Small Ruminant Research 221 (2023) 106947

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Ultrasonography and computer-assisted assessment of postpartum uterine echotexture and its relationship with peripheral oxidative stress biomarkers in goats

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ARTICLE INFO

Keywords:
Caruncle diameter
Endometrial echogenicity
Goats
Glutathione peroxidase, Malondialdehyde,
Puerperium
Superoxide dismutase
Ultrasonography

ABSTRACT

The postpartum (PP) period is a crucial factor for the resumption of reproductive capacity and the regular cycling of goats. Until now, there is a lack of information on monitoring the PP uterine involution using morphological assessment of uterine echotexture by ultrasonography. This study aimed to use ultrasonography and computer software analysis for morphometrical evaluations of postpartum uterine involution in 30 pleuriparous Egyptian Balady goats from Day zero (kidding day) till Day 21 PP. Uterine echotexture (pixel intensity; PI and integrated density; ID) was assessed by the computer-assisted image analysis software. In addition, the changes in peripheral oxidative stress biomarkers were monitored by measuring superoxide dismutase (SOD; U/mL), glutathione peroxidase (GPx; U/mL) activities, and malondialdehyde (MDA; nmol/mL) levels in the sera during the PP period. Results revealed a significant ($P < 0.001$) reduction in the transverse uterine diameter from 8.05 ± 0.58 cm at Day zero to less than 2 cm at Day 16 PP onward. The uterus returned to its apparently normal non-pregnant state with the absence of lochia by Day 16.6 ± 1.35 PP. There were significant (P < 0.0001) increases in the PI and ID of the endometrium with the progression of the PP days [Day 0: PI: 86.99 ± 8.61; ID (×1000): 82.09 ± 1.47 versus Day 12 PP: PI: 186.73 ± 5.62; ID (×1000): 355.17 ± 38.63]. Gradual elevations in the levels of SOD (0.33 ± 0.02 U/mL) and MDA (11.81 ± 0.21 nmol/mL) were found from Day 0 and reaching the maximum levels (SOD: 0.48 ± 0.12 U/mL; MDA: 15.31 ± 0.13 nmol/mL) at Day 6–7 PP. Concentrations of GPx increased gradually from Day 0 (65.23 ± 2.09 U/mL) till Day 21 PP (145.81 ± 2.99 U/mL). In conclusion, assessment of the changes in morphometrical and echotextural parameters of the uterus by ultrasonography as well as the oxidative stress biomarkers provided useful information in the progress and completion of the normal physiological PP uterine involution in goats.

1. Introduction

During gestation, the uterus is stretched several times its size by the developing fetus and placental structures. After parturition, the uterus decreases in size before the resumption of normal estrous cycles and fertility. The postpartum (PP) period involves a series of integrated anatomic and physiologic re-adjustments of both the uterus and endocrine system which are crucial factors for the resumption of reproductive efficiency in breeding animals (Greyling, 2000). Uterine involution is very important for the elimination of bacterial infection and the histological regeneration of the endometrium and regular cycling efficiency. So, the PP uterine involution is considered a momentary hindrance to animal fertility (Kiracofe, 1980). It depends mainly on the rate of myometrial contractions and endocrinological changes. In goats, uterine involution is completed by Day 28 PP based on decreases in the uterine mass and volume (rate of decrease is around 17–20% daily for the first 34 days PP) and the degree of recovery of the endometrial epithelium over the caruncular areas (Greyling and Van Niekerk, 1991a, 1991b).

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https://doi.org/10.1016/j.smallrumres.2023.106947
Received 21 June 2022; Received in revised form 27 January 2023; Accepted 25 February 2023
Available online 27 February 2023

Different methods were used to assess the postpartum uterine involution in farm animals including macroscopic assessment after slaughtering of animals on different PP days (Degefa et al., 2006) and ultrasonography (Zdunczyk et al., 2004; Meile et al., 2020; Lin et al., 2021). In large animals, monitoring uterine involution could be performed through transrectal palpation or ultrasonography (Lin et al., 2021). However, ultrasonography is a very useful and reliable method for observing uterine involution in small ruminants (Hauser and Bostedt, 2002; Zdunczyk et al., 2004) and sows (Meile et al., 2020) due to difficulties of rectal examination. Ultrasonography has many pivotal roles in the assessment of hemodynamics and morphometric changes in uterine contents during different reproductive events (Karen et al., 2014; Samir et al., 2016, 2017; Samir and Kandiel, 2019; Samir et al., 2019) and infertility problems (Ioannidi et al., 2020; Grahofer et al., 2020) in farm animals. Real-time B-mode ultrasonography is a good diagnostic technique for morphometric evaluation of PP uterine involution including evaluation of the uterine contents, size of caruncles, and uterine diameters (Hauser and Bostedt, 2002; Zdunczyk et al., 2004). In addition, it could be used to distinguish between normal and abnormal PP uterine states in bitches (Pharr and Post, 1992), ewes (Ioannidi et al., 2020), and sows (Grahofer et al., 2020).

