Jaboticabal, Brazil

Quantitative ultrasound elastography and biometry of the bitch uterus in the early puerperium after vaginal delivery and caesarean section

"Postpartum ultrasound in bitches"

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Abstract

The aim of this study was to evaluate and compare uterine tissue biometry and stiffness in bitches submitted to c-section or normal delivery during the postpartum period by ultrasound. Twelve healthy brachycephalic bitches weighing 10.5 ± 3.3 kg and age of 2.56 ± 0.89 years were evaluated. Sonographic evaluations were performed once a day immediately after delivery until 10th day postpartum. Uterine thickness (mm) and shear wave velocity (SWV; m / s) were evaluated by B-mode ultrasound and ARFI (Acoustic Radiation Force Impulse) elastography, respectively. Specifics softwares designed for qualitative and quantitative image analysis (Virtual Touch Tissue Quantification - VTQ ™ and Virtual Touch Tissue Imaging Quantification - VTIQ ™; Siemens®, Munique, Alemanha) were used to perform the analysis. The sonographic parameters were compared by ANOVA and Pearson correlations that were used to determine the relationship between the SWV's techniques. Statistical significance was set at 95% ($P < 0.05$). Uterine thickness ($P = 0.012$) in c-section (group 1 - G1) (15.26 ± 4.73 mm) was higher than in normal delivery (group 2 - G2) (12.53 ± 2.64 mm) from 1st to 5th puerperal day; the thickness of the uterine myometrium-endometrium, myometrium and endometrium presented gradual and significant involution, similar between both groups (G1 - 6.27 ± 1.66 mm; and G2 - 6.38 ± 2.05 mm ($P = 0.557$), from 1st to 7th day ($P < 0.0001$), myometrial thickness in G1 (3.63 ± 1.02 mm) and G2 (3.53 ± 1.06 mm) ($P = 0.854$), presented involution from the 1st to 9th ($P = 0.005$) and endometrial thickness in G1 (2.64 ± 1.04 mm) and G2 (2.76 ± 1.18 mm) ($P = 0.557$), from 1st to 6th day ($P = 0.003$). Myometrial SWV was higher than endometrial, regardless of the type of delivery. The VTQ ™ SWV of the myometrium ($P = 0.0411$) and endometrium ($P = 0.0043$) was similar on the days after delivery between groups and presented a gradual increase, with G1 myometrium SWV of 2.20 ± 0.79 m / s and G2 2.10 ± 0.72 m / s (P =

0.7846); the endometrial SWV in G1 of 1.70 ± 0.56 m / s and G2 of 1.60 ± 0.51 m / s (P = 0.8273). VTIQ ™ myometrial and endometrial SWV (P = 0.7519) were similar on the days after delivery between groups, with G1 myometrial SWV of 2.20 ± 0.43 m / s and G2 of 2.30 ± 0.37 m / s (P = 0.7048); the endometrial SWV in G1 of 2.00 ± 0.41 m / s and G2 of 2.00 ± 0.36 m / s (P = 0.7048). It was concluded that bitches with eutocic delivery had smaller uterine thickness and faster puerperal involution than animals submitted to c-section. Uterine tissue stiffness increased gradually during postpartum throughout uterine involution and was similar for bitches with normal delivery and c-section.

KEYWORDS: biometrics, canine, elastography, puerperium, uterine

1. INTRODUCTION

The postpartum phase is characterized by physiological changes that occur in the period following the end of delivery, in which the female's genital system will progressively return to normal condition and pre-pregnancy size. Uterine involution is accompanied by adequate uterine tone for loci elimination and epithelial regeneration (Voorwald & Tiosso, 2015).

Bitches exhibit serous-bloody vaginal discharge during puerperium, which lasts 1-6 weeks, being a period more susceptible to uterine diseases, in which pathological conditions may develop (Feldman & Nelson, 1986). Follow-up of uterine involution is essential for the establishment of future pregnancies, since failure to complete regression can lead to postpartum endometritis / metritis and placental retention (Hirt et al., 2000; Magata et al., 2013).

