UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

ULTRASONOGRAPHY B-MODE, ELASTOGRAPHY (ACOUSTIC RADIATION FORCE IMPULSE), COLOR DOPPLER AND HYSTEROSCOPY UTERINE IN POSTPARTUM IN SANTA INES SHEEP

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para obtenção do título de Doutor em Medicina Veterinária área de Reprodução Animal.

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TÍTULO DA TESE: ULTRASONOGRAPHY B-MODE, ELASTOGRAPHY (ACOUSTIC RADIATION FORCE IMPULSE), COLOR DOPPLER AND HYSTEROSCOPY UTERINE IN POSTPARTUM SANTA INES SHEEP

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AUTHOR CURRICULUM DATA

RENATA SITTA GOMES MARIANO – was born in Garça, São Paulo state, Brazil, on May 11th of 1990. In 2008 she joined the Faculty of Veterinary Medicine in Garça, concluding her academic experience on December 2012. In 2013, she began a Master student in Animal Reproduction Science, at Faculty of Agricultural and Veterinary Sciences – FCAV/Unesp in Jaboticabal under Prof. Dr. Wilter Ricardo Russiano Vicente. She concluded her Master's degree and on 2015 she began her PhD at the same university, and field, under Dr. Vicente supervisor. Where in 2017 she moved to the College Station, Texas, United States, to join researches on fetal programming at Texas A&M University during four months in Animal Science department under Dr. Rodolfo C Cardoso.

“I never lose. I either win or I learn.”

(Nelson Mandela)

“Though nobody can go back and make a new beginning, anyone can start over and make a new ending.”

(Chico Xavier)

To my Dad and Mom, with love.

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SUMMARY

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Certificamos que o Protocolo nº 12338/15 do trabalho de pesquisa intitulado **"Ultrassonografia modo B e Doppler uterino de ovelhas em diferentes momentos reprodutivos"**, sob a responsabilidade do Prof. Dr. Wilter Ricardo Russiano Vicente, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 06 de julho de 2015.

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ULTRASONOGRAPHY B-MODE, ELASTOGRAPHY (ACOUSTIC RADIATION FORCE IMPULSE), COLOR DOPPLER AND HYSTEROSCOPY UTERINE IN POSTPARTUM IN SANTA INES SHEEP

ABSTRACT – The aim of this study was to evaluate the uterine characteristics of Santa Inês sheep during the postpartum period, using mode B-mode ultrasonography, elastography ARFI (acoustic radiation force impulse), Doppler and hysteroscopy, with emphasis on the early diagnosis of reproductive alterations, evaluation of development and regression during this period**.** Twenty Santa Inês sheep were used and designated after clinical and obstetric evaluation. B-mode, Doppler and elastography ARFI evaluations were performed by transabdominal approach using the Siemens S2000 ultrasound system, with a multi-frequency and convex transducer of 5.0 to 8.0 MHz. Ultrasonography of the uterine structure was performed at immediate postpartum (M0) and sequentially every 48 hours, during 30 days, totaling 16 experimental samples.

Ultrasonography characteristics of the uterus (echogenicity, echotexture, and biometry), vascular parameters (color Doppler) and stiffness aspects (qualitative and quantitative elastography) of the uterine structures were evaluated during the postpartum. Hysteroscopy evaluation of the uterine involution of the sheep was performed as follows: immediate moment after lambing (M0) and sequentially every 6 hours, until the moment where the endoscopic access to the uterus through the cervix was no longer possible. Uterine biopsy was performed at the same moments. The experimental design will be completely randomized, with a significance level of 5% for tests performed.

Keywords: ovine, ultrasound, puerperium, uterus.

LIST OF ABBREVIATIONS

ARFI – Acoustic radiation force impulse

SD – standard deviation

% - percentage;

Kg – kilogram;

h – hour;

M0 – immediate postpartum moment

n – sample size

pp – days postpartum

SWV – Shear wave velocity

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CHAPTER 1 – GENERAL CONSIDERATIONS

1. Introduction

Puerperium is the period after completion of parturition and it occurs in a decreasing logarithmic scale, especially during the first week after lambing. After parturition, the uterus undergoes marked remodeling during involution; however, little is known about the hormonal, cellular and molecular mechanisms that regulate this process. Considering the requirement to establish early diagnostic methods for the evaluation of puerperal changes in sheep, monitoring of this period may be essential to avoid a decline in reproductive efficiency. There are conflicting reports regarding uterine involution in ewes, which may reflect differences in breeds and management. An understanding of the postpartum recovery process of the reproductive tract in the ewe is of increasing importance in production systems where more than pregnancy per year is desirable.

It was hypothesized that the use of imaging techniques allows the monitoring of uterine involution and physiological changes during the postpartum period in ewes.

In this study we review the puerperal period, the mechanisms underlying the ensuing of normal ovine reproduction, and the ultrasound assessment to evaluate this period in ewe as an experimental model or for application in veterinary medicine.

2. Literature Review

Improving reproductive efficiency in domestic farm animals is critically important to the livestock industry, ewes must be breed within 30 to 40 days after parturition (Abecia et al., 2017).

For intensive sheep production, the postpartum period is of most importance for the continuity of the reproductive function of females and may vary from 17 to 40 days (Zdunczyk et al., 2004; Fernandes et al., 2013), being influenced by breed, season, management, intercurrences during partum, number of births and age (Zdunczyk et al., 2004). Nutrition also is essential for reproductive efficiency of ruminants, affecting directly the duration of postpartum anestrus (Diskin et al., 2003; Rhind, 2004)

The postpartum period has also a reproductive and economic importance that is influential in achieving a satisfactory interval between partums and impact the number of offsprings produced per year (Sanchez et al., 2002), mainly under intensive accelerated production systems (Greyling, 2000; Ababneh and Degefa, 2005).

Indeed, the influences that contribute to a low productivity in the herds are associated with delayed uterine involution due to inflammatory and/or infectious processes of the uterus, disorders in endometrial regeneration, anestrus and delay in the onset (Sheldon et al., 2017). In the prepartum, there are risks of physical damage during the birth process and also an upsurge of microbial infections in the ewe. Therefore, uterine involution may be delayed in case of occurrences of dystocia, number of fetus and abortions (Sheldon et al., 2006; Benzaquen et al., 2007), as well as uterine prolapse and placental retention among other disorders (Gomes et al., 2014). Regarding uterine pathologies, it is also important to mention endometritis, pyometra and hydrometra/mucometra (Medan and El-daek, 2015).

Ultrasonography in small ruminants' production has been increasing the routine applicability and play a role being responsible for expressive benefits to the reproductive management, enabling puerperal evaluation and consequent uterine involution of sheep in real time, and being a practical tool (Lohan et al., 2004; Hajurka et al., 2005). Actually, it is the only non-invasive techniques that may reveal details of the progressive changes in the uterus of ewes (Ioannidi, 2017).

2.1 Anatomy and Physiology of Postpartum

The uterus of domestic animals consists in: the corpus or body and its endometrium, two horns and the uterine cervix. Uterine wall has three different layers: endometrium; myometrium, and perimetrium (Akers and Denbow, 2008). The perimetrium and the myometrium, share a similar echogenic structure, so an ultrasound differentiation is not possible. The uterine endometrium of ewes has both aglandular caruncular and glandular intercaruncular areas (Hafez, 2004; Wang et al., 2013). Myometrium is hormone sensitive and undergoes both hypertrophy and hyperplasia during pregnancy, progressively returning to its normal size during the postpartum period (Lowe and Anderson, 2015). Placentation in sheep (synepitheliochorial) involves growth and union of placental cotyledons with endometrial caruncles developing placentomes, which are the primary sites of conceptus-maternal exchange for gases and micronutrients, such as amino acids and glucose (Bazer et al., 2012). The ovine cervix is a long, fibrous tubular composed predominantly of connective tissue with an outer serosal layer and inner luminal epithelium. The lumen is highly convoluted and tortuous due to the presence of cervical rings and provide a physical barrier to external contaminants (Kershaw et al., 2005).

