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Monitoring Influence of PGF2α, Estrogen and Oxytocin on Postpartum Uterine Involution by Ultrasonography in Ewes

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***Abstract*:** The goal of this study was designed to investigate the effect of estrogen, oxytocin and PGFα on uterine involution post-partum (p.p). In addition, the times for ewes to go through involution by ultrasonographical rectal examination lead to assess the best time for insemination that ensure the highest fertilization rate to avoid endometrit is post-partum. This study carried out at sheep and goat's improvement station, state board of agricultural research in Agargof, during November 15,2020 to April 15, 2021. This study involved 30 ewes of 2-4 years old and 50-65kg body weight. Blood was collected to estimate progesterone concentration and the role of ovarian activity. Results showed a gradual decrease in uterine lumen and uterine diameter. The uterine horn diameter, at different weeks of post-partum in groups treated PGF2α was 40.63 mm at first week and decrease14.22 mm. The uterine involution in ewes treated with PGF2α on post-partum, facilitate uterine involution process in ewes and lead to remove uterine fluid (lochia) and decrease uterine size, and uterine lumen. The study proves progesterone concentration as indicator to detect Ewes' post-partum ovarian activity. The current study reveals that the ultra-sonographic approach is a viable option for uterine involution management.

***Keywords*:** Sheep, Uterine involution, Post-partum, PGF2α, Oxytocin, Ultrasonography

Involution of the uterus includes uterine shrinkage, tissue loss, and tissue healing. (Zdunczyk et al 2004). The planectomies deteriorate within the first week following delivery, and lochia, a viscous brown fluid generated by endometrial erythrocyte autolysis, fills the uterine canal. During the second week of caruncle involution, necrotic plaques are discharged, and the lochia becomes less dense and more translucent. (Rubianes et al 1996). The caruncles re-epithelialize throughout the third and fourth weeks, and the uterine tissue mass diminishes, as seen by the considerable drop in uterine weight (Hauser and Bostedt 2002). The diameter of the uterine lumen and caruncle in German Lend ewes were measured during the first 30 days after birth, and it was discovered that in most ewes, uterine regression occurs around day 17 postpartum. However, the process was delayed in ewes that had obstetric interventions, cesarean section, or retained fetal membranes. For macroscopic uterine involution and takes around 17 to 35 days. (Fernande et al 2013). At 22 to 24 days after delivery, uterine involution is visible, and by 30 days after delivery is complete. (Ababneh and Degefa 2005). Silent ovulations can occur in ewes and are caused by inadequate steroid synthesis by the ovarian follicles. (Degefa et al 2006). The hypothalamus appears to become resistant to E2 as a result of the high levels of E2, resulting in the absence of behavioral estrus before the first postpartum ovulation. The hypothalamus becomes less resistant to E2 after being exposed to P4 released during the first luteal phase postpartum, and responds to E2 exposure in subsequent cycles. (Godfrey et al 1998). The P4 concentration seldom reaches 1 ng/mL during these short cycles, which last 3–4 days. (Hayder and Ali, 2008). P4 receptors are downregulated and oxytocin receptors are upregulated when the uterus is not exposed to P4 and estrogen before the first postpartum ovulation. From the third to the tenth day following delivery, the uterus decreases significantly, accompanied by a decrease in the frequency and length of uterine contractions. (Noakes et al 2001). Lochia, which are in a variety of hues and sizes, but are present in the uterus during normal involution. (Tzora et al 2002). The objectives of this study was to employ a Trans rectal Ultrasonography technique to directly image the reproductive system (ultrasonography is often used for pregnancy detection) and to quantify hormone levels to investigate ovarian activity in sheep.

MATERIAL AND METHODS

This research was conducted at Ruminant Researches Station – Ministry of Agriculture 25 Km North West of Baghdad (Agurgof). A total of thirty post-partum local breed ewes aged (2-4 years) with an average body weight of (50-60 kg) with a history of one lambing at least were used in the

