Journal of Animal and Veterinary Advances 7 (11): 1418-1421, 2008
ISSN: 1680-5593

The Effect of Progestagen on the Changes of the Vaginal Flora Arising from Intravaginal Sponge Treatment and Susceptibility of the Vaginal Flora to Antibiotics in Ewes

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Abstract: The aim of this study, was to investigate the effect of progestagen on the changes of the vaginal bacterial flora with sponge treatment. Progestagen impregnated sponges (30 mg fluorogestone acetate) were inserted ewes (Group I, n = 12) for 12 days and, sponge without progestagen (blank sponge), served as control groups (Group II, n = 12), were inserted ewes for 12 days during the non-breeding season. Vaginal bacterial counts were evaluated on the vaginal flora samples obtained before the introduction of the sponges, at sponge withdrawal and after 48 h from withdrawal of sponge. The mean value for the colony forming units ($x10^3$ mL^{-1}) were 6.1 and 4.5 on the day of intravaginal sponge insertion and increased to 113.5 and 139.8 at sponge withdrawal ($p<0.05$), decreased 7.9 and 43.3 after 48 h withdrawal of sponge in Group I and II, respectively ($p<0.05$). The changes of the vaginal bacterial flora were not different statistically at the time of sponge withdrawal in progestagen and non-progestagen sponge groups. Although, there were not differences between at the time of sponge introduction and withdrawal of sponge in 2 groups, it was found a difference after 48 h removal of sponges with progestagen and without progestagen treatments groups ($p<0.05$). Amoxicillin/Clavunate, Ampicillin, Oxacillin, Trimethoprim/Sulfamethoxazole 1/19 and Tetracycline were more resistance than the other antibiotics according to results of the antibiotic susceptibility test. Intravaginal sponge treatments increased bacterial counts, but this increase returned normal values at probable estrous time in progestagen impregnated sponge treatment. Number of vaginal bacteria did not return normal values in the non-progestagen sponge treatment group after 48 h removal of sponge, because of ewes in this group naturally could not come into estrus. In this study, it was concluded that progesterone did not affect the number of bacterial counts in the vaginal flora except for changes caused by intravaginal sponge treatment.

Key words: Ewes, intravaginal sponge, progestagen, vaginal flora

INTRODUCTION

Sheep are polyoestrous animals dependent on seasons (Hafez, 1993). Several methods such as natural progesterone, synthetic progestagens, melatonin, prostaglandin F2α, or gonadotropin releasing hormone and isolated ram introduction have been used for estrus synchronization (Godfrey *et al*., 1999; Iida *et al*., 2004; Wildeus, 2000). To increase fertility in sheep progestagens are used to induction of estrus or estrus synchronization. Progestagens can be given by oral administration, subcutaneous or intravaginal insertion (Simonetti *et al*., 2000; Wildeus, 2000). Intravaginal sponges have been the traditional treatment of choice for estrous synchronization (Menchaca and Rubianes, 2001; Wildeus, 2000), but an abnormal hemorrhagic and putrid vaginal discharge can be seen at sponge withdrawal (Hashemi *et al*., 2006; Scudamore, 1988) and increases in the vaginal flora numbers can be observed (Amin, 1996). However, we have not found any literature about effects of progestagens impregnated sponge to the number of bacteria in vaginal flora. Suarez *et al*. (2006) reported that the presences of a foreign body, such as sponge in the vagina stimulated bacterial growth and local mucous secretion during sponge treatment and these changes generated a localized inflammation (Motlomelo *et al*., 2002). Abnormal vaginal flow or purulent mucous collection was correlated to a high incidence of unfertilized ova in superovulated and artificially inseminated ewes, with impaired embryo development and low pregnancy rates (Scudamore, 1988). After withdrawal of sponge, it was observed that bacterial populations

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returned to numbers similar to those observed before sponge insertion at estrous time (Amin, 1996; Suarez *et al*., 2006).