The adoption of image analysis software heralded the diagnostic perspectives of ultrasonography and reduce the interobserver variability of an ultrasound image through quantification of the changes in the echotexture of the organ (Samir et al., 2020; Elfadadny et al., 2021). Monitoring the changes in the uterine echotexture using a computer-image analysis of the pixel intensity, heterogeneity, uniformity, and integrity of the endometrium is considered a good indicator of histomorphology and chemical composition of the endometrium in animals (Polat et al., 2015; Ioannidi et al., 2017, 2020). Some literature on cows illustrated the importance of assessment of the uterine echotexture/ultrasonogram for evaluating the normal postpartum uterine involution and its correlation to ovarian cyclicity or infertility problems (Ioannidi et al., 2015; Polat et al., 2015; Ioannidi et al., 2017, 2020). In post-partum sows, subjective grayscale analysis of the uterus by ultrasonography is suitable for characterizing uterine echogenicity and echotexture and could help assess patterns of the sow's puerperium and for monitoring the therapeutic approaches (Grahofer et al., 2022). To the authors' knowledge, there is a lack of information on monitoring the PP uterine involution in goats using morphological assessment of uterine echotexture by ultrasonography.

Superoxide dismutase (SOD) is an essential enzyme that has vital antioxidant properties (Aljuhani et al., 2015). It functions as the first line of cell defense against oxidative damage caused by reactive oxygen species (ROS) by converting the superoxide radical into oxygen and hydrogen peroxide, thereby protecting cells from oxidation (Arockiaraj et al., 2014; Yan et al., 2014). Adequate levels of glutathione peroxidase (GPx) are also crucial in maintaining reduction-oxidation balance within the body tissues. Malondialdehyde (MDA) is considered the last product of the peroxidation of lipids and therefore changes in its concentrations could be used as a biomarker of oxidative stress (Castillo et al., 2005). Determining the activities of the peripheral SOD and GPx and measuring the MDA levels could be useful biomarkers in the evaluation of the reproductive performance of dairy cows (Castillo et al., 2005; Konvičná et al., 2015; Colakoglu et al., 2017). However, changes in these biomarkers and their relationship with the uterine echotexture were not fully monitored during the PP period in goats. Therefore, the current study aimed to evaluate the PP uterine involution in goats by assessment of uterine morphometrical changes, including the endometrial echotexture. In addition, it aimed to assess the changes in the peripheral oxidative stress biomarkers [SOD (U/mL) and GPx (U/mL) activities, and MDA (nmol/mL) levels] in the sera during the PP period.

2. Material and methods

This study was carried out on pleuriparous Egyptian Balady goats which are one of the indigenous goat breeds of Egypt and several neighboring countries. Balady goats are nonseasonal breeders and are reared mainly for meat production (Aboul-Ela and Chemineau, 1988; Degefa et al., 2006).

2.1. Animals

Thirty pleuriparous postparturient Egyptian Balady goats aged 1.5–3.0 years and weighing 27–35 kg were used in the current study. These goats were housekept at a goat farm located in the Kalyobia governorate, Egypt during the kidding season (2021). For breeding history, goats were synchronized for estrus using progesterone sponges and were then artificially inseminated (n = 35) using fresh diluted semen from pooled semen samples of two fertile bucks. Pregnancy diagnosis was confirmed in 30 goats by ultrasonography at 35 days after breeding. All goats had good general health conditions and did not suffer from any disease problems during the gestation period that lasted about 148–155 days. No interventions were conducted to assist the delivery process in all goats of this study (2.00 ± 0.37 kids/goat; mean ± SD). In addition, goats did not show any disease symptoms during the studied days of puerperium. Goats were fed a diet consisting of a pelleted ration (energy requirements of 6.39 MJ/kg diet and 14% crude protein) twice daily, while water and blocks of minerals licks were available ad libitum. All goats were housed under natural daylight conditions and were allowed for grazing for 1 h daily. The internal and external parasites were eradicated by using broad-spectrum anthelmintics [Doramectin 1% (Dectomax®; Pfizer, USA), one mL / 50 kg body weight]. This study was performed following the Guidelines of Cairo University for the Use and Care of Animals (Vet. CU. 20092021467).

2.2. Ultrasonographic examinations

The ultrasonographic examinations were carried out daily from Day zero (Day of kidding) till Day 21 PP in all goats using a real-time B-mode ultrasonography scanner (Aquila Vet C E 0344, Pie Medical, Esaote Europe B.V., Maastricht, Netherlands) equipped with a dual-frequency curved array probe (5 and 7.5 MHz) for transabdominal ultrasonography and with a dual-frequency, linear-array transducer (6.0 and 8.0 MHz) for transrectal ultrasonography. All ultrasonic examinations were recorded on high-grade videotape and a computer system for retrospective analysis.