experiment. They were housed in semi opened shade regarding the nutritional regime. Careful clinical examination was done to determine that animals are healthy and free from diseases. Before starting the experiment, all ewes were submitted to trans-rectal and trans-abdominal ultrasonography to be sure that the 30 local ewes were in advance pregnancy. All ewes were signed by different numbers and colors then the females. Animals preparation for ultrasonic examination experimental animals was prepared prior examination by fasting of feed for 12 hrs. to improve the accuracy of trans rectal and transabdominal scanning. The hair on the ventral abdomen was gently trimmed and shaven (Super-Max, Green, Feltham). Prior to scanning, enough gel was given to the probe to ensure good contact and remove air between the probe and the animal's skin, and the gel was also placed on the right side of the ewe The diameter of the uterine horn and uterine lumen were measured on all ewes once a week from day one to day 42 post-partum. The liner-array prostate probe (7.5 MHz) was used to do a trans-rectal examination. After the rectum had been cleared of feces, the transducer was gently inserted into the rectum after a suitable amount of ultrasonic gel had been applied. The transducer was moved medially and laterally to provide the best view of the uterus and the greatest diameter of the uterine horn was measured and recorded on the ultrasound screen (Godfrey et al 2004, Hayder and Ali 2008). Ultrasound testing were performed using a real-time B-mode scanner with a multi-frequency (3.5 and 5 MHz), linear, and sector array transducer that produced rectangular and conical pictures. Beginning one week after delivery, uterine involution was examined weekly with a trans-rectal ultrasound examination and continuing until the commencement of ovarian activity using B-mode ultrasonography. The blood was collected from the jugular vein by vacationer's gel tubes, every 1 week after parturition to 6 weeks in post-partum. For determination of progesterone level blood samples was centrifuged at 3600 rpm for 10 minutes. Sera were collected by micropipette in small plastic tubes and kept in -20C until assay for progesterone. The skin on the jugular vein was cleaned by 70% alcohol. The needle was inserted in jugular vein in a single drive by the right hand, cotton being kept in the left hand, the tube was pressed toward the needle and held tightly until the desired amount of blood was collected about 5ml. The jugular vein is then drained of 5 ml of blood into vacuum tubes (Gel and Clot Activator). ELISA Test System was used to test P4 concentration. The kit was provided by Monobind Inc. Certified Company, Lake Forest, USA. When the uterine diameter did not decrease any more for three consecutive tests, the involution was considered complete. (Ali et al 2001).

Statistical analysis: The Statistical Analysis System-SAS (2012) application was used to detect the effect of different components on research parameters.

RESULTS AND DISCUSSION

The progesterone concentrations appear to be high (Table 1). At the beginning of the postpartum period, the number of women in groups 1 and 2 stays low (0.33, 0.30 and 0.30 respectively) and is in agreement with Kaulfuss et al (1996) where progesterone concentration during the first week of parturition varied from 0.1 to 0.2 ng /ml. Progesterone levels were e significant on the third to six weeks (In ewes, the progesterone profile is regraded during the postpartum phase also is shown in Figure 1. The low progesterone levels were observed in the second weeks of parturition, which is consistent with Hussain et al. (2016), who reported that progesterone concentration remained at basal levels in post-partum, and that the results show differences in from the first to the sixth weeks. The changes could be attributed to the

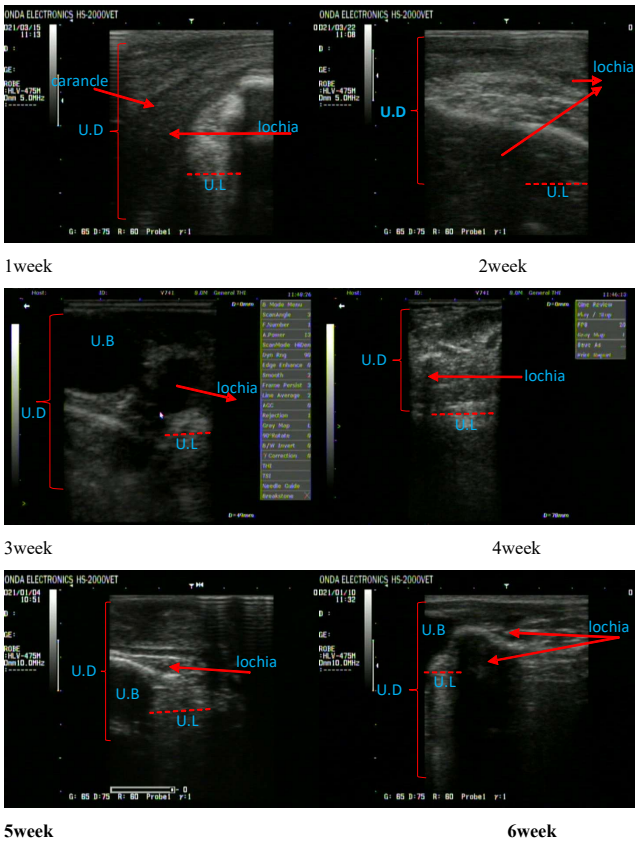

Fig. 1. Ultrasonography image of uterine involution in untreated control sheep at various postpartum periods (First to 6 week). The uterine wall and uterine lumen were easily separated by diverse ultrasonographyecho textures in the ultrasonography image of the postpartum uterus