Ultrasonography has an important role in clinical studies of canine reproduction for reproductive tract evaluation, being accurate, innocuous and providing real-time information on ovulatory process, pregnancy, embryonic loss and puerperium (England & Russo, 2006; Davidson & Baker, 2009; Yilmaz & Uçar, 2012). In contrast to other mammalian domestic species, ultrasonographic information of the uterus during postpartum in bitches is scarce (Yeager & Concannon, 1990; Ferri & Vicente, 2002; Ferri et al., 2003; Orfanou et al., 2009; Barbosa et al., 2013).

Elastography is a recent, non-invasive, fast and easy-to-perform ultrasound method for studying the elastic properties of tissue (Karaman et al., 2016). In addition, elastographic evaluations can be performed using the qualitative technique, compression or strain elastography designated "static or semi-static" and the quantitative technique, sonoelastography and ARFI which employs compression waves, as a "dynamic" technique (Feliciano et al., 2015a; Maronezi et al., 2019). Applications in gynecology and obstetrics have recently been documented (Stoelinga et al., 2014; Fuchs et al., 2013), in medicine there is only one study on uterine and cervical stiffness after placental delivery (Tanaka et al., 2011). In veterinary medicine, ARFI has been used to detect pathological changes in canine spleen, kidneys of cats as well as in liver, prostate gland, and testes of dogs (Holdsworth et al., 2014; Feliciano et al. 2015b,c; Garcia et al., 2015; Maronezi et al., 2015), canine and feline mammary glands (Feliciano et al., 2017; Feliciano et al., 2015), metastasis in canine lymph nodes (Silva et al., 2018a) canine and ovine fetal lung and liver (Simões et al., 2018; Silva et al., 2018b), however there are no elastographic studies reporting the use of ARFI for evaluation of changes in uterine stiffness during postpartum involution in animals.

With these precepts, it is hypothesized that: i) there are differences in uterine biometrics reduction during the puerperal phase of bitches submitted to normal / c-section delivery evaluated by B-mode; ii) alteration in uterine tissue stiffness by elastography during normal / c-section postpartum. Thus, the aim of this study was to evaluate and compare the biometry and the quantitative elastographic characteristics and reference ranges for shear wave velocitiy of canine uterine structure during ten days of postpartum in bitches submitted to c-section or normal delivery.

2. MATERIAL AND METHODS

All experimental procedures were approved by the Animal Ethic and Welfare Committee (Univ. Estadual Paulista) protocol N° 9.884/16. Twelve brachycephalic, primiparous or multiparous bitches (body weight 10.5 ± 3.3 kg and age of 2.56 ± 0.89 years), clinically healthy (after revision of clinical history, general examination, hematology and biochemical ALT, creatinine and blood glucose dosage), of different breeds (eight French bulldogs, two Pugs and two Shih-tzus) owned by commercial dog breeders were used in this study.

Females were evaluated daily to detect clinical and ultrasonographic signs indicative of parturition moment, using formulas to calculate gestational age (Gestational age (days) = (6 × parietal diameter) + (3 × abdominal diameter) + 30) (Beccaglia et al., 2016; Feliciano & Assis, 2019) and days for parturition (biparietal diameter = (mm - 25.11) / 0.61 (small size) and biparietal diameter = (mm - 29.18) / 0.7 (medium size)) (Luvoni & Grioni, 2000).

The bitches were selected and divided in two experimental groups: 1) group 1 - G1: animals submitted to c-section; and 2) group 2 - G2: animals with normal delivery. In general, pregnant females were monitored daily through obstetric assessments (including gestational ultrasound) to verify mucoid vulvar discharge, body temperature drop, maternal restlessness, fetal viability and maturity, pelvic and abdominal muscle relaxation, uterine contractions and cervical dilation, signs indicative of parturition proximity (Laliberte, 1986; Johnston, 1986).

