Effects of intrauterine administration of oxytetracycline on uterine involution in the ewe

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Summary

After parturition, the genital system is returning to its normal non-pregnant state. The reduction in the size of the genital tract is called involution. Involution of the ovine uterus after parturition was investigated following the intrauterine administration of oxytetracycline (2 gr). Under general anaesthesia and using strict asepsis the genital tract of ewes in 2nd month of gestation were 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 (Radio-opaque markers method). Then, for assessment of uterine involution sequential radiographs were taken on the day of lambing and 3, 7, 14, 28 and 42 days after parturition. Three measurements (uterine body length, gravid and non-gravid horn diameter) were made on each of the radiographs. Involution of the uterine body length and gravid horn diameter were completed by about 28 days after parturition in sterile water treated (Control) group (n = 6) whereas completion of the non-gravid horn involution was significantly more rapid (by about 14 days after parturition) than gravid horn ($P<0.05$). The mean of the uterine body length, gravid and non-gravid horn diameter of oxytetracycline administered (treatment) ewes (n = 4) at any stages after parturition were non significantly higher than the control group ($P>0.05$). The mean of three measurements of the treatment (n = 4) group at the day 42 were similar to the control group at the day 14 after lambing. However, the present study demonstrates that treatment with intrauterine oxytetracycline may prolong involution of the reproductive tract of ewes after parturition.

Key words: Uterine involution, Postpartum period, Oxytetracycline, Radiography, Ewe

Introduction

After parturition, the genital system is returning to its normal non-pregnant state. The reduction in the size of the genital tract is called involution (Arthur *et al*., 1996). For many years attempts have been made to observe the rate of uterine involution by the administration of exogenous drugs. Most of the studies have been conducted with using exogenous hormones in the cow (Foote and Hunter, 1964; Marion *et al*., 1968; Tian and Noakes, 1991b; Sheldon and Dobson, 2000; Sheldon *et al*., 2002) and in the ewe (Foote, 1971; Tian and Noakes, 1991a), but there is only little information of the effects of antibiotics on uterine involution (Onuma *et al*., 1990).

Oxytetracycline is a broad spectrum antibiotics used in human and veterinary medicine. Decreasing random migration and chemotaxis of the neutrophils (Majeski and Alexander, 1977), phagocytosis (Forsgren and Gnarpe, 1982) and anti-inflammatory properties of tetracycline that are unrelated to their antimicrobial activities (Golub *et al*., 1987) have been reported. There is strong evidence that tetracycline can inhibit the degradation of collagen by inhibition of collagenolysis (Golub *et al*., 1983, 1984, 1985). The degradation of collagen is likely to be the main change occuring in the uterine involution process (Harkness and Moralee, 1956).

The uterine involution of ewes have been assessed macroscopically at slaughter

(Foote *et al*., 1967; VanWyk *et al*., 1972b; Call *et al*., 1976; Rubianes and Ungerfeld, 1993; Rubianes *et al*., 1996). Also, comprehensive histological studies of the sheep uterus during postpartum period have been carried out by Uren (1935) and VanWyk *et al*., (1972a). Data on the postpartum uterine involution in ewe are often based on postpartal findings but Tian and Noakes (1991a) provided a useful radiographic method for identifying the various parts of the tubular genital tract by the surgical attachment of radio-opaque markers and the use of sequential radiographs, they employed this method to monitor uterine involution in ewes.

In the study described here, the modified method (Tian and Noakes, 1991a) was used to monitor the changes of uterus during postpartum period and to investigate whether intrauterine administration of oxytetracycline may influence the involution of the tubular genital tract of ewes after lambing.