The goats had been fastened for about eight hours before the examination. In the first week of PP, the does were scanned daily by transabdominal ultrasonography in the standing position, from both sides to enable a good visualization of the entire postpartum uterus. Thereafter, the does were examined daily by transrectal ultrasonography until Day 21 PP. In the transabdominal ultrasonography, the transducer was applied to the inguinal region of both sides after adding carboxymethylcellulose gel (Echo gel, IBE Co., Egypt). Transrectal ultrasonography was applied according to Haydera and Ali (2008) as the does were restrained in a standing position, the rectum was evacuated, and the transducer with some ultrasonic gel was introduced into the rectum. The transducer was moved medially and laterally to get the best view of the uterus. The non-pregnant uterus lies just cranial to the non-echogenic, fluid-filled bladder. After the uterus was imaged, the following parameters were measured: A) Transverse diameter of the previously gravid uterine horn (TUD; defined as the maximum diameter of the previous gravid horn). In the case where both uterine horns were largely due to multiple pregnancies, the mean of both horns was taken as one estimation. B) The caruncle size (CD): The largest 3–5 caruncles were measured by electronic caliber and the average was calculated. C) The day after which the caruncles were no longer visualized by ultrasound scanning was reported in each goat. D) Changes in the Ultrasonic echogenicity of the lochial content were reported. E) The time of the end or complete uterine involution was recorded when the transverse diameter of the uterus was < 2 cm, no further reduction in the uterine

diameter for three successive examinations, absence of lochial content, and difficulty to visualize the caruncles (Zdunczyk et al., 2004; Degefa et al., 2006; Haydera and Ali, 2008; Medan and El-Daek, 2015).

2.3. Uterine echotexture assessment

For evaluation of uterine echotexture (pixel intensity; PI, and integrated density; ID), a cross-section and a longitudinal section of the uterus were imaged by B mode ultrasonography for further assessment using computer-assisted image analysis software (ImageJ, MD, USA) as previously described (Polat et al., 2015). Ultrasonographic images were assessed using the spot technique. A total of 4 square-shaped spots (5 mm × 5 mm) were placed on each saved image of the endometrium (Fig. 1A), excepting artifacts. The PI of the endometrium represents the average pixel values within the selected area of the tissue based on a reverse scale of gray shades (1−255); so that number one is pointed to black and 255 is referred to as white (Samir et al., 2020). Integrated density is the sum of the values of the pixels in the selected part of an image.

2.4. Blood samplings, and antioxidant and lipid peroxidation biomarkers analysis

From the jugular vein, blood samples (5 mL) were withdrawn at a fixed time (08.00) daily from Day of kidding (Day 0) till Day 21 PP, and the separated sera were kept at − 20 °C until analysis of antioxidant and lipid peroxidation biomarkers. Monitoring the changes in the levels of SOD (U/mL), MDA (nmol/mL), and GPx; (U/mL) in goats during the PP periods was performed using commercial colorimetric test kits based on the manufacturer protocols. In brief, simple, rapid, colorimetric, and enzymatic activity assay kits were used for the assessment of the SOD (Superoxide Dismutase Activity Assay Kit; ab65354; BioVision Co., Abcam) and GPx (Glutathione Peroxidase Assay Kit; ab102530; BioVision Co., Abcam; with a sensitivity of 0.5 mU/mL) activities following the established protocols (www.abcam.com/assaykitguidelines) and as previously described reports (Li et al., 2021; El-Nobi et al., 2021). The MDA competitive ELISA Kit (MDA colorimetric Assay Kit; ab238537; BioVision Co., Abcam) was used for the determination of the MDA levels (Sohail et al., 2020). All assays were carried out in duplicates and the absorbance of each well on the microplate reader for SOD, GPx, and MDA assays was read using optical density at 450 nm, 340 nm, and 450 nm, respectively.

2.5. Statistical analysis

To assess the normality of data for all variable distributions, the Shapiro-Wilk test was used (Ghasemi and Zahediasl, 2012; Mishra et al., 2019). All measured parameters were normally distributed ($P > 0.05$). The obtained data were presented as means ± S.D. The analysis of variance (Repeated ANOVA) was used to test the significance of differences between means in all measured parameters. The transverse uterine diameter, caruncle diameter, and parameters of endometrial echotexture were subjected to linear regression analysis (Velleman and Welsch, 1981). To find out a clear relationship between peripheral oxidative stress biomarkers and uterine echotexture postpartum in goats, the correlation coefficient was tested. All statistical analyses were performed using GraphPad Prism 5 software (San Diego, CA, USA). Significance was assigned at $P < 0.05$.

