudo et al. (2010). These patterns may reflect the influence of various angiogenic factors that cause biochemical and vascular changes and are responsible for CL formation (Acosta and Miyamoto 2004).

Doppler ultrasound has been widely used in female reproduction programs (Coulam et al. 1999). It has also been applied to characterize the ovarian arteries in cattle undergoing superovulation programs. The results from that study showed that the arteries are different compared to cattle on a normal cycle (Honnens et al. 2008). It is important to first characterize the features of these vessels in normal canine species to serve as a basis for future studies. The purpose is to provide information about the physiology of ovulation in this species; contribute to reproductive biotechnology, and provide an experimental model for wild canids.

CONCLUSION

Thus, we conclude that ovarian ultrasound and uterine and ovarian artery Doppler measurements are specifically altered during the periovulatory phase. When considered together, the measurements could be used to more accurately define the ovulation date, however these assessments must be performed serially by an experienced professional.

Acknowledgements.- To Grande Canafístula Kennel, Difirresus e Vivenda dos Pinheiros Kennels, for allowing us to use their bitches and to Laboratory of Carnivore Reproduction for providing support and infrastructure.

Funding.- Financial support for this study was obtained from several Brazilian funding agencies: Coordenação de Apoio a Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) through individual research grants to LDMS and fellowship to CCB and LDMS. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of Conflicting Interests.- The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

Acosta T.J. & Miyamoto A. 2004. Vascular control of ovarian function: ovulation, corpus luteum formation and regression. Anim. Reprod. Sci. 82-83:127-140.

Alvarez-Clau A. & Liste R. 2005. Ultrasonographic characterization of the uterine artery in the nonestrus bitch. Ultrasound Med. Biol. 31:1583-1587.

Bergeron L.H., Nykamp S.G., Brisson B.A., Madan P. & Gartley C.J. 2013. An evaluation of B-mode and color Doppler ultrasonography for detecting periovulatory events in the bitch. Theriogenology 79(2):274-83.

Bicudo A.L.C., Mamprim M.J., Lopes M.D., Vulcano L.C. & Derussi A.A.P. 2010. Conventional ultrasound examination and Dopplerfluxometry of ovarian of bitch, during the follicular phase of the oestral cycle. Vet. Zootec. 17:507-518.

Bollwein H., Maierl J., Mayer R. & Stolla R. 1998. Transrectal color Doppler sonography of the A. uterina in cyclic mares. Theriogenology 49:1483-1488.

Bollwein H., Meyer H.H.D., Maierl J., Weber F., Baumgartner U. & Stolla R. 2000. Transrectal Doppler sonography of uterine blood flow in cows during the estrous cicle. Theriogenology 53:1541-1552.

Bollwein H., Weber F., Kolberg B. & Stolla R. 2002. Uterine and ovarian blood flow during the estrous cycle in mares. Theriogenology 65:2129-2138.

Borini A., Tallarini A., Sciajno R. & Maccolini A. 2004. Colour power Doppler in infertility and ART. Rev. Gynecol. Pract. 4:230-234.

Bouchard F.G., Solorzano N., Concannon P.W. & Youngquist R.S. 1991. Determination of ovulation time in bitches based on teasing, vaginal cytology, and elisa for progesterone. Theriogenology 35:603-611.

Coulam C.B., Bustillo M., Soenksen D.M. & Britten S. 1994. Ultrasonographic predictors of implantation after assisted reproduction. Fertil. Steril. 62:1004-1010.

Coulam C.B., Goodman C. & Rinehart J.S. 1999. Colour Doppler indices of follicular blood flow as predictors of pregnancy after in vitro fertilization and embryo transfer. Hum. Reprod. 14:1979-1982.

Domingues S.F.S., Caldas-Bussiere M.C., Martins N.D. & Carvalho R.A. 2007. Ultrasonographic imaging of the reproductive tract and surgical recovery of oocytes in *Cebus apella* (*Capuchin monkeys*). Theriogenonogy 68:1251-1259.

Eker K. & Salmanoglu M.R. 2006. Ultrasonographic monitoring of follicular development ovulation and corpora lutea formation in a bitch. Turk. J. Vet. Anim. Sci. 30:589-592.