Materials and Methods

Experimental ewes

Ten healthy, three-year-old parous Makuii ewes, which had lambed normally indoors, were used. The ewes were fed grass hay ad libitum and bedded on wood shavings, during pregnancy and after parturition. All the ewes suckled their lambs until several weeks after the experiment ended. The 10 ewes, which concieved were divided at random into two groups for antibiotic treatment (4) and for control (6). Within 24 hrs of lambing ewes in the treatment group were infused with 2 gr intrauterine oxytetracycline hydrochloride 20% which had been diluted in 20 cc sterile water (Damloran Pharmaceutical Co., Borujerd, Iran) and the ewes in the control group received 20 cc sterile water. The identity of the control ewes remained unknown to the authors until the data had been analysed. For assessment of uterine involution sequential radiographs were taken on the day of lambing and 3, 7, 14, 28 and 42 days after lambing. Uterine involution was measured by a modification of the radiographic method described by Tian and Noakes (1991a) in which radio-opaque markers were attached to the uterus and cervix.

Attachment of radio-opaque markers

Under general anesthesia and using strict asepsis the genital tract of ewes in the 2nd month of gestation were 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 (Mersilk, Ethicon Ltd., UK) and atraumatic needle, ensuring that the lumen was not penetrated. The 4 mm shot (A) was attached at the level of the cranial end of the cervix, the 7 mm shot (B) was sutured to the dorsal intercornual ligament, and the 6 mm (C) and 3 mm (D) shots were sutured to the gravid and non-gravid uterine horn wall directly opposite to the shot B, so that the distances between them and shot B were comparable with the diameters of gravid and non-gravid horns at the point of the external bifurcation.

Radiography

Two radiographs, a dorsoventral and a lateral view, were taken at each examination with the ewe restrained in a yoke. The distance between the head of the X-ray tube and the film cassette was 85 cm for each radiograph. The setting of kilovoltage (kv) was 80 and milliamps X time (mAs) was regulated automatically by apparatus (Toshiba, KCD-10 M, 6 AIT, Tokyo Shibara Electric Co., Ltd.). Since the two radiographs were taken with the X-ray beams at right angles to each other, the absolute distance between markers could be calculated by using pythagorus's theorem (Tian and Noakes, 1991a).

Statistical analysis

Results are expressed as mean ±SEM. Significance was assigned at $p<0.05$. The data for the length of the uterine body and the diameter of the uterine horns were analysed after logarithmic transformation. Repeated measures analysis of variance was used to detect differences in the diameter of horns and length of uterine bodies at each examination within control or treatment group and least significant difference (LSD) test was used for multiple comparisons.

Independent t-test was used to detect differences between the control and treatment group.

Results

Three measurements were made on each of the radiographs. The distance between markers A and B gave a measurement of the length of the uterine body, the distance between markers B and C gave a measurement of the diameter of the previous gravid horn, and between markers B and D gave a measurement of the diameter of the previous non-gravid horn. The mean (±SEM) values of three measurements made on six occasions are showed in Table 1 for oxytetracycline administered group (treatment) and sterile water administered group (control).

Uterine body length and gravid horn diameter decreased ($P<0.05$) sharply during the first 14 days after parturition in the control and treatment group. Although there were no significant differences between the measurements taken on days 14, 28 and 42 but the three measurements slowly decreased until day 28 after lambing. The mean of uterine body length, gravid and non-gravid horn diameter of oxytetracycline administered ewes (treatment) at any stages after parturition were higher than control group (Figs. 1a, b and c). The mean uterine body length, gravid and non-gravid horn measurements of oxytetracycline treated groups decreased until day 42 after lambing. The mean of three measurements of the oxytetracycline treated ewes at day 42 were similar to sterile water treated animals at day 14 after lambing ($P>0.05$). However, the time for the uterine body length and horns diameters to complete involution in treatment group tended to be longer than control group.

Discussion

Tian and Noakes (1991a) provided a useful radiographic method that it avoids the need to slaughter ewes at laparatomy for assessing the changes in the size of the genital tract after lambing, because the sequential changes in the size of the genital tract could be monitored in live animals frequently and with minimal disturbance. One disadvantage of their method was inability to differentiate the right from the left horn and also, inability to identify the previous gravid horn in radiographic views. In contrast to study of Tian and Noakes (1991a), who