3. Results

By ultrasonography, the uterus appeared as a large sac filled with caruncles and lochial content between days 0 and 2 PP. The lochial content first appeared as anechoic (black color) at day zero (kidding day) with an abundant amount. By progressing PP days, the lochial content inside the uterus decreased in amount and increased in its echogenicity (Fig. 1B & C).

Changes in the TUD in goats during the PP were illustrated in Table 1. The transverse diameter of the uterus decreased linearly ($P < 0.0001$) with the PP period. It was 8.05 ± 0.58 cm at Day zero, reduced continuously (about 50%) to 4.00 ± 0.73 cm on Day 6 PP, and reached less than 2 cm (1.96 ± 0.15 cm) at Day 16 PP onward. From Day zero to Day 2 PP, caruncles had a crescentic or elliptical appearance and were crowded (near to each other). The echogenicity of caruncles changed from hypoechoic structures (grey) from Day zero to Day 4 PP to hyperechoic structures (whitish grey) from Day 5 onward (up to 9–15 days PP) (Fig. 2). After Day 12 PP, it was difficult to be observed by ultrasonography. Changes in the CD during the PP in the present study were illustrated in Table 1. There were significant ($P < 0.0001$) decreases in the CD by the progression of the PP. It was 2.76 ± 0.10 cm at Day zero, decreased gradually, and reached 1.18 ± 0.28 cm on Day 5 PP. The caruncles were difficult to be observed by ultrasonography after Day 12 PP, and their diameter reached 0.33 ± 0.04 cm. In addition, there was a negative linear correlation between the PP days and the TUD/cm ($r^2 = -0.928$, $P < 0.001$), and the CD/cm ($r^2 = -0.912$, $P < 0.001$), where days postpartum (Y) can be determined by the following equations: $Y = 20.593 - (8.5 \times CD)$, $r^2 = 0.832$ and standard

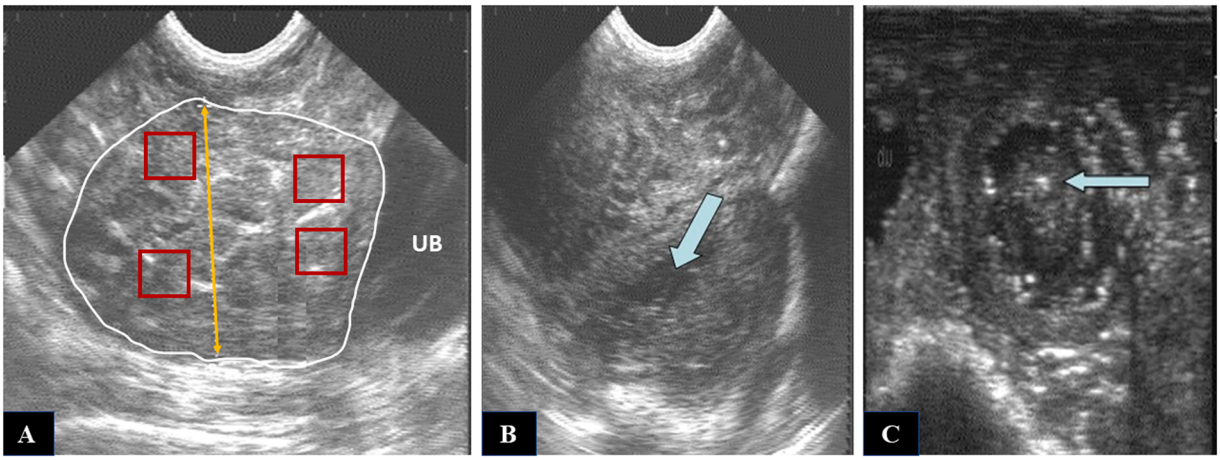

Fig. 1. A. A postpartum uterus of a goat as imaged by B-mode ultrasonography to monitor changes in the transverse uterine diameter (yellow arrow), and to assess the echotexture of the endometrium by computer image analysis software. Changes in the echogenicity appearance of the lochial content inside the uterus from Day 1 postpartum as imaged by transabdominal ultrasonography **(**Fig. 1**B)** to Day 11 postpartum as imaged by transrectal ultrasonography **(**Fig. 1**C)**.

Table 1
Monitoring changes in the transverse uterine diameter (TUD/cm), caruncle diameter (CD/cm), pixel intensity (PI), and integrated density of the endometrium of goats (n = 30) during postpartum (PP) periods using ultrasonography.

