Iranian Journal of Veterinary Research, University of Shiraz, Vol. 6, No. 2, Ser. No. 12, 2005

Evaluation of uterine involution using radio-opaque markers during the postpartum period in Makuii ewes

Babaei, H.[1*] and Ayen, E.[2]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of kerman, Kerman, Iran; [2]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran

[*]**Correspondence:** H. Babaei, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of kerman, Kerman, Iran. E-mail: hombabaei@yahoo.com

Summary

After completion of the third stage of parturition and expulsion of the placenta, the uterus started to involuting until it reaches to normal size. The rate of uterine involution after parturition was studied in 6 healthy Makuii ewes. During second month of pregnancy the genital tract was exteriorized through a posterior midline laparatomy under general anesthesia and then four non-toxic split shots with different sizes were sutured on the serosal wall of the uterine body and horns. After parturition, the distance between markers was measured by sequential radiography. The mean length of the uterine body declined until 28 days after lambing but statistically maximum reduction was seen at about 14 days after parturition ($P<0.05$). Also, the mean diameter of gravid and non-gravid horn rapidly declined until 14 days postpartum ($P<0.05$) but reduction countinued until 42 days postpartum ($P>0.05$). The difference between the mean diameter of the gravid and non-gravid horn was not significant between days 14-42 (because of relatively small reduction in size). There were a high correlation between the measurements taken at the time of laparatomy and determined by radiography (4 days after surgery) for the mean length of uterine body ($r = 0.89$), the mean gravid horn diameter ($r = 0.91$) and non-gravid horn diameter ($r = 0.79$). In conclusion, after final statistical analysis of sequential radiographic views by using a repeated measurement analysis of variance, involution of the uterus in Makuii ewes was completed about 28 days postpartum for the uterine body and about 14 days for both the gravid and non-gravid horn. Radio-opaque marker is a useful method to study changes of the uterine size after parturition in live ewes.

Key words: Ewe, Postpartum, Uterine involution, Radiography

Introduction

After completion of the third stage of parturition and expulsion of the placenta, involution of the uterus beginning and restoring to its normal non-pregnant size and physiological function. Uterine involution in ewes has been assessed histologically (VanWyk *et al*., 1972a), macroscopically after slaughter (Foote *et al*., 1967; VanWyk *et al*., 1972b; Call *et al*., 1976) and with radio-opaque markers method in live ewes by Tian and Noakes (1991). Although these reports have provided useful information, the methods described could not make it possible to monitor the rate of involution in individual (live) animals except in the method used by Tian and Noakes (1991).

The process of involution is regulated by myometrial contractions and consists of discharge of lochia, elimination of bacterial infections and regeneration of the endometrium (Jainudeen and Hafez, 1993). Histologically, there is exfoliation of the degenerating caruncle resulting from changes in the vascular and connective tissue, which lead to ischaemic necrosis and change in collagen content of the uterus (Harkness and Moralee, 1956; Gier and Marion, 1968; VanWyk *et al*., 1972a). The periods reported for completion of involution ranges from 17 (Foote *et al*., 1967) to 60 days (Honmade, 1977). But many reports are in agreement with the day about 28 after lambing (VanWyk *et al*., 1972b; Kiracofe, 1980; Tian and Noakes, 1991; Godfrey *et al*., 1998).

In ruminant dams the process is delayed by abnormal parturition and puerperal complications (Huszenicza *et al*., 1987; Hussain and Daniel, 1991) and is governed, in addition, by age and parity (Marion *et al*., 1968; Huszenicza *et al*., 1987; Izaike *et al*., 1989), season (Marion *et al*., 1968), suckling and milking (Izaike *et al*., 1989), milk yield (Menge *et al*., 1962; Huszenicza *et al*., 1987; Izaike *et al*., 1989), nurition and weight fluctuation after parturition (Menge *et al*., 1962). There is, however, no report available on the uterine involution of native Iranian ewes like Makuii breed. The present study, therefore, was undertaken to investigate uterine involution during postpartum period in Makuii ewes with a radiograph method (radio-opaque markers) described by Tian and Noakes (1991).

Materials and Methods

Experimental animals

Six healthy (after clinical and paraclinical examination), three-year-old parous Makuii ewes, which had lambed (single) normally indoors were used. The ewes throughout the study suckled the lambs. Sequential radiographs were taken within 24 hrs after lambing and 3, 7, 14, 28 and 42 days postpartum. Uterine involution was measured by a modification of the radiographic method described by Tian and Noakes (1991) in which different sizes of the radio-opaque markers are attached to the uterine horns and internal os of cervix. The ewes were fed hay ad libitum and bedded on wood shavings, during pregnancy and after parturition.