In ovines, gestation lasts on average 150 days and may vary due to interference of maternal, fetal and genetic factors. If this does not occur, a new estrous cycle will begin at an average interval of 17 days (Oliveira et al., 2013). During the beginning of pregnancy, the uterus is located in the pelvic cavity, similarly to non-pregnant status. In mid-gestation and later stages of gestation, it becomes an abdominal organ.

After lambing and before subsequent gestation, four events must occur concomitantly: uterine involution, endometrial regeneration, elimination of bacterial contamination and return of ovarian cycling. The uterine involution begins immediately after lambing and involves physical shrinkage, necrosis and sloughing of caruncles, and also the regeneration of the endometrium. Sloughing of the uterine caruncles contributes to reduction in weight of the uterus because they take over half of the weight of the uterus. There is initially regeneration of the endometrium in the inter-caruncular areas and then by centripetal growth of the cells over the caruncle (Sheldon, 2004, 2008).

As puerperium advances, the involution processes also involve regressive changes in the uterus, as endometrial regeneration, and cervix (Bajcsy, 2005). There is a progressive decreasing in vaginal discharge and in uterine and cervical diameters. It occurs due to vasoconstriction and peristaltic contractions (Leslie, 1983; Wehrend and Bostedt, 2003), remodeling of caruncles, regeneration of endometrial tissue, reduction in uterine blood flow and endometrial vascularity, as well as reduction of smooth muscle mass (Guilbault et al., 1984; Slama et al., 1991). The cervical canal is still widely opened immediately after birth, but elements of the soft birth canal still provide a certain degree of closing, because in a physiological situation the surrounding tissues and organs of the abdominal and pelvic cavity compress the cervix.

A balanced and coordinated endocrine system is important for normal reproductive function, that is, the returning of ovarian cycling. This involves homeostasis among gonadotrophin releasing hormones (GnRH) from the hypothalamus; follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) from the adenohypophysis; prostaglandin F2-alpha (PGF2α) from the uterus and the gonadal steroids (Kota et al., 2013). After parturition, steroid hormone concentrations decrease to basal values and there is an increase in plasma FSH concentration within days of calving that stimulates the emergence of the first postpartum follicular wave (Sheldon, 2008). Thus, the ability to achieve maximum reproductive efficiency depends on a thorough understanding of postpartum hormonal changes.

The postpartum fertility of sheep depends on uterine physiological involution and the resumption of cyclic ovarian activity (Lamraoui et al., 2017). However, reestablishment between the time of complete uterine involution and the return to cyclic activity in the postpartum period is not totally elucidated (Hayder and Ali, 2008; Nasciutti et al., 2011).

2.2 Postpartum assessment by means of ultrasonography

To maximize sheep production, it is necessary to adopt tools that enable reproductive monitoring in order to improve reproductive performance and herd productivity (Sharkey et al., 2001).

In small ruminants, due to the impossibility of performing rectal palpation, accurate evaluation of the internal organs of the reproductive tract becomes unreasonable (Oliveira et al., 2013). Uterine involution studies have revealed different intervals to complete this process. Most studies were assessed by hormonal dosages (Ishwar, 1995), radiography (Tian and Noakes, 1991), laparotomy (Rubianes et al., 1996) or at the moment of slaughter (Zdunczyk et al., 2004). The cons of the above techniques include invasiveness, reduced accuracy and difficulty to apply in clinical conditions (Ioannidi et al., 2017).

In sheep, ultrasonography is routinely used for pregnancy diagnosis, but there is insufficient information utilizing this technique for the evaluation of the uterine regression in this species. It has been reported as a practical and efficient tool assessment the uterine involution during post-partum period in cows (Sheldon and Ownes, 2017), mares (Griffin and Ginther, 1991), goats (Ababneh and Degefa, 2005; Badawi et al., 2014; Fasulkov, 2014) and sheep (Hauser and Bostedt, 2002; Zdunczyk et al., 2004; Hayder and Ali, 2008; Nasciutti et al., 2011, Gomes et al., 2014; Ioannidi et al., 2017)

Uterine characteristics that can be assessed by ultrasonography of the uterus during postpartum are asymmetry of the organ, distention of uterine lumen, presence, quantity and texture of uterine content, thickness of uterine wall, and localization of inflammatory on the uterine wall, texture of uterine wall, alterations in uterine wall vascularization and confirmation of uterine involution completion (Ioannidi et al., 2017).

Ultrasonography has a role in differentiating the normal or abnormal uterus during postpartum (Medan and El-daek, 2015). It also provides additional information on physiological and pathological processes in the uterus, which may contribute to the development of new methods for the treatment of reproductive disorders in ovines (Jaśkowski et al., 2013). The ultrasound also offers possibilities to diagnose abnormalities in the uterine regression like accumulation of lochia in the uterine lumen or the presence of retained foetal membranes leading to a prolonged phase of involution, placental retention among other disorders and other such endometritis, hydrometra, uterine infection and hemorrhage (Hauser and Bostedt, 2002).

Ultrasound examination of the female reproductive system in small ruminants can be conducted using two approaches: transabdominal and transrectal (Zdunczyk et al., 2004). To evaluate the uterine involution in sheep, it is recommended in the first week post-partum the transabdominal evaluation, because it is more sensitive than the transrectal, as the uterus remains in the cranioventral abdominal portion until the eighth postpartum day (Hauser and Bostedt, 2002). As the uterus involution is in progress, the use of transrectal ultrasonography permits more objective measurement of the uterine horns diameter and visualization of the uterine lumen (Sheldon, 2004).

Uterus and its content in a normal uterus can be easily visualized during the early stage of the puerperium appearing enlarged, with a heterogeneous echotexture (Hauser and Bostedt, 2002), and diameter can be up to 10 cm (Fernandes et al., 2013). As the uterine size decreases progressively, it may be more difficult to image it. Ababneh and Degefa (2005) reported difficulty to image the uterus after the 13th day post-partum.

Research reports evaluating by ultrasound the uterus during the postpartum period have shown different intervals to complete uterine involution, between 17-35 days postpartum (Ali et al., 2001; Hauser and Bostedt, 2002; Zdunczyk et al., 2004; Ababneh and Degefa, 2005; Badawi et al., 2014; Fasulkov, 2014; Medan and El-Daek, 2015; Elmetwally and Bollwein, 2017. Gomes et al. (2014) reported a reduction of uterine depth slower in ewes with twin parturition, as compared to singleton parturition, and a uterine depth decrease in all ewes during postpartum period, observing the sharpest drop from day 1 to day 16 after parturition, corresponding to more than 50% of total uterine regression. Accordingly, Hauser and Bostedt (2002) reported uterine size decreasing by 50% of its size, 5 days postpartum. However, Rubianes and Ungerfeld (1993) reported that 97% of the uterine involution in the animals evaluated occurred approximately day 17 postpartum. Other researchers found that this involution occurs in a longer period, at 28 days (Regassa and Noakes,1999) or between the fourth and fifth week postpartum (Hayder and Ali, 2008).

According to Ababneh and Degefa (2005) the nonexistence of intrauterine fluid 4-7 days postpartum on ultrasound imaging demonstrate rapid regression of the uterus, and the involution of uterus may be characterized by a small cross-sectional diameter of the horns and absence of lochia in the uterus by ultrasonography (Zdunczyk et al., 2004).

The ability to measure changes in blood flow during postpartum period provides a promising diagnostic tool for assessing the status of uterine involution during this period. It has been used for several years to identify postpartum uterine involution in women, reporting an association between uterine blood flow and delayed uterine involution (Mulic-Lutvica et al., 2007; Guedes-Martins et al., 2015). It has been used to assess uterine blood flow during the postpartum period in cows (Krueger, 2009; Heppelmann et al., 2013), sheep and goats (Elmetwally and Bollwein, 2017; Ioannidi et al., 2017). Preliminary finds in ewes have indicated that blood flow and diameter of the uterine artery decrease progressively, starting soon after lambing. Changes are of greater magnitude during the first post-partum week and the results became significant subsequently to the 20th day post-partum compared to findings immediately after lambing (Ioannidi et al. 2015). Elmetwally e Bollwein (2017) reported significantly decrease in uterine blood flow during the postpartum period in goats and sheep, especially during the first nine days postpartum, and these important changes can be explained because of loss of metabolic requirements of the fetus and placenta.