PP Days	TUD/cm	CD/cm	Endometrial PI	Endometrial ID (×1000)
Day 0	8.05 ± 0.58	2.76 ± 0.10	72.13 ± 4.09	76.45 ± 3.27
Day 1	6.92 ± 0.58	2.17 ± 0.11	86.99 ± 8.61	82.09 ± 1.47
Day 2	6.50 ± 0.45	1.96 ± 0.33	104.89 ± 6.11	89.27 ± 10.96
Day 3	5.68 ± 0.62	1.61 ± 0.37	106.68 ± 3.70	109.24 ± 13.81
Day 4	4.97 ± 0.91	1.43 ± 0.23	122.97 ± 5.43	127.51 ± 9.01
Day 5	4.34 ± 0.79	1.18 ± 0.28	131.00 ± 8.21	140.28 ± 14.63
Day 6	4.00 ± 0.73	1.04 ± 0.12	147.31 ± 5.89	168.23 ± 20.26
Day 7	3.60 ± 0.41	0.99 ± 0.06	155.45 ± 5.36	174.35 ± 30.11
Day 8	3.01 ± 0.55	0.75 ± 0.14	165.87 ± 4.26	199.69 ± 20.81
Day 9	2.65 ± 0.49	0.62 ± 0.07	165.30 ± 4.88	208.61 ± 15.43
Day 10	2.52 ± 0.48	0.55 ± 0.03	168.06 ± 8.25	238.78 ± 24.39
Day 11	2.39 ± 0.47	0.43 ± 0.09	176.15 ± 5.09	300.22 ± 39.12
Day 12	2.26 ± 0.42	0.33 ± 0.04	186.73 ± 5.62	355.17 ± 38.63
Day 13	2.18 ± 0.34	ND	180.65 ± 6.79	333.97 ± 11.52
Day 14	2.14 ± 0.31	ND	172.82 ± 5.82	312.31 ± 13.21
Day 15	2.02 ± 0.20	ND	189.74 ± 7.49	344.47 ± 5.57
Day 16	1.96 ± 0.15	ND	182.31 ± 5.33	295.08 ± 8.20
Day 17	1.90 ± 0.08	ND	187.32 ± 6.17	279.60 ± 10.33
Day 18	1.87 ± 0.06	ND	178.81 ± 5.22	256.11 ± 7.01
Day 19	1.91 ± 0.07	ND	174.52 ± 4.23	238.77 ± 5.08
Day 20	1.85 ± 0.11	ND	173.58 ± 5.60	211.57 ± 6.11
Day 21	1.86 ± 0.09	ND	172.72 ± 4.63	214.48 ± 4.75

Notes: All data are mean±SD. The measurements of the TUD/cm and CD/cm were assessed by electronic calibers of the ultrasonography, while the changes in the endometrial PI and ID were assessed by software image analysis or ultrasonogram. All measured parameters are significant at least at $P < 0.05$.

error was ± 2 days, Y = 18.842 – (2.688 ×TUD), $r^2 = 0.861$ and standard error was ± 2 days, and Y = 17.024 – (0.446 ×CD) – (2.215 ×TUD), $r^2 = 0.908$ and standard error was ± 1.50 days.

We also noticed three distinct phases for the whole period of PP uterine involution in the present study, phase 1 (rapid involution phase) extended from the time of parturition (Day zero) till Day 5–6 PP, during which both TUD and CD measurements reduced sharply. Phase 2 (gradual involution phase) extended up to Day 12 PP, during which the reductions in both TUD and CD were gradual. Phase 3 showed a static or slow involution rate and the TUD was less than 2 cm. The process of uterine involution in goats of this study was completed ultrasonically by Day 16.6 ± 1.35 PP when the TUD reached < 2 cm, with an absence of lochial content, and difficulty to visualize the caruncles.

Results of the endometrial echotexture were illustrated in Table 1. There were significant (P < 0.0001) increases in the PI and ID of the endometrium by the progression of the PP days. Increases in the values of the PI and ID were obvious during the period from Day 1 (PI: 86.99 ± 8.61; ID (×1000): 82.09 ± 1.47) till Day 12 PP (PI: 186.73 ± 5.62; ID (×1000): 355.17 ± 38.63). Later, significant decreases in the endometrial PI and ID were observed till Day 21 PP (PI: 172.71 ± 4.63; ID (×1000): 214.48 ± 4.75).

The present study showed significant (P< 0.001) changes in the oxidative stress biomarkers (Table 2). Gradual elevations in the levels of SOD and MDA were found from Day 0 (0.33 ± 0.02 U/mL and 11.81 ± 0.21 nmol/mL, respectively) and reached the maximum levels on Days 6–7 PP (0.48 ± 0.12 U/mL and 15.31 ± 0.13 nmol/mL, respectively). Then, the levels of MDA showed gradual decreases till Day 21 PP (10.12 ± 0.13 nmol/mL), while transient decreases in the levels of SOD were observed till Day 15 PP (0.37 ± 0.05 U/mL) followed by re-increases again till Day 21 PP (0.49 ± 0.09 U/mL). Concentrations of GPx increased gradually from the kidding day (65.23 ± 2.09 U/mL) till Day 21 PP (145.81 ± 2.99 U/mL). In this regard, there were significant correlations between the pixel intensity of the endometrium and SOD (0.45, P < 0.01) and GPx (0.67, P < 0.001) activities, and MDA (0.79, P < 0.001) levels.