Attachment of radio-opaque markers

Under general anesthesia and using strict asepsis the genital tract was exteriorised through a posterior midline laparatomy and four non-toxic split aluminium shots with different sizes were sutured to the wall of the uterine horns and internal os of the cervix, with No. 0 silk and atraumatic needle, care was taken not to penetrate to the lumen of the genital tract. One 4 mm diameter shot (A) was attached to the internal os of the cervix, one 7 mm diameter shot (B) was sutured to the dorsal intercornual ligament and a 3 mm (C) and a 6 mm (D) diameter shots were attached to the wall of non-gravid and gravid uterine horns directly opposite to the shot B, so that the distance between the shot A and B were measured as the length of the uterine body and the distance between the shot C and B, and shot D and B at the base of the horns after the point of the bifurcation were measured as the diameter of the non-gravid and gravid horn, respectively (Fig. 1).

All of the ewes were in 2nd month of gestation. At the time of laparatomy to attach the markers, we could easily diagnose the gravid horn. The direct measurements of the uterine body length and the gravid and non-gravid horn diameters were made with a sterile ruler on each of the uterus.

Radiographic procedure

Two radiographs, a dorso-ventral and a lateral view were taken 4 days after surgery and at each examination after lambing. Two points as constant marks were considered in all of the ewes. One mark was in the dorsal midline, at the point of intersection of a line between the tuber coxae and the longitudinal axis of the vertebral column, during dorso-ventral radiography and the other one was on the left flank just cranial to the point of the stifle joint during lateral radiography. This ensured that the genital tract was in the line with the X-ray beam. To have a constant distance between genital tract and X-ray tube, during radiography (especially lateral radiography), ewes restrainted carefully standing in the middle of a yoke and on the other hand the distance from head of the X-ray tube to the cassette on the lateral and dorso-ventral radiography was kept constant at 85 cm. The setting of kilovoltage (KV) was 80 and milliamps x time (mAs) was regulated automatically by apparatus (Toshiba, KCD-10 M, 6AIT, Tokyo Shibara Electric Co., Ltd.). Care was taken to radiate beam at right angle upon the film cassette. Three measurements were made on each radiograph. At dorso-ventral radiograph, distance between the centers of markers A and B (uterine body length), and the distance between the centers of markers B and C (non-gravid horn diameter) and between markers B and D (gravid horn diameter), but at lateral radiograph, the vertical distance between horizontal line passed from the marker B and other shots were measured. Since the two radiograph were taken at right angle to each other, the absolute distance between the shots were calculated by using Pythagorus's theorem (Tian and Noakes, 1991).

Statistical analysis

The postpartum uterine horns and bodies measurements were analyzed after logarithmic transformation by using repeated measurement analysis of variance, and least significant difference (LSD) test was used for multiple comparisons. All values are reported as means ± SEM and results were considered significant at $p<0.05$.

Results

The markers could be readily identified at all of the radiographic views. It was easily possible to differentiate the left and right uterine horns in the lateral and dorso-venteral radiographs, because we carefully noted the direction of the gravid and non-gravid horns during surgery and also, we had used shots with different sizes on the uterine walls.

Uterine body length, gravid and non-gravid horn diameter during the 42 days experimental period are presented in Table 1. Final analysis of results showed that the mean uterine body length decreased ($P<0.05$) sharply during the first 14 days after parturition (19.32 ± 2.58, 15.35 ± 1.79, 11.49 ± 2.2, 7.23 ± 1.3 cm at days 1, 3, 7 and 14, respectively, mean ± SEM). The mean of the uterine body length at day 28 was lower than day 14 ($P>0.05$). Again, the mean uterine body length increased at day 42 postpartum (6.06 ± 0.72, 6.52 ± 0.71 cm at days 28 and 42, respectively, mean ± SEM, $P>0.05$).

Involution of the gravid and non-gravid horns was more rapid at the first 14 days after lambing ($P<0.05$) but there was slight reduction from day 14 until day 42 after parturition in the mean diameter of the gravid (3.69 ± 0.54, 3.41 ± 0.36, 3.24 ± 0.63 cm, respectively, mean ± SEM, $P>0.05$) and non-gravid horns (3.51 ± 0.45, 3.43 ± 0.54, 3.29 ± 0.37 cm, respectively, mean ± SEM, $P>0.05$). Because of relatively small reduction at the diameter of the horns at the late stages of the uterine involution, differences between gravid and non-gravid horns were not statistically significant. After 42 days postpartum, the ewes were slaughtered and some follicles with different sizes and even corpus luteum were seen on their ovaries.

The mean length of uterine body, diameter of the gravid and non-gravid horns measured directly during laparatomy did not have any statistical difference in comparison to the radiography. Although a high correlation was observed between them (r = 0.89, r = 0.91, r = 0.79, respectively).