Females who had uterine contractions longer than two hours and no onset of labor or no progression or retention between fetuses (Pretzer, 2008), with a fetal heart rate of $\leq$ 160 beats per minute characterizing fetal distress and dystocic delivery were referred for cesarean section (Gil et al., 2014).

For c-section, as pre-anesthetic medication metoclopramide (0.5 mg / kg) was administered, anesthetic induction was performed with propofol at dose required for intubation, followed by epidural with lidocaine (4 mg / kg) and subsequent maintenance with isoflurane. In the immediate postoperative period, tramadol (4 mg / kg) and benzylpenicillin (20,000 IU / kg) were instituted; and dipyrone (25 mg / kg) every 12 hours for 5 days for postoperative treatment. The surgical technique was based on hysterotomy performed on the uterine body near the bifurcation, hysterorrhaphy was performed in two planes with invaginating continuous sutures, using needled caprofyl 3-0; Caprofyl 0 and 2-0 were used for muscle and intradermal suture, through separate and continuous suture, respectively (Fossum et al., 1997; Gilson, 2007).

The morphophysiological development of the puppies was verified weekly by clinical examinations until the 60th day postpartum, when the pups were sold.

Ultrasonographic uterine evaluation (Acuson S2000™ ultrasound scanner; Siemens®, Munich, Germany, equipped with a 9.0 MHz linear transducer) was performed in an acclimatized room at approximately 21◦ C every 24 hours, starting after parturition and extended up to 10th day postpartum. To perform ultrasonographic exams, a wide trichotomy of the abdominal region was previously realized. The transducer was positioned in the caudal abdominal region and all adjustable settings of the ultrasonic device (e.g., depth, gain, mechanical index and focal zones) were optimized and then left unchanged for the entire study period. All ultrasonographic examinations were performed by a single experienced operator (5 years) to reduce evaluation time and stress endured by lactating animals.

Postpartum uterine tissue was measured in longitudinal section by ultrasound, assessing: uterine diameter (from serous to serous), uterine wall thickness (from serous to lumen), endometrium and myometrium (Figure 1). The presence of intraluminal content was also evaluated.

Following B-mode ultrasound, quantitative elastographic (ARFI) of the uterine wall (myometrium and endometrium) were obtained using two specific software designed for tissue stiffness image analyses: 1) VTQ™ (Virtual Touch™ Tissue Quantification; Siemens®, Germany); and 2) VTIQ™ (Virtual Touch Tissue™ Imaging Quantification; 2D-SWE technique, Siemens®, Germany) previously validated for the present application by Feliciano et al. (2014). Real-time quantitative elastographic attributes, or shear wave velocities (SWV m/s) of the tissues, were obtained placing an electronic calliper with fixed dimensions (VTQ™; 5 x 5 mm; Figure 2) and (VTIQ™; 1 x 1 mm; Figure 3) within the parenchyma of each organ at three different locations (cranial, caudal, central), with the depth ranging from 0.5 to 2.0 cm.

Statistical analysis was performed using the R® statistical software (R Foundation for Statistical Computing; Vienna, Austria), using a block (bitches) randomized experimental design, with parcels subdivided in time (postpartum days). Residual normality (Shapiro test) and homoscedasticity of variances (Barlett's test) were previously tested. The variation between measurements of the three regions of interest in each uterine portion was studied by the Bland-Altman concordance test. SWV averages and uterine thickness were compared between the types of parturition evaluated and postpartum days by the analysis of variance (ANOVA) with repeated measures. The parameters that resulted significant at ANOVA test were tested the adjustments to mathematical regression models (linear, quadratic and cubic) or orthogonal contrasts. The relationship between the elastographic techniques used was studied by the Pearson correlation test. The statistical significance was set at 95% (*P* value < 0.05).

3. RESULTS

All animals had normal gestations and there were no apparent abnormalities in post-partum examinations. A total of 70 foetuses was born (5.83 ± 1.33 puppies /bitch), the mean litter size in French bulldog was (6.12 ± 1.24 puppies/bitch), in the Pug (4.50 ± 2.12 puppies /bitch) and in the Shih-tzu (6.00 ± 0 puppies /bitch). B-mode ultrasonography and ARFI were performed without difficulty and did not cause evident morphological changes for mother and puppies during the first 60 days after delivery. All bitches nursered and puppies from all litters were weaned at 45 days postpartum.