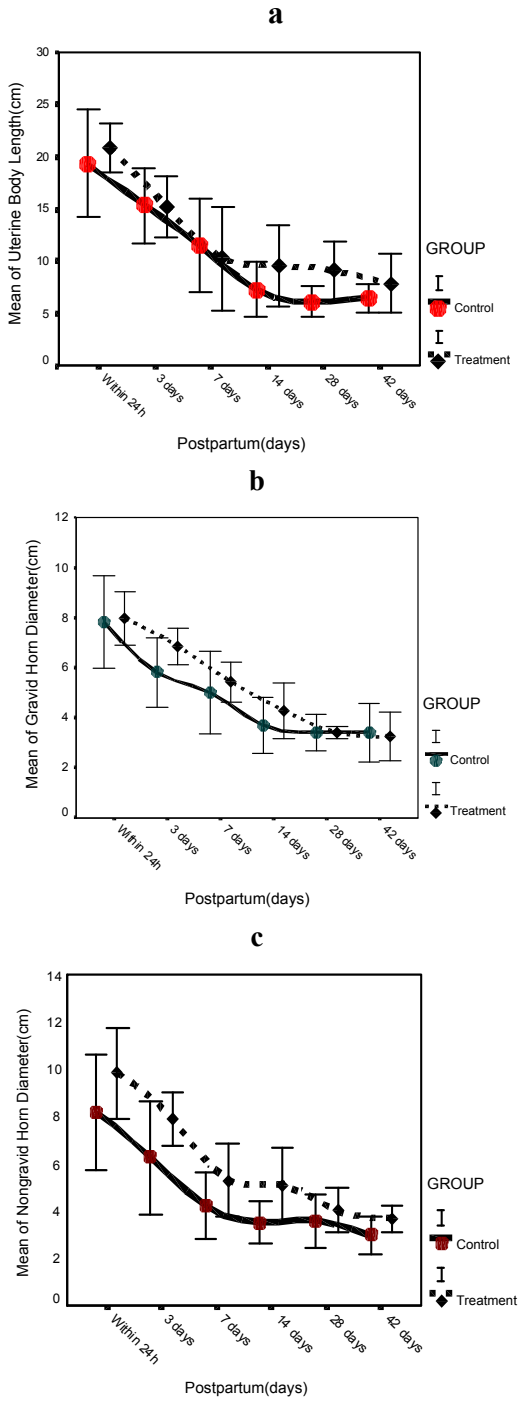

Fig. 1: Mean (±SEM) of uterine body length (a), gravid horn diameter (b) and non-gravid horn dimeter (c) in ewes with sterile water (Control, n = 6) and oxytetracycline (Treatment, n = 4) treated group

Table 1: Mean (±SEM) dimensions of the uterus of ewes administered intrauterine oxytetracycline (Treatment) or sterile water (Control) at six stages after parturition

Group	Uterine Measurement (cm)	Days from parturition					
		Within 24hrs	3	7	14	28	42
Control n = 6	Uterine body length	19.32[a] (2.58)	15.34[b] (1.79)	11.49[c] (2.2)	7.24[d] (1.3)	6.06[d] (0.73)	6.45[d] (0.65)
	Gravid horn diameter	7.83[a] (0.92)	5.81[b] (0.7)	5[bc] (0.83)	3.69[d] (0.55)	3.41[cd] (0.37)	3.41[cd] (0.59)
	Non-gravid horn Diameter	8.19[a] (1.23)	6.27[b] (1.2)	4.23[c] (0.68)	3.51[cd] (0.45)	3.43[d] (0.54)	3.29[d] (0.37)
Treatment n = 4	Uterine body length	20.8[a] (1.15)	15.19[b] (1.49)	10.28[bc] (2.46)	9.57[c] (1.97)	9.07[c] (1.45)	7.85[c] (1.4)
	Gravid horn diameter	7.97[a] (0.52)	6.85[b] (0.36)	5.43[bc] (0.39)	4.29[cd] (0.56)	3.39[d] (0.12)	3.26[cd] (0.48)
	Non-gravid horn diameter	9.82[a] (0.97)	7.89[b] (0.55)	5.3[c] (0.77)	5.08[d] (0.77)	4[cd] (0.47)	3.94[cd] (0.19)

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

determined the uterine involution by a radiographic method, in the present study it was possible to differentiate the previously gravid horn from non-gravid horn in the lateral and dorso-ventral radiographs because the two markers of gravid and non-gravid horn (C and D, respectively) were not the same size.