Table 1: Mean (±SEM) dimensions of the uterus of ewes at six stages after parturition

Day after lambing	1	3	7	14	28	42
Uterine body length (cm)	19.32 ± 2.58^{a}	15.35 ± 1.79^{b}	11.49 ± 2.2^{c}	7.23 ± 1.3^{d}	6.06 ± 0.72^{d}	6.52 ± 0.71^{d}
Gravid horn diameter (cm)	7.83 ± 0.91^{a}	5.81 ± 0.70^{b}	5 ± 0.82^{bc}	3.69 ± 0.54^{d}	3.41 ± 0.36^{cd}	3.24 ± 0.63^{cd}
Non-gravid horn diameter (cm)	8.19 ± 1.22^{a}	6.27 ± 1.20^{b}	4.23 ± 0.68^{c}	3.51 ± 0.45^{cd}	3.43 ± 0.54^{cd}	3.29 ± 0.37^{d}

[a]Means that do not have common letter in the same horizontal row are different ($P<0.05$)

Discussion

The method in this study already used by Tian and Noakes (1991) to measure the effects of exogenous hormones on uterine involution. Radio-opaque markers method was used again by Ayen and Noakes (1997).

Fig. 1: Genital tract of a ewe after slaughtering showing position of the radio-opaque markers sutured on the internal os of the cervix (A), dorsal intercornual ligament (B), non-gravid and gravid uterine horns during pregnancy (C and D)

It has been used by Regassa and Noakes (1999) to evaluate relationships between uterine involution with acute phase proteins. All those studies and the present study confirmed that the radio-opaque markers method used for assessing the changes in the size of the genital tract before and after lambing is reliable.

During surgery the gravid and non-gravid horns were identified and two different sizes of split shots attached on them. This made us enable to differentiate between the left, right, pregnant and non-pregnant horns in the lateral and dorso-ventral radiography. This is in contrast to the method of previous studies using the same radiographic technique (Tian and Noakes, 1991; Regassa and Noakes, 1999). Tian and Noakes (1991) had used the similar sizes of split shots on the gravid and non-gravid uterine horn walls. Regassa and Noakes (1999) had used different sizes of the split shots on the gravid and non-gravid uterine horn walls. Regassa and Noakes (1999) had used different sizes of the split shots too, but in contrast to the study described here attachment of the shots to the genital tract had been made when the ewes were non-pregnant.

The mean (±SEM) uterine body length were slightly larger at 42 days than 28 days postpartum (6.52 ± 0.71 vs 6.06 ± 0.72, respectively; $P>0.05$). A similar result was obtained by Tian and Noakes (1991) and Regassa and Noakes (1999), who suggested that it was probably, uterine involution was being measured in conscious living ewes in which the measurements might be influenced by changes in uterine tone and position. Tian and Noakes (1991) suggested that changes in uterine dimensions might indicate some degree of ovarian rebound, perhaps with folliculogenesis or even ovulation. This hypothesis was confirmed by observation of some follicles and corpus luteum on the ovaries of the ewes in the present study.

In the present study, the end of the uterine body involution was determined to be at approximately 28 days postpartum, which is confirming the reports of the previous studies using, either sequential slaughtering (Basset, 1963; VanWyk *et al*., 1972a, b; Rubianes *et al*., 1996) or the same radiographic technique by Tian and Noakes (1991) and Regassa and Noakes (1999). Also, In the study described here involution of the gravid and non-gravid horn were completed by 14 days after parturition, although after day 14 its regression process was relatively low untill day 42. This corresponds to the study by Rubianes and Ungerfeld (1993) who observed uterine weight and length of uterine horns decreased ($P \leq 0.01$) during the first 17 days and then remained constant. Hauser and Bostedt (2002) observed a rapid decline in uterine diameter from the point of parturition to day 11 postpartum. During this period, more than 80% of the size of the uterus regressed. According to the histological studies by VanWyk *et al*., (1972a) an enormous decrease in mass of the postpartal uterus occurs during the first 12 days following parturition in sheep. They reported that uterine involution in ewe was completed by 28 days postpartum. Tennant *et al*., (1967) reported that in the cow the rate of involution of the gravid horn is most rapid during the initial 3-4 week period following parturition but, further gradual reduction in mean uterine horn diameter continues afterwards. This data are in agreement with the present study that a rapid decline was seen in the uterine body length and the gravid and non-gravid horn diameters during the first 14 days after parturition. Conclusions regarding the time required for a complete uterine involution vary according to the breed, the reproductive status, the method of the study and the time of the year when the observation was made. Moreover, none of the previous studies on uterine involution has so short investigation intervals (20 examination in 42 days).

The time for completion of the uterine involution in Makuii ewes must be almost similar to the other studies. Many reports are in agreement with 28 days after lambing. In the present study, the end of the uterine involution was also determined at approximately 28 days postpartum. However, further studies using a large number of ewes with a single or twin pregnancy and more examination intervals with different sex of foetus, will provide more useful observations and informations on the study of uterine involution.