B-mode uterine thickness was higher ($P = 0.012$) during postpartum in c-section animals (group 1 - G1) (15.26 ± 4.73 mm) compared to normal delivery (group 2 - G2). (12.53 ± 2.64 mm), in which involution was considered significant in G1 from the 1st to the 5th puerperal day and in G2 from the 2nd day (Figure 4).

The thickness of the myometrium, endometrium and myometrium-endometrium presented gradual and significant involution, similar between both groups. The myometrial in G1 of 3.63 ± 1.02 mm and G2 of 3.53 ± 1.06 mm ($P = 0.854$), with involution from the 1st to the 9th day ($P = 0.005$), characterized by linear regression: (MedMiom = 4.463 - 0.1660 DPP; $P < 0.0001$ and R2 = 21%); the endometrial in G1 of 2.64 ± 1.04 mm and G2 of 2.76 ± 1.18 mm ($P = 0.557$), with involution from the 1st to the 6th day ($P = 0.003$), characterized by linear regression: (MedEndom = 3.347 - 0.1257 DPP; $P = 0.04$ and R2 = 10%) (Figure 5).

The thickness of the myometrium-endometrium in G1 of 6.27 ± 1.66 mm and G2 of 6.38 ± 2.05 mm ($P = 0.557$), with involution from the 1st to the 7th day ($P < 0.0001$) characterized by linear regression: (MedME = 7.856 - 0.2923 DPP; $P = 0.001$ and R2 = 22%) (Figure 6).

In normal delivery group there was rapid involution and it was not possible to calculate a regression formula to estimate uterine size over time ($P = 0.3960$). In the c-section group there was progressive involution, calculated by regression: (MedUterus = 19.3 - 0.8 DPP; $P = 0.0001$ and R2 = 84%).

In the assessment of tissue stiffness by the VTQ™ quantitative elastographic technique (Virtual Touch™ Tissue Quantification; 2D-SWE technique, Siemens, Germany), myometrial ($P = 0.0411$) and endometrial ($P = 0.0043$) SWV were similar in the days after delivery between groups and presented a gradual increase. The G1 myometrial SWV was 2.20 ± 0.79 m / s and G2 was 2.10 ± 0.72 m / s (P = 0.7846), characterized by linear regression: (ComVelMiom = 1.68 + 0.1 DPP; P <0.0021 and R2 = 54%); endometrial SWV in G1 was 1.70 ± 0.56 m / s and G2 was 1.60 ± 0.51 m / s (P = 0.8273), characterized by linear regression: (ComVelEndom = 1.32 + 0, 07 DPP; P <0.0005 and R2 = 49%) (Figure 7).

In the assessment of tissue stiffness by the VTIQ ™ quantitative elastographic technique (Virtual Touch™ Tissue Imaging Quantification; 2D-SWE technique, Siemens, Germany), myometrial and endometrial SWV (P = 0.7519) were similar in the days after delivery between groups. Being the SWV of the myometrium in G1 of 2.20 ± 0.43 m / s and G2 of 2.30 ± 0.37 m / s (P = 0.7048); that of the endometrium in G1 of 2.00 ± 0.41 m / s and G2 of 2.00 ± 0.36 m / s) (P = 0.7048) (Figure 8).

Comparing the uterine structures, the mean myometrial SWV by VTQ ™ and VTIQ ™ was higher than the endometrial SWV, regardless of the type of delivery, showing a gradual increase in the first 10 days postpartum.

4. DISCUSSION

Based on the results of the present study, the use of B-mode and ARFI ultrasonography in uterus of brachycephalic bitches, performed once a day immediately after delivery until 10th postpartum day, was feasible and applicable, causing no evident changes in maternal health, as described in elastographic studies of women (Tanaka et al. 2011) and biometric in bitches (Ferri e Vicente; 2002; Ferri et al., 2003; Barbosa et al. 2013).