There was a rapid decline in gravid uterine horn diameter and uterine body length of the control as well as the treatment ewes from the time point of parturition to day 14 postpartum (Figs. 1a, b and c). During this period, more than 90% of the size of the uterus is regressed. This is according to histological studies by VanWyk *et al*., (1972a) and ultrasonographic studies by Hauser and Bostedt (2002). Rapid decline in the size of the uterus is presumably caused by contraction of the myometrium accompanied by vasoconstriction and loss of fluids (Botha, 1976). The same results have been reported by Buch *et al*., (1955), Morrow *et al*., (1966) and Tennant *et al*., (1967) in cow that the rate of involution of the gravid horn is most rapid during the initial 3-4 week period following parturition.

In the present study, the end of the uterine involution was determined at approximately 28 days postpartum. This corresponds to the study by VanWyk *et al*., (1972a), Tian and Noakes (1991a) and Godfrey *et al*., (1998). But, it is in contrast of other authors (Foote and Call, 1969; Rubianes and Ungerfeld, 1993; Rubianes *et al*., 1996; Hauser and Bostedt, 2002). Conclusions regarding the time required for a complete uterine involution vary according to the method of their studies, the reproductive status of the control group, the breed, the time of the year when the observation is made and the degree of involvement of horns in placentation.

In contrast to the study of Onuma *et al*., (1990), who reported the positive effects of oxytetracycline on uterine involution, our results show that uterine involution in oxytetracycline treated ewes tended to be longer than in the sterile water treated group. Uterine involution has been showed to be due to the degradation of collagen after parturition (Harkness and Moralee, 1956) and possibly, exogenous drugs must have a profound effect on this process. Tetracyclines are known to inhibit collagenases activity from several cell types, such as neutrophils, macrophages, osteoblasts and chondrocytes (Rifkin *et al*., 1993). Tetracyclines can inhibit collagenases by a mechanism independent of the antimicrobial efficacy of the drug (Golub *et al*., 1987). It seems that there is direct inhibition of the enzyme by the drug. The anticollagenase properties of the tetracyclines have been explained by existence of a cation-binding mechanism (Golub *et al*., 1987). Tetracyclines can also inactivate other proteases (i. e., Elastase, Cathepsin G, Serine proteases) (Sorsa *et al*., 1993). Collagenase (Harkness and Moralee, 1956; Woessner, 1962; Jeffrey *et al*., 1971; Ryan and Woessner, 1971) and other

proteases (Shimada *et al*., 1985) are important regulators of the extracellular matrix during uterine involution.

It has been suggested that in cattle, in which bacterial contamination during postpartum period is very common, uterine involution may be an inflammatory process (Kindahl *et al*., 1992), evidence for this suggestion has been provided by demonstration of an acute phase protein response early during the puerperium in cows (Alsemgeest *et al*., 1993) and ewes (Scott *et al*., 1992). Golub *et al*., (1983, 1984, 1985) have published a series of studies describing anti-inflammatory properties of tetracycline that are unrelated to their antimicrobial activities. Anti-inflammatory drugs reduce the biosynthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). $PGF_{2\alpha}$ and its synthetic analogues are known to have both ecbolic and luteolytic effects and they have been showed to hasten uterine involution (Kindahl *et al*., 1982).

In conclusion, these results suggest that intrauterine infusion of oxytetracycline during the puerperal period was associated with some degree of uterine subinvolution. It seems that exogenous drugs with a profound effect on the degradation of collagen may influence the rate of uterine involution. Intrauterine administration of antibiotics suppress uterine leukocyte activity (Dhaliwal *et al*., 2001) or even may have a direct effect on collagenase activity through which may affect the rate of uterine involution. Further investigations are need to clarify the effects of other antibiotics on the rate of uterine involution after parturition.

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