3. Conclusion

The use of ultrasonography complement the monitoring of uterine involution in ewe, since uterine structures cannot be assessed by rectal or abdominal palpation in this species. This is a promissory tool to distinguish the pathological from the normal puerperium and thereby avoid unnecessary invasive procedures. Thus, the early diagnosis of intercurrences could improving the survival rate of obstetric patients and reproductive life, as well reducing the occurrence of infertile animals due to pathological postpartum. It is also important the knowledge obtained from ultrasonography examinations can help us better understanding the physiology of the postpartum period.

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CHAPTER 2 – B-MODE, DOPPLER AND ACOUSTIC RADIATION FORCE IMPULSE (ARFI) ELASTOGRAPHY EVALUATION IN POSTPARTUM SHEEP

ABSTRACT – To evaluate the uterine involution by ultrasonography during postpartum of healthy ewe, twenty adult multiparous Santa Ines ewes were selected. Ultrasonography postpartum evaluation (B-mode, color Doppler and ARFI elastography) of the uterine structure was performed at immediate postpartum (M0) and sequentially every 48 hours, during 30 days, totaling 16 experimental samples. The echotexture did not present significant variations ($p>0.05$) remaining homogeneous in most evaluations during the postpartum period, and echogenicity of the uterus increased ($p = 0.0452$). A progressive and remarkable decrease of the uterine total diameter were observed ($p<0.0001$), especially during the first days postpartum. The measurement of uterine wall gradually decreased, as well the endometrial, myometrium and lumen diameter progressively decreased ($p<0.0001$) as the days progressed. Uterine blood flow was observed in all animals, and decreased during postpartum period, being significantly lower ($p= 0.0225$) on the 30th day postpartum. On qualitative elastography, the uterine parenchyma was not deformable and the images presented as homogeneous dark areas. On quantitative elastography, the mean shear velocity values of the uterine wall did not differ during the postpartum period. The B-mode, color Doppler and ARFI elastography of the uterus in healthy ewe could be easily performed. This is the first study that evaluate the stiffness of uterine wall parenchyma in animals, providing baseline data about quantitative e qualitative stiffness of the normal uterus, and may be a useful tool for the early diagnosis of uterine alterations during the postpartum period, using the reference parameter established for the assessment of uterine integrity during postpartum period. Thus, the early diagnosis of intercurrences could improving the survival rate of obstetric patients and reproductive life, as well reducing the occurrence of infertile animals due to pathological postpartum.

Keywords: puerperium, uterine involution, uterine stiffness, sheep.

1. Introduction

The postpartum period, also known as the puerperium, is defined as the time between parturition and completion of uterine involution (Sheldon and Owens, 2017; Sheldon, 2004). The uterine involution involves intense modifications as contraction of muscle fibers, catabolism, physical shrinkage, necrosis, sloughing of the caruncles and regeneration of the uterine ephithelium (Sheldon et al., 2008; Bajcsy, 2005). During involution, the size of the uterus diminishes and both the myometrium and endometrium are restored and so the position, then the uterus will be prepared for a next conception (Noakes, 2001).

The physiological events that take place during the postpartum period influences next conception and pregnancy and provide a reproductive and economic importance that is significant in achieving a satisfactory interval between partums (Sanchez et al., 2002). Postpartum fertility in ewes depends on uterine physiological involution and restoration of cyclicity (Takayama et al., 2010). However, few information is available taking into account the reestablishment between the time of complete uterine involution and the return to cyclical activity (Hayder and Ali, 2008; Nasciutti et al., 2011). Thus, we believe monitoring postpartum period allows an early diagnosis of alterations, and an efficiently treatment of uterine diseases, to limit their negative effect on fertility.

Ultrasonography is the greatest non-invasive technique, which may reveals details of the progressive changes in the uterus of ewes (Badawhi, 2014), plays a key role to distinguish the normal to abnormal postpartum uterus, and permits an objective measurement and visualization of the uterine horns and lumen diameter (Medan and El-Daec, 2015). The advantage of using non-invasive methods is that they can be applied under practical conditions, allowing analysis of either physiological or pathological events (Bajcsy, 2005).

B-mode ultrasonography and color Doppler may be an useful tool to evaluate obstetrics and gynecology disorders, as well as uterine involution. Studies in humans describe the ability of Doppler to measure changes in uterine blood flow rates during the postpartum period, being a promising diagnostic tool for assessing uterine involution (Brackleyet al., 1998; Mulic-Lutvica et al., 2007; Guedes-Martinset al., 2015).

Elastography, a new technique based on ultrasonography recently been introduced in obstetrics and gynecology (Hernandez-Andrade et al., 2013), is used to evaluate stiffness in a tissue using a short acoustic push pulse in the target tissue (Karaman et al., 2016). This enable accurate assessment of stiffness intrauterine pathologies and it is consider a way to "imaging palpation**" (**Woźniak et al., 2016).

The advantages of acoustic radiation force impulse (ARFI) elastography include the repeatability of objective measurements, and the ability to evaluate qualitative and quantitative information of tissue stiffness without requirement for external compression (Sporea et al., 2012; Alan et al., 2016). The elasticity of soft tissues is measured to investigate differential diagnosis of many diseases, such as inflammation, fibrosis, and tumoral tissues (Karaman et al, 2016; Tan et al., 2013). However, there are no elastographic studies of uterus during postpartum involution in ewes using the ARFI technique, which could provide even more physiologic and pathological information.

Considering the necessity to carry out studies to establish the physiological patterns of postpartum that enable early diagnosis methods for evaluation of possible puerperal changes in sheep, monitoring this period becomes essential. This avoid a decline in reproductive efficiency, infertility in animals with high breeding values and delay in the return to cyclicity.

The aim of this study was to describe the physiological changes throughout uterine involution, evaluating the uterine regression and tissue stiffness during postpartum involution by B mode ultrasonography, color Doppler and ARFI elastography to elucidate the mechanism of physiological uterine involution and uterine characterization during the postpartum period in ewe.

2. Material and methods

Ethical aspects

All animal procedures were approved by the Animal Ethics and Welfare Committee of the Faculty of Agricultural and Veterinary Sciences, Univ. Estadual Paulista (Unesp), Jaboticabal, Brazil (protocol N ° 12338/15).

Animals

Twenty adult multiparous healthy Santa Ines ewes, aged 3.1 ± 1.1 years, weighing 45.4 ± 4.3 kg and exhibiting a mean body score of 3 (scale 1-5, Jefferies 1961), were selected for this study following clinical background evaluation, physical examinations, hematological tests and ultrasonography of the reproductive system.

Ewes were maintained in an elevated sheep house at the Animal Reproduction Department, fed with corn silage, balanced commercial concentrate, mineral salt and water *ad libitum* during pregnancy and throughout the postpartum period. Only the animals that had non-intercurrent full-term normal delivery were used in this study.

Experimental Protocol

The animals were brought to the ultrasound laboratory, maintained in quadrupedal station, and no sedation was required throughout the entire duration of scanning. A wide trichotomy of the abdominal region was performed to facilitate the examinations and coupling gel applied thereupon. The ultrasonographic postpartum evaluations (B-mode, Doppler and elastography) of the uterine structure of the sheep was performed as follows: immediate moment (M0) and sequentially every 48 hours, during 30 days, totaling 16 experimental samples.

B-mode and color Doppler ultrasonography

Ultrasonography evaluations started on B-mode using the ACUSON S2000® (Siemens®, Munich, Germany) ultrasound system, and were performed by the same experienced operator using a convex multi-frequential transducer (4C1®; 1-4.5 MHz; Siemens®, Munich, Germany). The transducer was positioned on the right or left inguinal area. Imaging starts by locating the bladder, which were used as an acoustic window to facilitate examination, especially after the first week postpartum. Images were obtained on the longitudinal and the transverse ultrasonographic planes. When uterus was located, the characteristics of the uterine wall such as ecotexture (homogeneous or heterogeneous) and echogenicity (hypoechoic, hyperechoic or isoechoic compared to the adjacent tissues, or with a mixed appearance), thickness of the uterine layers (myometrium and endometrium), diameter of lumen and uterine body, and characteristics of uterine contents (anechoic or hypoechogenic with or without cellular debris) were evaluated.