4. Discussion

The PP period is a crucial factor for the resumption of reproductive cyclicity and the breeding of goats (Greyling, 2000). In most previous studies, the PP uterine involution in goats was evaluated macroscopically or histologically by slaughtering goats at different postpartum intervals (Fasanya et al., 1987; Degefa et al., 2006). In the present study, ultrasonography was applied as a non-invasive, accurate, and useful aid for evaluating the progress of PP uterine involution in goats. Ultrasonography could be potentially used to distinguish between normal and abnormal postpartum uterine states as the uterine wall and luminal contents can be imaged in detail (Pharr and Post, 1992). The clinical application of ultrasonography in previous literature was restricted mainly to the detection of accumulation of lochia and retained fetal material in the uterine lumen (Yeager, Concannon, 1990; Pharr and Post, 1992; Hesselink and Taverne, 1994). Indeed, visual evaluation of the PP uterus and its content by ultrasonography is useful for assessing normal uterine involution. However, the ultrasonographic images may be affected by the experience of the operator and the setting of the device. To overcome these limitations, the assessment of endometrial echotexture by ultrasonogram will decrease interobserver variability and enhance the accuracy of ultrasonographic evaluation of the uterus because it depends on a computer image analysis software to quantify the echotexture of the uterus (PI and ID). In the current study, we are fully monitoring, for the first time, the morphometrical changes of the uterus by ultrasonography and assessing the changes in the echotexture of the endometrium by image analysis software during the post-partum period in goats. The results of the present study add to the base of knowledge because they provided full detailed information about the normal histomorphological changes of the uterus during the PP in goats. Such data are considered good reference values for the physiological status of the uterus during the PP and could be potentially helpful in the assessment of pathological uterine changes (such as endometritis) in PP goats.

In the present study, the uterus appeared as a large sac between days 0 and 2 postpartum. The echogenicity of both lochial content and caruncles changed by progressing of days postpartum. The echogenicity of lochial content varied from anechoic to hypoechoic fluid during the PP postpartum. The lochia was observed from Day 4–7 days PP, while it was not observable by ultrasonography on Day 9–14 PP. Similar findings were noticed in goats (Ababneh & Degefa, 2005) and sheep (Hauser & Bostedt, 2002; Zdunczyk et al., 2004). Uterine contractility during the early PP is responsible for the evacuation of the uterine contents and the hastening of the involution process. In sheep and goats, the lochia was completely cleared between days 12 and 15 PP (Degefa et al., 2006; Elmetwally and Bollwein, 2017). Under the effect of the uterine involution, the TUD reduced, and the luminal contents increased in echogenicity due to the expulsion of the fluid remnants, leaving cellular

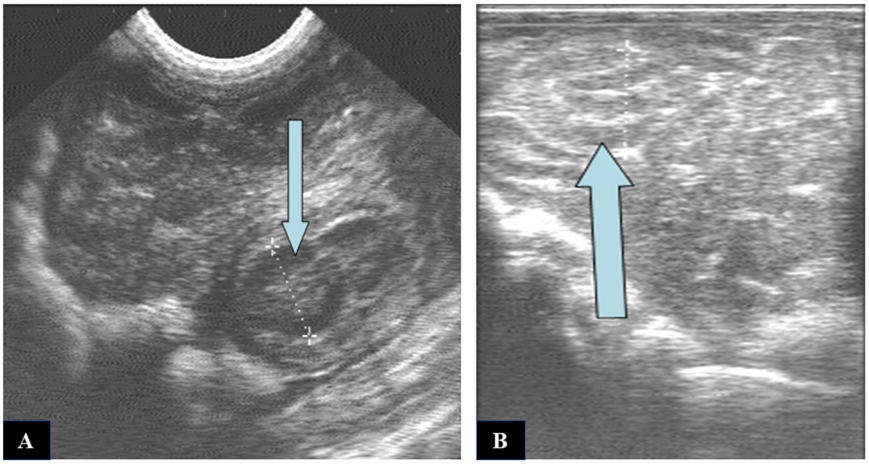

Fig. 2. The uterus of the goat on Day 2 postpartum (A) and Day 11 postpartum (B) as imaged by B-mode transabdominal and transrectal ultrasonography, respectively. Notice the changes in the echogenicity appearance of caruncles by the progression of the postpartum period.

Table 2
Monitoring changes in the levels of superoxide dismutase (SOD; U/mL), malondialdehyde (MDA; nmol/mL), and glutathione peroxidase (GPx; U/mL) in goats (n = 30) during postpartum (PP) periods.