Progesterone suppresses specific components of the immune system and natural killer cell activity, while it has a mainly positive influence on other nonspecific components. In addition to this, progesterone increases antibody-dependent cellular cytotoxicity of polymorphonuclear leucocytes and expression of complement receptors and phagocytosis of peritoneal macrophages. In the uterus, mainly immunosuppressive effects of progesterone are described (Scheibl and Zerbe, 2000). Progestagens could effect the components of the immune system that respond to bacterial growth (Suarez *et al*., 2006). Immunosuppression by progesterone may be likened to the immunosuppressant activity of glucocorticoids (Pineda, 2003). Although, the increasing of the number of vaginal bacteria raised from a reaction occurring against sponge perceived as a foreign object, it is not known clearly, the effect of progestagens or polyurethane sponge on the vagina. Because of this confusing situation, intravaginal sponges with progestagen and without progestagen were used in the study. In this way, the effectiveness of progestagen hormone or polyurethane sponge was to reveal individually on the changes of the vaginal flora caused by intravaginal sponge treatment in ewes.

MATERIALS AND METHODS

The experiment was conducted under natural conditions during the non-breeding season. A total of 24 ewes (2 and 4 years old) divided into 2 groups (n = 12). Ewes were selected randomly for this study. The ewes grazed on natural pasture all day and water was offered *ad libitum*. Intravaginal sponges (30 mg FGA; Chronogest, Intervet International B.V., Boxmeer, Netherlands) were inserted into the vagina for a 12 days period in ewes of Group I (n = 12). Group II (n = 12); served as control group for 12 days period and ewes in this group received blank sponges, which were polyurethane sponge and did not include progestagen. Vaginal flora samples were collected from the vagina, using sterile hyssops by direct contact of the anterior vagina and samples were transported in the Stuart transport medium for bacteriological examination. Samples were collected from two groups immediately prior to the introduction of sponges, at the time of sponge withdrawal and at 48 h after sponge withdrawal.

Bacterial count and antibiotic susceptibility: The samples were collected from the vagina, at approximately 2 cm from the vulvae lips, using sterile hyssops by direct contact, without rubbing for bacterial count from 2 groups. The first of the hyssops was vigorously vortexed for total bacterial counts in 1 mL sterile Phosphate Buffered Saline (PBS), pH 7.4, for 1 min in order to suspend the bacteria. The resultant suspension was serially diluted and the bacteria were counted on Blood Agar Plates and incubated for 48 h at 37°C. The second hyssop was used to determine the bacterial susceptibility of the vaginal flora to different antibiotics before sponges insertion. Antimicrobial susceptibility test was performed on BD Diagnostic Instrument Systems. Amikacin, amoxicillin/clavunate, ampicillin, aztreonam, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime sodium, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, nitrofurantoin ofloxacin, oxacillin, rifampin, tetracycline, trimethoprim/sulfamethoxazole, penicillin G and vancomycin had been used in the sensitivity tests of the isolated bacteria. BD Diagnostic Instrument Systems (Sparks, MD, USA), which contains a serial conventional kromantojenic and florojenik biochemical substances, were used in accordance with the instructions of the producer firm in the sensitivity tests of antibiotic.

Statistical analysis: The results of the vaginal bacterial counts (CFU mL^{-1}) were analysed by student t-test. The statistical model was consisted with the interaction between groups and times. The SPSS 13.0 statistics program was used for the analysis of data. Differences were considered significant at a level of $p<0.05$.

RESULTS

At the withdrawal of the sponges, a vaginal flow with different quantity was observed in all ewes in the study groups. The presence of sponge in the vaginal cavity, increased the local mucous secretion, which was characteristic of purulent or hemorrhagic mucous collection. At the time of sponge introduction and removal of sponge, the number of total vaginal bacteria was not significantly different between progestagen sponges group and non-progestagen sponges group ($p>0.05$). However, the bacterial counts after 48 h removal of sponge were significantly different between groups ($p<0.05$). Vaginal bacterial counts (CFU mL^{-1}) according to, sponge treatment are presented in Table 1. The mean value for the colony forming units ($x10^3$ mL^{-1}) were 6.1±0.8 and 4.5±0.7 on the day of intravaginal sponge insertion and increased to 113.5±39.3 and 139.8±56.9 at sponge withdrawal, decreased 7.9±0.8 and 43.3±30.8 ($p<0.05$) after 48 h withdrawal of sponge in Group I and II, respectively.