Color Doppler were used to determine the characteristics in uterine blood flow such as presence or absence of vascularization, type of flow (arterial, venous or turbulent and mixed) and type of vessel (peripheral, central, or diffuse), as well as flow changes during the postpartum period

ARFI elastography

Following B-mode ultrasound, same portions studied were considered to accomplished analysis of the elastographic parameters, and a specific software designed for qualitative and quantitative image analyses (Virtual Touch Tissue Quantification® - VTTQ; Siemens®, Munich, Germany) was used. For the adjustment of the quantitative elastographic technique and the values for the shear wave velocity (SWV) of the uterine tissue, the caliper was placed within the uterine wall (endometrium/myometrium), and a minimum of three samples to obtain the mean, with a depth ranging from 0.5 to 5.0 cm were obtained in each portion evaluated. The values of the quantitative evaluation were expressed in the shear wave velocity (SWV - m/s).

Each tissue stiffness evaluated qualitatively by elastogram grayscale image of this studied was calculated by analyzing the relative displacements of tissue elements due to an acoustic pressure pulse. For the interpretation of the formed elastographic image, white regions indicated less rigid tissue (more elastic/softer or more deformable regions) than the dark regions (more rigid structures/harder/not deformable).

Statistical analysis

This is a descriptive observational study with repeated measures. Statistical analysis was performed using the software R, version 3.3.0 (R® foundation for statistical computing, Austria). Data were tested initially for normality (Shapiro test) and homoscedasticity of variances (Barlett test). The variables resulting from the different analyzes were compared between the times by analysis of variance (ANOVA) and Tukey's post test, or by the Kruskal Wallis test and Dunns post-test otherwise. Correlation studies (Spearman or Pearson) and regression (linear and non-linear) were performed in relation to the moments. The qualitative characteristics were compared by the Chi-square test. Differences were considered significant when p-value < 0.05 (5%).

3. Results

All animals included in this study had a normal birth with no obstetrical complications. Uterine ultrasonography could be performed in all animals (n=20), and the uterine characteristics were determined without any difficulties in all animals evaluated by transabdominal approach. No intercurrences were observed during the experimental period and the animals showed no signs of discomfort during exams.

Some qualitative variables varied during the involution process in relation to time. The uterine echogenicity increased, turn into hypoechoic then isoechoic (p = 0.0452), and after 20 days of partum hyperechoic echogenicity was no longer observed. The echotexture did not present significant variations (p>0.05) remaining homogeneous in most evaluations during the postpartum period.

In the present study, the uterine contents were present in all animals until the 8th day postpartum (pp), and decreased (p=0.0215) gradually by the 22nd day pp, when it was no longer observed. The uterine contents were hypoechogenic at the first days pp and turn into anechoic after the 10th day (p=0.0335), presenting less than 10% debris at this time.

Furthermore, a progressive and remarkable reduction of the total uterine diameter were observed (p<0.0001; r2=0.7696), especially during the first days postpartum, and after the 8th day pp it decreased progressively until the end of the evaluations. The measurement of uterine wall thickness gradually decreased (p<0.0001; r2=0.7199) with the advance of days, reducing significantly after 6 days pp.

The endometrial thickness progressively decreased (p<0.0001; r2=0.8460) as the days progressed, diminishing after 10 days and becoming almost imperceptible at the ultrasound after 24 days. The myometrium thickness gradually decreased (p<0.0001; r2=0.8471) with the development of days, reducing significantly after 12 days pp, turning into almost undetectable after 20 days postpartum. The diameter of the lumen reduced with the progress of the days (p=0.0002; r2=0.8123), dropping significantly after 8 days p.p.

The changes in uterine measurement during the involution process in relation to time are shown in Figure 1.

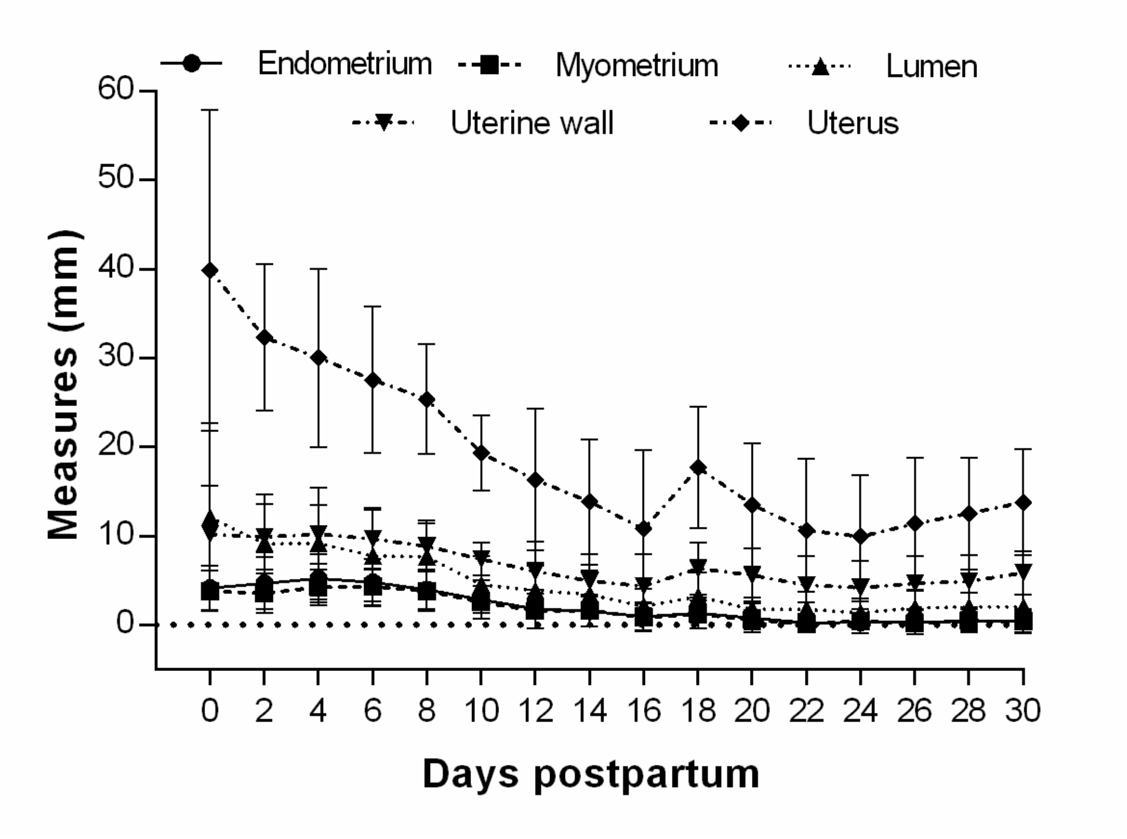

Figure 1. Graphic illustration showing the relation between uterine measurements (uterine wall thickness; biometry of lumen uterine body) and postpartum days in healthy ewes uterus.

Regarding to the color Doppler evaluations, the uterus was highly vascularized in all animals, and the flow was easily notable in the first days after parturition (Figure 2A). However, it decreased during the postpartum period, being less evident after the 18th day, and lower (p= 0.0225) on the 30th day (Figure 2B). However, the type of flow, that was mostly mixed and the type of vessel, peripheral, did not present significant variations (p>0.05) during the postpartum period.