The type of delivery influenced the diameter of the uterine body, measured at B-mode of 12.53 ± 2.64 mm (normal delivery) and 15.26 ± 4.73 mm (c-section) equivalent to the value reported by Barbosa et al. (2013) of 1.11 ± 0.16; 1.24 ± 0.31 cm, respectively, being higher in bitches submitted to c-section compared to normal delivery, highlighting reduction during the first week postpartum.

Differences in uterine involution between the two types of delivery underscore lower rates and rapid involution in the normal delivery group, which may be explained by the hormonal and physiological changes that are higher in eutocic labor compared to c-section, due to the more effective action of PGF2α, which has a vasodilation function and stimulates oxytocin to produce uterine contractions (Hoffmann et al., 1996; Barbosa et al., 2013).

Ferri et al. (2003) comment on whether surgery has an influence on the pattern of uterine regression during the first seven days postpartum compared to normal delivery. According to Mijten et al. (1997), inadequate manipulation and trauma of uterine tissues during surgery and complications such as hemorrhage and ischemia are more important than the type of thread and suture applied. Differently, Voorwald & Tiosso (2015) mention that the surgical incision performed during c-section has no effect on puerperal progression.

The sonographic characteristics of the myometrial and endometrial stratification verified during the first two weeks postpartum corroborate the histological descriptions, that is, the myometrium is thick and with intertwined bundles of collagen fibers between muscle bundles of the longitudinal and external circular muscle layers; and the endometrium is composed of primary folds formed by the swollen lamina propria and secondary folds related to rough coating, with proliferation of collagen fibers and slight infiltration of mononuclear cells of the endometrial lamina propria (Al-Bassam et al.,1981). The uterine lumen was evidenced during the study period with normal locus expulsion discarding pathological processes, consistent with Barbosa et al. (2013).

Comparing the myometrial and endometrial diameter individually, the first one (3.53 ± 1.06 mm) was larger than the second (2.76 ± 1.18 mm), both presenting significant and gradual involution in all females, corroborating with Yeo et al. (2007) who found a rapid decrease in myometrial (4.70 to 3.33 mm) and endometrial (8.80 to 3.63 mm) thickness in miniature Schnauzer bitches during 7 days after eutocic delivery. There are no literary statements to compare current results with the postpartum period after c-section, as well as the thickness of the myometrium-endometrium.

Nursing may also have a positive influence on female uterine involution, as uterine contractions occur in association with milk release during the immediate postpartum period (Chua et al., 1994), generated by neurohypophysis oxytocin release in response to the stimulation of the mammary gland and the auditory promotion of the vocalization of the newborn (Uvnas-Moberg & Eriksson, 1996). Myometral contractions promote decrease in uterine volume, particularly lumen and uterine wall thickening (Grunert & Birgel, 1989). Yeager & Concannon (1990) described that throughout the puerperium, the size of the organ changes and decreases progressively, between one to four days, the musculature declines the ability to react to contraction inducing stimulation with involution of 60% of its volume compared to the end of pregnancy (Landim-Alvarenga, 2006b), concomitant to regenerative changes in glandular and epithelial structures (Orfanou et al., 2009; Chu et al., 2002).

To date, there are no reports on the use of ARFI to assess changes in uterine stiffness during postpartum in animals. Tanaka et al. (2011) were the first researchers to quantify involution and change in uterine stiffness in women with normal childbirth, using uterine SWV VTQ ™ before delivery, immediately and 1 and 2 hours after placental expulsion (1,81 ± 0,60; 3,04 ± 0,76; 3,12 ± 0,95 e 2,72 ± 0,81 m/s, respectively), noting that there is an association of strong uterine contraction, initiated immediately after delivery, followed by increased uterine body rigidity over time, similarly to the present study, which recorded a gradual increase in myometrial and endometrial SWV VTQ ™ (2,10 ± 0.72, 1.60 ± 0.51 m / s, respectively) on the days after delivery.