Acknowledgement

This study was made possible through the support of Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran.

References

1- Ayen, E and Noakes, DE (1997). Displacement of the tubular genital tract of the ewe during pregnancy.Vet. Rec., 141: 509-512.

2- Basset, EG (1963). Some effects of endogenous hormones on muscles and connective tissue, with special reference to the ewe. *Proceedings of the Newzealand Society for Animal Production*. 23: 107-120.

3- Call, JW; Foote, WC; Eckre, CD and Hulet, CV (1976). Postpartum uterine and ovarian changes, and estrus behaviour from lactation effects in normal and hormone treated ewes. Theriogenology. 6(5): 495-521.

4- Foote, WC; Call, JW and Hulet, CV (1967). Effect of lactation and hormone treatment on ovulation, oestrus and uterine involution in the ewe. J. Ani. Sci., (Suppl. 1), 32: 48.

5- Gier, HT and Marion, GB (1968). Uterus of the cow after parturition: involutional changes. Am. J. Vet. Res., 29(1): 83-96.

6- Godfrey, RW; Gray, ML and Collins, JR (1998). The effect of ram exposure on uterine involution and luteal function during the postpartum period of hair sheep ewes in the tropics. J. Ani. Sci., 76: 3090-3094.

7- Harkness, RD and Moralee, BE (1956). The time-course and route of loss of collagen from the rat's uterus during post-partum involution. J. Physiol., 32: 502-508.

8- Hauser, B and Bostedt, H (2002). Ultrasonographic observations of the uterine regression in the ewe under different conditions. J. Vet. Med. A., 49: 511-516.

9- Honmade, D (1977). Postpartum changes in the uterus of the ewes. Zhivotnovodstvo, 3: 62-63 (Cited in Animal Breeding Abstract's 45: 384).

10- Hussain, AM and Daniel, RCW (1991). Bovine endometritis: current and future alternative therapy. J. Vet. Med. A., 38: 641-651.

11- Huszenicza, G; Molnar, L; Solti, L and Haraszti, J (1987). Postpartal ovarian function in Holstein and crossbred cows on large scale farms in Hungary. J. Vet. Med. A., 34: 249-263.

12- Izaike, Y; Suzuki, O; Okano, A; Shimada, k; Oishi, T and Kosugiyama, M (1989). Influences of parity, milk yeild and suckling stimulation on uterine involution in beef cows. Jpn. J. Anim. Reprod., 35: 45-49 (in Japanese with English abstract).

13- Jainudeen, MR and Hafez, ESE (1993). Reproductive failure in females. In: Hafez, ESE (Eds.), *Reproduction in farm animals*. 6th. Edn., Philadelphia. Lea and Febiger. PP: 261-286.

14- Kiracofe, GH (1980). Uterine involution: its role in regulating postpartum intervals. J. Anim. Sci., (Suppl. 2), 51: 16-25.

15- Marion, GB; Norwood, JS; Gier HT (1968). Uterus of the cow after parturition: factors affecting regression. Am. J. Vet. Res., 26: 71-75.

16- Menge, AC; Mares, SE; Tyler, WJ and Casida, LE (1962). Variation and association among postpartum reproduction and production characteristics in Holstein-Friesian cattle. J. Dairy Sci., 45: 233-241.

17- Regassa, F and Noakes, DE (1999). Acute protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. Vet. Rec., 144: 502-506.

18- Rubianes, E and Ungerfeld, R (1993). Uterine involution and ovarian changes during early postpartum in autumn-lambing corriedle ewes. Therio. 40: 366-372.

19- Rubianes, R; Ungerfled, C; Vinoles, B; Carabajal, B; De Castro, T and Ibarra, D (1996). Uterine involution time and ovarian activity in weaned and suckling ewes. Can. J. Anim. Sci., 76: 153-155.

20- Tennant, B; Kendrick, JW and Peddicord, RG (1967). Uterine involution and ovarian function in the postpartum cow. A retrospective analysis of 2338 genital organ examinations. Cornell Vet., 57: 543.

21- Tian, W and Noakes, DE (1991). A radiographic method for measuring the effect of exogenous hormone therapy on uterine involution in ewes. Vet. Rec., 129: 463-466.

22- VanWyk, LC; VanNiekerk, CH and Belonje, PC (1972a). Involution of the postpartum uterus of the ewe. J. S. Afr. Vet. Assoc., 43: 19-26.

23- VanWyk, LC; VanNiekerk CH and Belonje, PC (1972b). Further observations on the involution of the postpartum uterus of the ewe. J. S. Afr. Vet. Assoc., 43: 29-33.