PP Days	SOD (U/mL)	MDA (nmol/mL)	GPx (U/mL)
Day 0	0.33 ± 0.02	11.81 ± 0.21	65.23 ± 2.09
Day 1	0.37 ± 0.02	12.65 ± 0.11	79.19 ± 3.12
Day 2	0.41 ± 0.04	13.54 ± 0.03	85.37 ± 2.39
Day 3	0.43 ± 0.03	14.71 ± 0.18	91.31 ± 1.07
Day 4	0.45 ± 0.09	14.95 ± 0.09	102.87 ± 4.52
Day 5	0.47 ± 0.06	14.65 ± 0.23	111.95 ± 4.11
Day 6	0.48 ± 0.12	15.11 ± 0.12	105.00 ± 5.80
Day 7	0.44 ± 0.06	15.31 ± 0.13	114.19 ± 6.24
Day 8	0.43 ± 0.07	15.09 ± 0.05	120.57 ± 3.31
Day 9	0.41 ± 0.04	14.37 ± 0.07	125.38 ± 2.63
Day 10	0.42 ± 0.01	14.44 ± 0.03	131.06 ± 4.35
Day 11	0.39 ± 0.05	13.95 ± 0.09	134.15 ± 3.30
Day 12	0.37 ± 0.11	13.12 ± 0.04	136.73 ± 6.12
Day 13	0.38 ± 0.06	12.87 ± 0.14	137.59 ± 3.09
Day 14	0.39 ± 0.02	12.44 ± 0.03	137.90 ± 4.87
Day 15	0.37 ± 0.05	13.75 ± 0.09	139.54 ± 1.31
Day 16	0.40 ± 0.15	12.02 ± 0.05	140.31 ± 3.66
Day 17	0.43 ± 0.08	11.67 ± 0.14	139.32 ± 5.38
Day 18	0.46 ± 0.06	11.55 ± 0.08	143.09 ± 4.27
Day 19	0.45 ± 0.07	10.95 ± 0.06	143.62 ± 2.21
Day 20	0.46 ± 0.11	10.32 ± 0.07	144.46 ± 3.19
Day 21	0.49 ± 0.09	10.12 ± 0.13	145.81 ± 2.99

Notes: All data are mean±SD. All measured parameters are significant at least at $P < 0.05$.

debris and these findings agreed with results reported in PP sheep (Zdunczyk et al., 2004).

In the present study, the involution rate of the uterus is passed by three phases (rapid involution phase (Day 0–6 PP), gradual involution phase (Day 7–12 PP), and nearly static or slow involution phase (Day 13–18 PP). Our findings agreed with Degefa et al. (2006), who found the three phases of uterine regression completed at Day 7 PP, 13 PP, and 19 PP, respectively. In the present study, half of the uterine size reduction (as estimated by the TUD) was completed on Day 6 PP. This result agreed with the findings of Ababneh and Degefa (2005) reported that the majority of uterine involution occurred in goats for one week of PP. Moreover, rapid decreases in the uterine diameter (>50%) were noticed in Nubian goats between day 3 to day 14 PP (Badawi et al., 2014). Studies in sheep reported a decrease in uterine size reached more than 50% in Farafra sheep during the first 2 weeks of PP (Hayder and Ali, 2008), and reached 80% during the first 11 days of PP in German sheep (Hauser and Bostedt, 2002). However, rapid decreases in the uterine diameter (>50%) ranged from day 3 to day 14 PP in Nubian goats (Badawi et al., 2014). The differences could be attributed to species, breed, parity, seasonality, and the variation in climate and management. In addition, there was a negative correlation between the postpartum days and the transverse diameter of the uterus (TUD) ($r^2 = -0.924$, $P < 0.001$) and caruncle size (CD) ($r^2 = -0.912$, $P < 0.001$). Similarly, Degefa et al. (2006) found a high negative correlation between the weight of the uteri in goats ($r = -0.915$) and PP days.

In the current study, the end of uterine involution detected by ultrasonography occurred by Days 16.6 ± 1.35 PP when the transverse diameter of the uterus reached < 2 cm (1.87 ± 0.05 cm) in the absence of lochial content, and difficult to visualize the caruncles. Degefa et al. (2006) mentioned that the uterus resumed normal non-gravid macroscopic and microscopic characteristics by Day 19 PP in goats. Complete uterine involution was achieved at approximately Day 22 PP in various breeds of goats such as Nubian goats (Makawi and Badawi, 2007; Badawi et al., 2014) and Shiba goats (Takayama et al. 2010). The uterine involution was completed on Day 18 PP in Boer x German Improved Fawn goats (Elmetwally and Bollwein, 2017) and between Days 18 and 22 PP in West African Sahelian goats (Zongo et al., 2015). However, complete uterine involution in Boer goats was reported by Day 27.9 PP (Greyling and Niekerk, 1991b), and a longer PP period (> 30 days PP) was reported in Boer (Greyling, 2000), Anglo-Nubian, Saanen (Freitas et al., 2004), and Nilotic goats (Atta et al., 2012). The variation in the time of the end of PP uterine involution reported in goats may be attributed to the breed's differences, methods of assessment, and the effect of seasons (Rubianes et al., 1996).