Comparing the uterine structures, the mean SWV by VTQ ™ and VTIQ ™ of the myometrium was higher than the endometrial SWV, regardless of the type of delivery, presenting a gradual increase. This behavior can be explained by the histophysiological changes that occur in the process of uterine involution, in which the smooth muscles of the myometrium decrease in length, with thickening of the wall, formation of folds in the mucosa and consequent decrease in organ volume, particularly the lumen, with intense production of locus; similarly the endometrium suffers a decrease in surface, with formation of folds and production of locus (Landim-Alvarenga, 2006b). The authors consider it advisable to use any of the software referenced here.

This is the first study that evaluates changes in physiological uterine elasticity in female dogs, defining normal patterns for the species, with the precept that pathological conditions may alter tissue stiffness and develop during the postpartum period, when they are more susceptible to disease, such as endometritis / metritis, fetal or placental retention, postpartum haemorrhage caused by genital tract trauma or placental subinvolution (Feldman & Nelson, 1986; Pharr et al., 1992). For this reason, the results of this study may provide support for early diagnosis and therapeutic follow-up of canine females with postpartum uterine alterations, post c-section uterine healing dynamics and information that may imply the prediction of possible complications in future pregnancies, such as rupture of gestational uterus (Ferri et al., 2003), preserving the reproductive life of breeders and reducing zootechnical, genetic and economic losses of kennels and breeders derived from castration, morbidity and maternal mortality.

5. CONCLUSION

Canine uterine biometric and elastographic evaluation was feasible. The results reveal that female dogs with eutocic delivery had smaller uterine thickness and rapid puerperal involution than animals submitted to c-section. Postpartum uterine tissue stiffness gradually increased with uterine involution and was similar for bitches with normal delivery and c-section. This suggests that the recorded standards are significant and provide important unpublished information, validating the techniques and reference values in the investigation of intrauterine integrity. Moreover, it is a prosperous tool for the recognition and differentiation of the pathological pueperium in mammalian species of veterinary interest.

ACKNOWLEDGEMENTS

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for the research grant and productivity scholarship award (processes 140189/2017-3 and 309199/2017-4), Sao Paulo Research Foundation (FAPESP) for the thematic project scholarship (process 2017/14957-6) and Jair Matos from Siemens Healthineers for technical assistance.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures Legends:

Figure 1. High resolution ultrasonographic image in longitudinal section of the uterine structure (myometrium (M), endometrium (E) and uterine diameter (U)) in a bitch on the 3rd puerperal day.

Figure 2. Image of the quantitative ARFI VTQ ™ elastography, in longitudinal section, of the canine uterine endometrium (green caliper) on the 4th postpartum day (SWV of 1.07 m / s and 1.8 cm depth).

Figure 3. Image of the quantitative ARFI VTIQ ™ elastography, in longitudinal section, of the canine uterine myometrium (M) and endometrium (E) on the 9th postpartum day.

Figure 4. Graphical representation of the mean total uterine diameter during the physiological postpartum period of normal delivery and c-section in 12 brachycephalic bitches.

Figure 5. Graphical representation of the myometrial (A) and endometrial (B) mean diameter during the physiological postpartum of normal delivery and c-section in 12 brachycephalic bitches.

Figure 6. Graphical representation of the mean diameter of the uterine myometrium-endometrium during the physiological postpartum period of normal delivery and c-section in 12 brachycephalic bitches.

Figure 7. Graphical representation of the SWV (m / s) mean of the myometrium (A) and endometrium (B) by the VTQ ™ quantitative elastographic technique during the physiological postpartum of normal delivery and c-section in 12 brachycephalic bitches.

Figure 8. Graphical representation of the SWV (m / s) mean of the myometrium (A) and endometrium (B) by the VTIQ ™ quantitative elastographic technique during the physiological postpartum of normal delivery and c-section in 12 brachycephalic bitches.