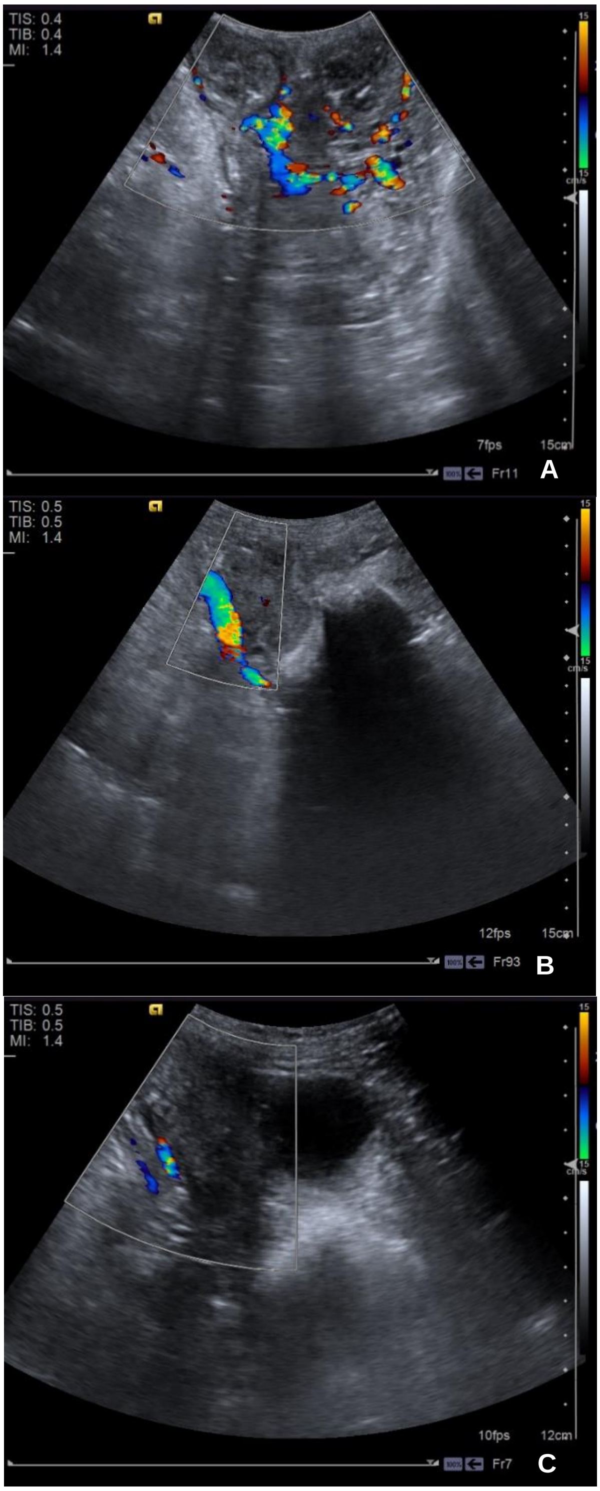

Figure 2: Ultrasound color Doppler image of uterine blood flow during postpartum in healthy ewes. A: 4 days; B: 18 days; C: 30 days pp.

Based on the qualitative elastography of the uterine tissues, all the animals showed that the uterine tissue is hard (dark regions), meaning they were not deformable during uterine involution (Figure 3). In 84% of the elastography assessments presented homogeneous parenchymal echotexture (Figure 3) while 16% heterogeneous echotexture, and there is no relation to the postpartum moment evaluated (p = 0.9010).

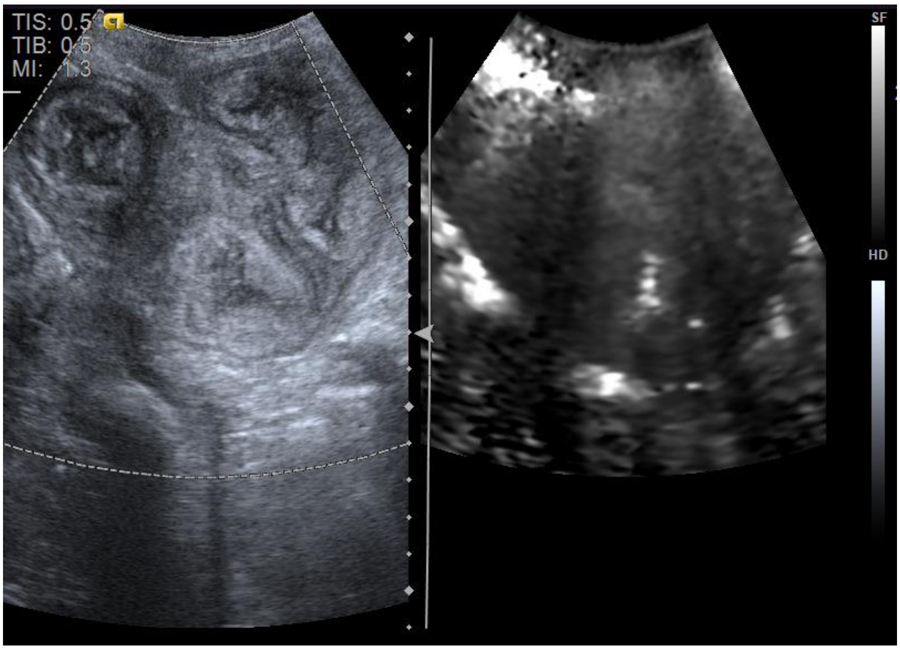

Figure 3. Ultrasound image of postpartum healthy ewe uterus during its qualitative ARFI elastography analysis. Note the B-mode image (left) and the image of the elastography (right) of the uterus showing a homogeneous and dark (hard) image, respectively.

Regarding the quantitative ARFI method, the shear velocities (Figure 4) of the uterine wall did not differ during the postpartum period. The SWV of the uterus and the depth evaluated were constant during postpartum assessment (P = 0.2176 and 0.1027, respectively), and no correlation was observed between SWV and depth of assessment (p = 0.9930) (Table 1).

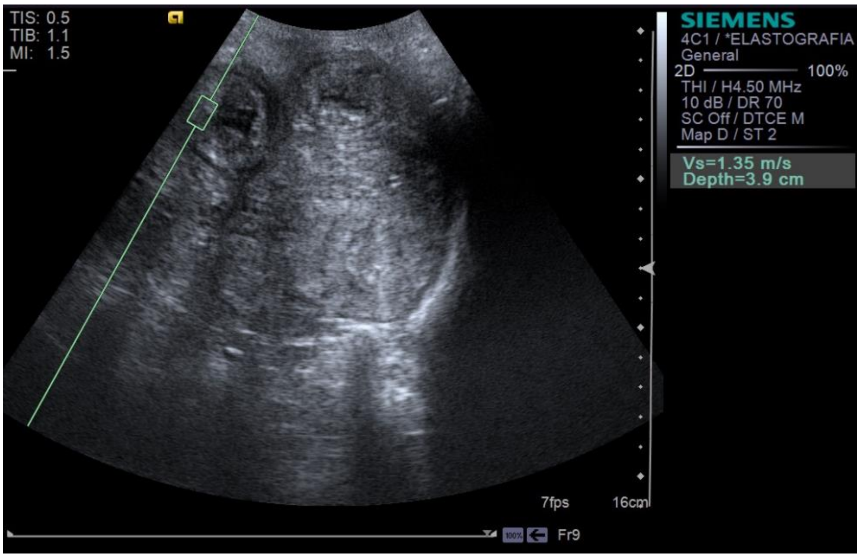

Figure 4. Ultrasound Image of postpartum healthy ewe uterus during its quantitative ARFI elastography analysis. Note the measurement of the shear velocity of uterine wall, with the presence of a caliper for the portion assessed.

Table 1. Mean values (±SD) (m/s) for shear waves velocities (SWV) and depth of different moments (days pp) during the postpartum involution in healthy ewes using acoustic radiation force impulse (ARFI) quantitative elastography (Jaboticabal, 2018).

Variable	SWV		Depth	
Days pp	Mean	SD	Mean	SD
0	1.5325	0.3818	3.514	1.2
2	1.4408	0.3386	3.312	1.494
4	1.5613	0.2285	2.887	1.83
6	1.5724	0.3434	2.808	1.479
8	1.58	0.3807	2.838	1.671
10	1.6347	0.3095	2.476	0.828
12	1.4305	0.2099	2.555	0.916
14	1.5293	0.2711	2.896	0.938
16	1.3992	0.2327	3.484	1.097
18	1.5067	0.3085	3.062	0.861
20	1.4291	0.1814	2.727	0.724
22	1.4577	0.1574	2.692	1.274
24	1.3577	0.1843	3.271	1.667
26	1.4375	0.1733	3.136	1.317
28	1.3638	0.16	2.658	1.213
30	1.3593	0.1342	2.958	1.193

4. Discussion

In this study, we showed the efficacy and the executability of B-mode, color Doppler and ARFI elastography as a safe and non-invasive method for evaluating the assessment of physiological uterine involution during postpartum in sheep. It was established reference data for values of uterine biometry, uterine wall stiffness and blood flow in healthy animals during this period.