Table 1. Progesterone concentration during post period in ewe progesterone (ng/ ml) (Mean ±SE)

Mean group	1 week	2 week	3 week	4 week	5 week	6 week	LSD value
Group 1	0.33±0.02Ac	1.93±0.64Ab	0.51±0.11A	2.02±0.70Ab	2.94±1.07Aa	0.34±0.03Bc	0.375 *
Group 2	0.30±0.00Aa	0.35±0.02Ba	0.31±0.01Ba	0.34±0.02Ba	0.34±0.02Ba	0.40±0.03Ba	NS
Group 3	0.30±0.00Ac	0.37±0.07Bc	0.35±0.05ABc	0.30±0.00Bc	3.00±0.70Ab	3.82±0.94Aa	0.381 *
LSD value	0.104 NS	0.287 *	0.194 *	0.295 *	0.402 *	0.391 *	---

Means with different capital letters in the same column and small letters in the same row are significantly differenti. * (P≤0.05).

Table 2. Uterine diameter at different period post-partum(mm)(Mean ±SE)

Week of examination	Group 1 treated PGF2α	Group 2 treated estrogen +oxytocin	Group 3 control	LSD value
1 week	40.63±1.49Ab	45.45±1.20Aa	43.30±1.02Aab	4.395*
2 week	31.20±1.63Ab	39.95±1.32Ba	41.95±0.80Aa	4.982*
3 week	22.76±0.62Bb	26.08±0.75Cb	34.04±1.21Ba	4.772*
4 week	17.22±0.67Cc	22.55±0.53Cb	29.56±1.07BCa	4.251*
5week	14.22±0.55Cb	16.26±0.46Db	28.13±0.97Ca	3.988*
6week	13.1±0.53Db	15.53±0.44Db	27.62±1.47Ca	4.846*
LSD value	4.027 *	5.179 *	4.522 *	----

See Table 1 for details

period of parturition, which could be in season or out of season as well as breed distinctions. The hypothalamic-pituitary-adrenal axis was activated, resulting in the development of estrogen synthesis and a decrease in progesterone levels, as well as a decrease in progesterone levels. The three groups' values differed significantly recorded utilizing before the typical luteal phase, small peaks of progesterone have been identified in postpartum ewes. They peak was at 0.5 ng/ml and then fade out after two weeks. From the moment of delivery to four weeks after delivery, the uterine diameter decreases rapidly (Table 2). There is no information about uterine involution after childbirth. The postpartum uterine diameter assessed by trans-rectal ultrasonography in the three groups is shown in Table 1. In groups 1 and 2, uterine involution occurred 35 days after delivery, while in group 3 it occurred 28 days after delivery. The third group completed uterine involution in a shorter amount of time than the first and second groups. The diameter of the uterine horn decreased considerably in all groups within the first four weeks after parturition. The purpose of the study is to provide information that can be used to improve reproductive efficiency through the use of ultrasonography. The percent decline in uterine diameter (mm) was 40.63, 31.20, 22.76, and 17.22 respectively during 4 weeks (Table 2). At the early days pp. of this inquiry, the uterine contents were present and exhibited a hypo echogenic feature, confirming Hauser and Bostedt's findings (2002). The current study is critical and provides information that will aid in enhancing reproductive efficiency. Understanding postpartum changes in the uterus and ovaries is critical to achieving maximal reproductive efficiency in ewes. Furthermore, the findings of this study demonstrated that real-time B-mode ultrasonography is a viable approach for evaluating uterine involution in post-partum ewes with uterine regression. It's also necessary to diagnose ovarian activity. The findings demonstrate that progesterone concentration is a strong predictor of luteal function throughout the postpartum period

CONCLUSION

The current study concluded that the ultrasonography approach is a viable option for uterine involution management and that uterine involution was completed more quickly in groups treated with PGF2, as measured by uterine diameter and lumen, as well as a decrease in lochia, than in other groups.

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