Ultrasonograms of the uterine endometrium has a pivotal role to diagnose the normal histomorphological changes of the endometrium during postpartum periods (Polat et al., 2015). In the current study, values of the PI and the ID of the endometrium increased linearly with the PP, and the maximum values were attained on Day 12 PP. These changes may be attributed to the rapid stage of involution of the uterus (Degefa et al., 2006) which results in decreased blood perfusion (Elmetwally and Bollwein, 2017) and expulsion of the lochial contents. Uterine blood flow decreased significantly during the first nine days of PP, and the reduction in blood flow volume was obviously (70%) on day 6 PP (Elmetwally and Bollwein, 2017) due to myometrium contraction during the PP (Van Camp, 1991; Tan et al., 1996). Decreased blood flow may induce decreases in the uterine transverse diameter (TUD/cm) and caruncle diameter (CD/cm) by more than 50% and expulsion of most lochia during the first week of postpartum goats. Interestingly, there was a close correspondence between the reductions in the TUD and CD, and the increases in the endometrial echotexture (PI and ID). Badawi et al. (2014) reported a parallel reduction of the uterine lumen corresponding to the rapid regression of the TUD during the early PP period. Using two

approaches of ultrasonography (transabdominal and transrectal ultrasonography) for evaluating the morphometrical changes of the involuting uterus during the studied days of puerperium might be a limiting factor in this study. Indeed, the transabdominal ultrasonography gave a good visualization of the entire postpartum uterus for better evaluating the involuting uterus during the first week of the PP period (from Day zero to Day 7 PP).

Puerperium is one of the most stressful periods in dairy animals because it is associated with increased oxidative stress (Trevisan et al., 2001). In the present work, increased levels of MDA that were found in the first week of the PP goats may indicate increased levels of lipid peroxidation as one of the important consequences of oxidative stress in goats. Many literatures reported similar findings in dairy cows (Mudron, Konvicná 2006; Saleh et al., 2007; Konvičná et al., 2015). The reduced energy intake during the periparturient period, especially the first week's PP, may increase fat mobilization, which is related to the generation of lipid peroxides and reactive oxygen species (ROS). The imbalance between the production of ROS and the defense ability of biological systems to scavenge these reactive intermediates causes oxidative stress (Trevisan et al. 2001). Therefore, nutritive antioxidants may be important during this period for the efficient reduction of oxidative stress (Konvičná et al., 2015). In the current study, the SOD levels gradually increased during the early puerperium, which might be a result of high superoxide generation during this critical time. Our findings agreed with previous reports on dairy cattle (Gaál et al., 2006; Konvičná et al., 2015). However, the GPx showed continuous elevations during the whole studied period. The findings of the present study may refer to the existence of oxidative stress in postpartum goats, especially during the first week because of significant elevations of antioxidant biomarkers. Therefore, administrations of antioxidants may be helpful during the early PP period in goats to improve the antioxidant system and reduce the incidence of many metabolic diseases.

5. Conclusion

Taken together, the assessment of the changes in the morphometrical and echotextural parameters of the uterus by ultrasonography provided useful information in the progress and completion of the normal physiological postpartum uterine involution in goats. Concurrently, there were postpartum changes in the oxidative and antioxidant biomarkers in goats.

Animal ethical statement

All experimental procedures were performed following the guidelines of animal ethics raised by the Faculty of Veterinary Medicine, Cairo University, Egypt for the use of animals.

Funding

This research did not receive any specific grant from funding agencies in the public or commercial sectors.

CRediT authorship contribution statement

Haney Samir: Conceptualization, Project administration, Methodology, Investigation, Visualization, Data curation, Formal analysis, Validation, Writing the original draft, Writing - review & editing. **Faten Radwan, Mohamed A.I. El Sayed, Ahmed R.M. El-Khawagah, Mohamed Kandiel, Hossam R. El-Sherbiny**: Visualization, Data curation, Validation, Writing – review & editing. **Aly Karen**: Visualization, Validation, Writing – review & editing. **Hossam R. El-Sherbiny**: Writing – review & editing.

Conflict of interest

We hereby declare that none of the authors has any financial or personal relationship with other people or organizations that could inappropriately influence the content of this paper.

Acknowledgment

We are grateful to acknowledge veterinarian Afify Ez and Dr. Sherif Fathy (the staff responsible for the goat farm) for allowing the use of animals.

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