Shortly after the moment of the partum, there are several physiological uterine modifications for the return of the uterus to enter in a new reproductive cycle (Elmetwally and Bollwein, 2017), such as degeneration of the caruncles, reduction in the size of the uterus and recovery of myometrium and endometrium (Bajcsy, 2005). Initially, the uterine wall is covered with caruncles (Van Wyk et al., 1972a), which could physiologically explain the increased in echogenicity of the uterine wall in B-mode found in this study. In addition, the echogenicity of the uterus may be related to uterine tone and hormonal changes (Viñoles-Gil et al., 2010; Degefa, 2003).

Studies reported a difficult to observe caruncles (Ababneh and Degefa, 2005; Kähn, 2004; Fasulkov, 2014; Zongo et al. 2015) and differentiation from uterine layers (Hauser and Bostedt, 2002) by ultrasound around 15 days postpartum, reflecting the images found in this study of hypoechogenicity and isoechogenicity, and no more hyperechoic images after 20 days postpartum. Similarly, Phar and Post (1992) described that during the period of initial uterine involution in bitches, the endometrium showed a hyperechoic characteristic, while the myometrium was hypoechoic and, at the end of this period, Yeager and Concannon (1990) described the uterus as homogeneous and hypoechoic. In felines, myometrium and endometrium were also observed as a hyperechoic characteristic during initial uterine involution, and then the uterine wall became isoechoic compared to adjacent tissues (Ferreti et al., 2000), corroborating the data found in our study with sheep during the same period.

A study using B-mode ultrasonography to evaluate uterine regression in ewe, reported that a delay of the separation process of the fetal membranes from the caruncles is characterized by a hyperechoic border of the caruncles with the central site more hypoechoic (Hauser and Bostedt, 2002), supporting our results that the variation in the pattern found in our study may reveal an alteration in the normal course of development of uterine involution.

Regarding to B-mode echotexture, a similarity of echotexture in perimetrium and myometrium was observed in ewes (Hauser and Bosted, 2002), as well as similar echotextures of caruncles and the endometrium in goats (Zongo et al., 2015), dificulting differentiation by ultrasonography during the first weeks of postpartum, corroborating to our findings that the parenchymal echotexture did not present significant variations remaining homogeneous during the postpartum period.

In the present study, the uterine contents were present and with a hypoechogenic characteristic at the first days pp., corroborating with finds described by Hauser and Bostedt (2002), Ababneh and Degefa et al. (2005) and Badawi et al. (2014), and it decreased gradually by the 22nd day postpartum, when it was no longer observed. This finds corresponds to a study conducted by van Wyk et al. (1972), who found only little amounts of fluid in the uterus up to day 20 pp. The debris observed during the initial days pp. in the uterine lumen may also be tissues, debris and blood normally present within the uterus during the postpartum (Shen et al., 2003). Hauser and Bostedt (2002) suggested that little amounts of fluids seem to be physiological in postpartum uterus.

A remarkable reduction in the uterine diameter was observed during the first days pp, however, after this period the reduction was decreased. Similarly, results were reported in ewes by Ioannidi et al. (2017), Elmetwally and Bollwein (2017), Zduńczyk et al. (2004), and Hauser and Bostedt (2002). The ultrasonography revealed to be a useful and reliable method to observe the uterine involution in sheep. Indeed, in our study it was possible to evaluate the measurements of thickness of myometrium, endometrium and the biometry of uterine body. The thickness of uterine wall, myometrium and endometrium decreased with the advance of days, which is may related to the uterine contractility during the early postpartum period, and the regeneration of the uterine layers (Ioannidi et al., 2016). The uterine wall thickness gradually decreased, and reduced significantly at the 6th day pp., related to data reported by Fasulkov (2012), which significant differences in the uterine wall thickness was observed by the 9th day postpartum in goats.

The present study presents reference data on the changes in uterine blood flow during the postpartum period in healthy ewes, assessed by color Doppler ultrasonography during the uterine involution process in this period. Our results agreed with findings in ewes, whereupon the uterine blood flow decreased during the postpartum period (Elmetwally and Bollwein, 2017; Elmetwally et al., 2016). This reduction has been associated with the great decrease in the uterine size after parturition, and may be related to the important uterine blood flow changes right after the partum, due to reduction of the metabolic requirements for pregnancy (Elmetwally and Bollwein, 2017). Similar results were reported in cows (Heppelmann et al., 2013), mares (Lemes et al., 2016), as well as in women during the initial postpartum (Van Schoubroeck et al., 2004).

According to our knowledge, studies reporting the use of elastography ARFI for evaluation of changes in uterine stiffness during postpartum involution have not been reported in animals. However, a study conducted by Tanaka et al. (2011) in humans evaluated the stiffness of the uterus and cervix before, immediately after, and 1 and 2 h after placental delivery, while our study assessed the uterus during 30 days. Additionally, the great repeatability of the procedure, every 48h, has been demonstrate, highlighting the importance and the consistence of the presented data in this study, as well as proves that the elastography ARFI does not have showed any issues for the animal health.

In this study, the uterine parenchyma exhibited as rigid (dark) and homogeneous tissue indicating that was not easily deformable. This is consistent with data of a recent study conducted by Frank et al. (2016) in the evaluation of normal uterine tissue in non-pregnant humans, emphasizing that normal uterine tissue is relatively homogeneous in elastographic evaluation. From the uterine characterization in the physiological puerperium, it reinforces the suggestion that a different elastographic pattern is potentially indicative of alterations, pathological or not, in the uterine parenchyma.

Shear wave elastography is based on the concept that shear waves move faster through more rigid regions in a tissue. It has been used successfully in placenta of normal and pre-eclamptic pregnancies in women (Cimsit et al., 2015; Kiliç et al., 2015; Wu et al., 2016) and in the evaluation of cervix in pregnant women (Gennisson et al., 2011; Carlson et al., 2014; Hernandez-Andrade et al., 2014). Additionally, SWV was considered as valuable method to objectively quantify the cervical stiffness and as a complementary diagnostic tool for preterm birth and for labour induction success in pregnant sheep, as animal model (Peralta et al., 2015). In our study, similarly to the qualitative results that demonstrated a consistent pattern of uterine wall stiffness, the quantitative data (SWV) were also constant in relation to the elasticity of the uterine wall in the involution process. These quantitative values obtained by ARFI of uterus in healthy animals are, to the authors' knowledge, the first that have been described in veterinary medicine. One published study using ARFI (Tanaka et al., 2011), in attempt to quantify endometrium and myometrium in healthy humans, described difference between the means of the SWV in layers, but the authors reported data as not representative to set a reference range.

In veterinary research, as mentioned previously, recent studies have described elastography ARFI with significant results, but this is the first study to evaluate the uterine wall parenchyma in animals. The results of the present study suggest that, even though during the postpartum involution the size of the uterus change over the time (Van Wyk et al., 1972b), the elasticity of the uterus undergoes little changes, not being significant. We believe that our study provides important information about the validation of the ARFI technique, and the stiffness of the normal uterus may be useful as a reference parameter for the assessment of uterine integrity during postpartum period.