England G.C.W., Moxon R. & Freeman S.L. 2012. Delayed uterine fluid clearance and reduced uterine perfusion in bitches with endometrial hyperplasia and clinical management with postmating antibiotic. Theriogenology 78:1611-1617

England G.C.W., Yager A.E. & Concannon P.W. 2003. Ultrasound imaging of the reproductive tract of the bitch. Disponível em <www.ivis.org>

Ferreira A.M., Pires C.R., Moron A.F., Araújo Júnior E., Traina E. & Mattar R. 2007. Doppler assessment of uterine blood flow in recurrent pregnacy loss. Int. J. Gynecol. Obst. 98:115-119.

Honnens A., Niemann H., Paul V., Meyer H.H.D. & Bollwein H. 2008. Doppler sonography of the uterine arteries during a superovulatory regime in cattle Uterine blood flow in superovulated cattle. Theriogenology 70:859-867.

Köster K., Poulsen Nautrip C. & Gunzel-Apel A.R. 2001. A Doppler ultrasonographic study of cyclic changes of ovarian perfusion in the Beagle bitch. Reproduction 122:453-461.

Lévy X. & Fontbonne A. 2007. Determining the optimal time of mating in bitches: particularities. Revta Bras. Reprod. Anim. 31:128-134. Disponível em <www.cbra.org.br>

Nargund G., Bourne T., Doyle P., Parsons J., Cheng W., Campbell S. & Collins W. 1996. Association between ultrasound indices of follicular blood flow, oocyte recovery and preimplantation embryo quality. Hum. Reprod. 11:109-113.

Pan H.A., Wu M.H., Cheng Y.C., Wu L.H. & Chang F.M. 2004. Quantification of ovarian stromal Doppler signals in poor responders undergoing in vitro fertilization with three-dimensional power Doppler ultrasonography. Am. J. Obstet. Gynecol. 190:338-344.

Polisca A., Zelli R., Troisi A., Orlandi R., Brecchia G. & Boiti C. 2012. Power and pulsed Doppler evaluation of ovarian hemodynamic changes during diestrus in pregnant and nonpregnant bitches. Theriogenology 79(2):219-24.

Silva L.D.M., Onclin K. & Verstegen J.P. 1996. Assessment of ovarian changes around ovulation in bitches by ultrasonography, laparoscopy and hormonal assays. Vet. Radiol. Ultrasound 37:313-320.

Steer C.V., Campbell S., Pampiglione J.S., Kingsland C.R., Mason B.A. & Collins W.P. 1990. Transvaginal colour flow imaging of the uterine arteries during the ovarian and menstrual cycles. Hum. Reprod. 5:391-395.

Steer C.V., Tan A.L., Mason B.A. & Campbell S. 1994. Midluteal-phase vaginal color Doppler assessment of uterine artery impedance in a subfertile population. Fertil. Steril. 61:53-68.

Szatmári V., Sótonyi P. & Vörös K. 2001. Normal duplex doppler waveforms of major abdominal blood vessels in dogs: a review. Vet. Radiol. Ultrasound 42:93-107.

Tan S.L., Zaidi J., Campbell S., Doyle P. & Collins W. 1996. Blood flow changes in the ovarian and uterine arteries during the normal menstrual cycle. Am. J. Obstet. Gynecol. 175:625-631.

Uchoa D.C., Silva T.F.P., Mota Filho A.C. & Silva L.D.M. 2012. Intravaginal artificial insemination in bitches using frozen-thawed semen after dilution in powdered coconut water (ACP-106c). Reprod. Domest. Anim. 47:1-4.

Wallace S.S., Mahaffey M.B., Miller D.M., Thompson F.N. & Chakraborty P.K. 1992. Ultrasonographic appearance of the ovaries of dogs during the follicular and luteal phases of the estrus cycle. Am. J. Vet. Res. 53:209-215.

Yang J.H., Wu M.Y., Chen C.D., Jiang M.C., Ho H.N. & Yang Y.S. 1999. Association of endometrial blood flow as determined by a modified colour Doppler technique with subsequent outcome of in-vitro fertilization. Hum. Reprod. 14:1606-1610.

Ziegler W.F., Bernstein I., Badger G., Leavitt T. & Cerrero M.L. 1999. Regional hemodynamic adaptation during the menstrual cycle. Obstet. Gynecol. 94:695-699.