5. Conclusions

The data presented in this study emphasize that ultrasonography is promissory tool to distinguish the pathological from the normal puerperium and thereby avoid unnecessary invasive procedures. It was was possible to describe the physiological changes throughout uterine involution, evaluating the uterine regression development and tissue stiffness during postpartum involution by B mode ultrasonography, color Doppler and ARFI elastography. We provided valuable information to elucidate the mechanism of physiological uterine involution and uterine characterization during the postpartum period in ewe, in order to identify and diagnose potential causes delaying involution. This can be of particular importance to apply in intensive reproductive management, where conception of females is essential, helping in reproductive performance and consequently an economic gain. It is also important to mention that the knowledge of the physiological course of uterine regression is a pre-requisite to diagnose pathologies in practice

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CHAPTER 3 - POSTPARTUM EVALUATION IN EWE: STUDY OF UTERINE AND CERVICAL INVOLUTION BY HYSTEROSCOPY AND HISTOLOGY

ABSTRACT – To evaluate the time of cervical closure during postpartum in healthy animals, and determine morphophysiological characteristics of this structure observed through hysteroscopy and histology of the uterus and cervix during this period, twenty adult multiparous Santa Ines ewes were selected. Hysteroscopy evaluation of the uterine involution of the sheep was performed as follows: immediate moment after lambing (M0) and sequentially every 6 hours, until the moment where the endoscopic access to the uterus through the cervix was no longer possible. Uterine biopsy was performed at the same moments. Regarding the time of cervical involution, 30% of the animals had a cervical closure between 12-18 hours. The most frequent external os type was papilla 7/20 (35%), followed by flap 5/20 (25%). Histological evaluations showed changes during the postpartum evaluation. This is the first study to evaluate the uterine involution through hysteroscopy in healthy ewes, and may be a useful tool for the early diagnosis of uterine alterations during the postpartum period, avoiding the use of invasive procedures to the assessment of this period.

Keywords: cervix, puerperium, sheep, endoscopy.

1. Introduction

Uterine involution and postpartum fertility may apply limitations on female reproductive performance and fertility (Greyling and van Niekerk, 1991; Greyling, 2000; Ababneh e Degefa, 2005) mainly under intensive accelerated production systems, and this is economically important in small ruminants. Therefore, little has been known about the time, pattern of cervical closure and the macroscopic and histological carachteristics of this physiologic process during postpartum.

Uterine cervix is firm and closed during the pregnancy due to a high content of connective tissue, which is surrounded by bundles of smooth muscle cells (Myers et al., 2015). Shortly before and during parturition, there is a remodelletion of the connective tissue of the cervix (Winkler et al., 2003; Engelen et al., 2007).

The cervix is an important barrier against the invasion of bacteria in the uterine cavity (Bekana et al., 1997), and the closure of the cervical canal after parturition is important for a successful new pregnancy. When this process is incomplete or delayed, as is the case of retention of the placenta, it predisposes to the development of other pathological processes such metritis, endometritis, and it can leads to lower conception rates, increased of partums intervals and also infertility (Engelen et al., 2007).

Endoscopy is a non-invasive tool widely used in reproduction in humans as a diagnostic and treatment technique, since it allows the direct observation and evaluation of the vaginal cavity and related structures (Sardo et al., 2016). However, applications in veterinary medice have not yet been so extensively established, but it has greatest potential that veterinary medicine will likely parallel the trends in human medicine (Katic and Dupre, 2016).

In veterinary medicine, several applications for vaginoscopy or histeroscopy have been described in cows (Franz, 2008; Madoz et al., 2010), ewes (Easley et al., 2017), mares (Ferrer et al., 2012) and bitches (Lévy, 2016), with the purpose of diagnosis and small procedures as well as providing an opportunity to take visually guided biopsies, proving to be highly effective (Easley et al., 2017). Vaginal examination also allows to detect damage to the wall of the vagina and cervix, indicative of obstetric injuries, vaginitis, and cervicitis (Sheldon et al., 2018).

The purpose of the present study was to evaluate the time of cervical closure during postpartum in healthy animals and to determine characteristics morphophysiological of this structure observed through hysteroscopy and histology of the uterus and cervix during this period in sheep.

2. Material and Methods

Ethical aspects

All animal procedures were approved by the Animal Ethics and Welfare Committee of the Faculty of Agricultural and Veterinary Sciences, Univ. Estadual Paulista (Unesp), Jaboticabal, Brazil (protocol N ° 12338/15).

Animals

Twenty adult multiparous healthy Santa Ines ewes, aged 3.1 ± 1.1 years, weighing 45.4 ± 4.3 kg and exhibiting a mean body score of 3 (scale 1-5, Jefferies 1961), were selected for this study following clinical background evaluation, physical examinations, hematological tests and ultrasonography of the reproductive system.

Ewes were maintained in an elevated sheep house at the Animal Reproduction Department, fed with corn silage, balanced commercial concentrate, mineral salt and water *ad libitum* during pregnancy and throughout the postpartum period. Only the animals that had non-intercurrent full-term normal delivery were used in this study.

Experimental Protocol

The animals were maintained in quadrupedal station, and no sedation was required throughout the entire duration of the exam. The postpartum evaluation of the uterine involution of the sheep was performed as follows: immediate moment after lambing (M0) and sequentially every 6 hours, until the moment where the endoscopic access to the uterus through the cervix was no longer possible.

A vaginal speculum was lubricated and inserted into the vagina for the identification of the uterine cervix. Then, it was grasped and pulled with an atraumatic Babcok grasper to visualize the external orifice of the uterus cervix. Through the speculum, a 4 mm diameter, 0∘, 30 cm rigid laparoscopic (Scope electronic optical, GDI Brazil) connected to a canula for endoscopy 5.5 mm (GDI Brazil) was inserted via the cervix in attempt of passage and characterization of the uterus and cervical orifice.

Upon entering the vagina, visible structures were identified and classified the following parameters according to Kershaw et al. and Grunert et al. (2005): format of the cervical extern os (duckbill, slit, rose, papilla, flap), mucosal staining, cervical canal opening (1-5), vaginal and cervical moisture (1-dry;2-slightly moist;3-medium;4-very moist;5-collection of mucus). The examination was video recorded and possible intercurrences and particularities were noted.

Uterine biopsy was performed videoassisted with a 2.2 mm laparoscopic flexible biopsy forceps (Huger®, China). When the passage of the optic through the cervix was possible, uterine fragment was collected of the uterine wall, tracing it to its detachment through the working channel of the cannula endoscope.

Histology

Once collected, uterine fragments were fixed in 10% formalin solution, buffered with phosphates (0.15 Molar), pH 7.2, for 48 hours. Subsequently the tissues were dehydrated in solutions of increasing concentration of alcohol, diaphanized in xylol, and included in paraffin according to the routine histological technique. For the preparation of the slides the blocks were cut at the thickness of 4μm and stained with Hematoxylin and Eosin and analyzed by light microscopy (Tolosa et al., 2003).

Statistical analysis

Statistical analysis was performed using the software R, version 3.3.0 (R® foundation for statistical computing, Austria). Hysteroscopy variables were compared between postpartum days by exact-Fisher test, quantitative variables by Friedman test and Dunss post-hoc. Differences were considered significant when P-value < 0.05.

3. Results

All animals (n=20) of this study had a normal birth with no obstetrical complications and were evaluated every 6 hours after delivery until the cervical closure, established as the moment when it was inaccessible the passage of the optic through the cervix, trying to avoid iatrogenic lesions.

If the cannula did not pass easily through the cervix on the first attempt, the cannula was re-positioned and further attempts made. In two animals, the access to the cervix and uterus was not possible. The vaginal wall and cervical canal were easily visualized. However, it was not possible in all the animals and moments collect the uterine tissue through the biopsy. There were no complications due to the examination, but one animal evaluated in this study had uterine prolapse on day 5 postpartum.

For the animals evaluated, 25% had cervical closure between 6-12 hours, 30% of 12-18 hours, 25% of 18-24 hours and 10% of 24-30 hours. In 10% of the animals, it was not possible to evaluate the cervical closure (Table 1).

Table 1. Correlation of number and percentage (%) of animals evaluated and time of cervical closure observed trough endoscopy during the postpartum involution in healthy ewes. Jaboticabal, 2018.

Cervical closure time	**Number of animals**	**Percentage**
6h-12h	5	25%
12h-18h	6	30%
18h-24h	5	25%
24h-30h	2	10%
No access to the uterus	2	10%

The external os was classified according to the shape, according to Kershaw et al. (2005). The most frequent external os type was papilla 7/20 (35%), followed by flap 5/20 (25%). The remaining types, duckbill (3/20; 15%), slit (3/20; 15%) and rose (2/20; 10%) were the least common. A small lesion in the external cervical os was observed in 3 animals. The mean of the cervical canal open was 2.61±1. The staining mucosal of the animals, showed a normal to hyperemic mucosal, mainly bright pink equally saturated. The mean of the moisture was 2.55±1 (vaginal), and 3.27±1 (cervical).

Regarding the histology, 15 samples of uterine tissue were obtained from nine animals, and it was processed and evaluated. The histological findings were compatible with uterine and cervical tissue. The histoarchitectural characteristics observed in the animals were: marked hyperemia of the submucosa vessels, Inflammatory cell infiltration mostly constituted by neutrophils, and apoptosis of the epithelial cells of the endometrial glands. The histoarchitectural pattern found in this study is shown in Figure 1.

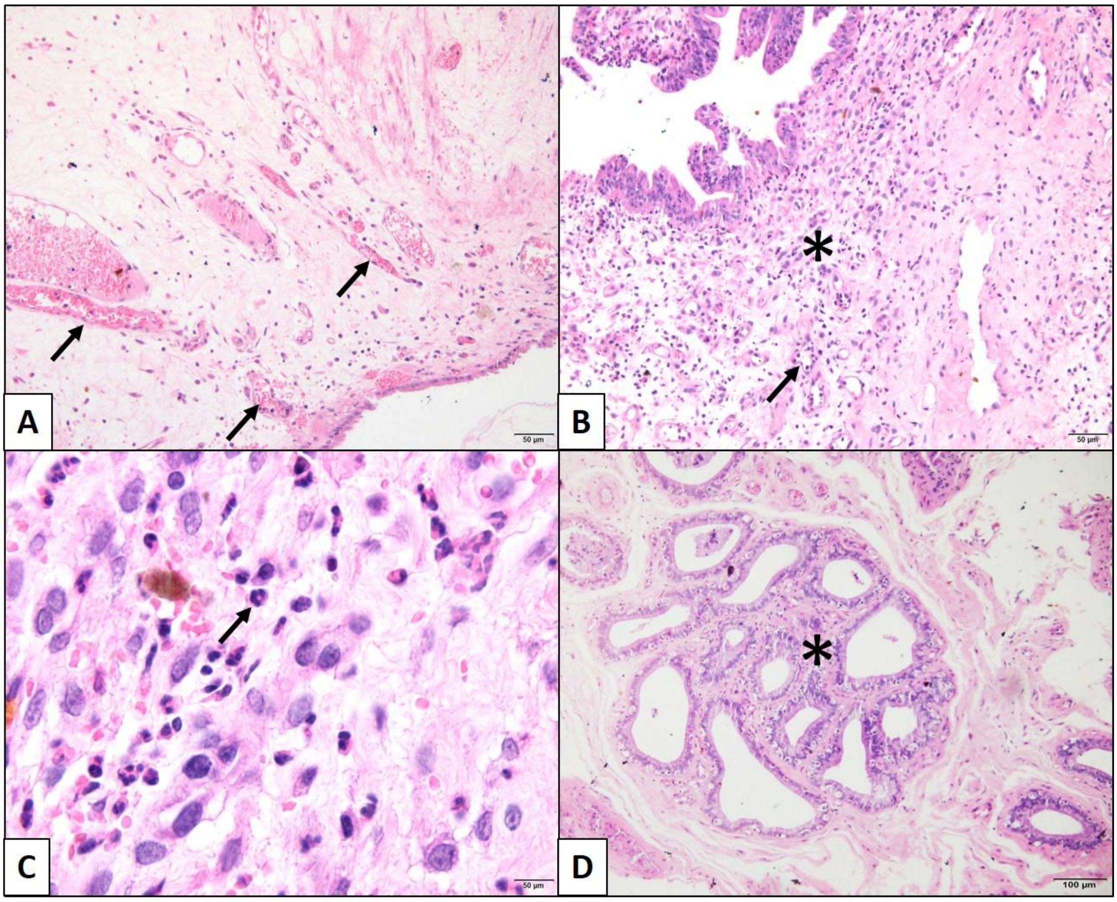

Figure 1 – Photomicrographs of the histological evaluation of ewes uterus in the postpartum period. A) Cervical region showing moderate hyperemia of the vessels in the submucosal (arrows) and discrete neutrophilic inflammatory infiltrate (Bar = 50μm). B) Uterine epithelium showing increased vascularization (arrow) and marked neutrophilic inflammatory infiltrate in the endometrium (*) (Bar = 50μm). C) Endometrial region presenting the predominance of neutrophils in the inflammatory infiltrate (arrow) (Bar = 50μm). D) Endometrial glands with epithelial cells in apoptosis (*) (Bar = 100μm). Hematoxylin-Eosin.

4. Discussion

Various techniques have been proposed for the study of uterine involution in ewes, and most of the disadvantages of these techniques include invasiveness, reduced accuracy, difficulty to apply in clinical conditions and need to sacrifice the experimental animals. In our study, the hysteroscopy in ewes has shown to be a noninvasive procedure, and atraumatic during normal, gently and proficient procedures.

It was not possible in all animals and moments to collect the uterine tissue through the biopsy, due to the anatomical conformation of the sheep cervix, which is highly variable between animals, and may explain the difficult to access the uterus (Kershaw et al., 2005), as well as the behavior of some animals postpartum made the procedure difficult. Similar limitations were recorded in a previous study in bubalines, when the circular annular rings in the cervix presented resistance to the passage of the hysteroscope (Chaudhary et al., 2014).

We believe the placenta present during hysteroscopic evaluations, as well as large amounts of fluid through the cervix in the postpartum immediate moment (M0), made difficult the passage of the endoscopic through the cervix and the visualization to the uterus, and affect the success rates in the procedure. Madoz et. Al. (2010) describe the use of a disposable plastic sleeve to cover the endoscopy during postpartum hysteroscopy.

Moreover, the rigid endoscope does not enable to gain access and passage through the cervical rings with no insufflation, when cervix appeared closed, as same as observed in a study conducted by Watts and Wright (1995) to investigate uterine diseases in bitches. However, same authors have reported uterine tear during postpartum hysteroscopy due to the pressure of the insufflation, because after parturition the uterine wall may be weakened. Even though some authors have described the use of a rigid endoscopy to examination of the uterus in cows (Madoz et al., 2010) and buffalos (Chaudhary al., 2013), they also reported it does not allow exploration of the whole uterus, mainly endometrium, and limits exploration.

The results of this study indicate the most frequent external os type in Santa Ines sheep was papilla followed by flap, similarly the most common finds in ewes reported by Halbert et al. (1990) and Kershaw et al. (2005) in cervices evaluated after slaughter. Dun (1955) suggested that the classification of the cervical os might change at parturition, increasing in size and complexity.

The opening of the cervix favors the entry of bacteria leading to an immediate cellular immune response (Sheldon, 2004), and besides the physiological infection developed, the uterus starts a regenerative process (O'shea and Wright, 1984), which may explain the inflammatory cell infiltration observed in the histological analyses in our study. Gray et al. (2003) reported a significant increase of neutrophils and macrophages from the first to seventh postpartum days in ewes. Therefore, it suggests that that increase it is physiological according to the changes of the intrauterine environment, being directly associated with phagocytosis of placental structures remaining from postpartum (Nasar et al., 2002; Gray et al., 2013).

In addition to these changes, Gray et al. (2013) described a marked reduction in vascularity in both caruncular and intercaruncular areas of the uterine wall during involution, controverting our finds, whereupon the uterine epithelium showing increased vascularization. This results may be explained by the increase of the uterine blood flow observed through color Doppler in ewes during postpartum (Elmetwally et al., 2016).

5. Conclusion

These data provide the first evidence that hysteroscopic examination of the uterus in ewes can be used to determine the progress of the involution, and may reveal in evidence details in which cannot be assessed through rectal palpation, and with more detail than obtained by ultrasonography.

The technique we have described for evaluate of the uterine involution will be a valuable procedure which will assist in early diagnosis and treatment of uterine disease in ewes, thus avoiding invasive procedures. For future studies, the technique could be improved.

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