Table 1: Vaginal bacterial counts expressed as colony forming units mL^{-1} (CFU mL^{-1}) at the time of sponge introduction, removal of sponge and after 48 h wihtdrawall of sponge

	Vaginal bacterial counts (CFU mL^{-1}) at the time of sponge treatment		
Groups (n = 12)	At sponge introduction	At removal of sponge	After 48 h removal of sponge
Progestagen sponge	$6.1\pm0.8\times10^{3(a)}$	$113.5\pm39.3\times10^{3(b)}$	$7.9\pm0.8\times10^{3(a)}$
Blank (non-progestagen) sponge	$4.5\pm0.7\times10^{3(a)}$	$139.8\pm56.9\times10^{3(b)}$	$43.3\pm30.8\times10^{3(c)}$

a,b,c: Means in the same column with different superscripts (a, b, c) differ significantly ($p<0.05$)

DISCUSSION

Suarez *et al*. (2006) reported that hormonal changes status such as estrous cycle could be effect on vaginal bacterial population especially when progesterone levels were high. During this period, progesterone can be effect immune system. As a know progestagen impregnated sponges are used to induce estrous during non-breeding season in ewes. To realize, the effect of progesterone on bacterial flora in vagina, sponges with progestagen and without progestagen were used to ewes in anestrus period. The present study was planned in anestrous time for the reason of avoiding the possible confounding effect on the initial vaginal bacterial population by different hormonal influeces such as estrous cycle status.

The use of intravaginal sponges stimulates a localized inflammation with the accumulation of a less foul-smelling fluid and a significant increase in bacterial load (Motlomelo *et al*., 2002; Romano, 2004). It was thought that the presence of a foreign body such as sponge in the vagina increased the local mucous secretion. Suarez *et al*. (2006) hypothesized that the bacteria present at the time of intravaginal sponge insertion and its by-products could later promote further inflammation. The volume of vaginal flow and the bacterial load reached peak from sponge insertion to day 5 after insertion of the intravaginal sponges. It then remained constant until sponge withdrawal (Ungerfeld and Rubianes, 1999, 2002). On the other hand, Suarez *et al*. (2006) reported that the diversity of growing colonies clearly decreased from day 5 to day 13 after intravaginal sponge treatment. Some researcher (Pineda, 2003; Scheibl and Zerbe, 2000) reported that progesterone has immunosuppressive effect. The effect of progesterone was mentioned in this and similar studies about bacterial counts but its efficiency and importance statistically were not researched sufficiently. Although, progesterone had immunosuppressive effect, we did not found an importance difference on the vaginal flora between progestagen impregnated and non-progestagen groups.

Bacterial counts of the vaginal flora increased significantly ($p<0.05$) at sponge withdrawal and a drastic decrease was recorded after 48 h withdrawal of sponge in progestagen impregnated sponge group. It was thought that predominant presence of polymorphonuclear leucocytes might contribute to the very fast clearance of the bacterial load observed after sponge withdrawal (Suarez *et al*., 2006).

The qualitative features of the bacterial load changed as the sponge insertion period. Some researchers (Guerra *et al*., 2002; Suarez *et al*., 2006) recommend the use of antibiotics with sponge treatments prior to insertion for preventing vaginal infection provoked following the use of intravaginal sponges. However, Ahern (1976) reported that the vaginal flora not differs significantly following the administration of oleandomycin and tetracycline in the sponge. In this study, several antibiotic resistant bacteria colonies being recorded in 2 treatment groups. According to the antibiotics susceptibility test, Amoxicillin/Clavunate, Ampicillin, Oxacillin, Trimethoprim/Sulfamethoxazole 1/19 and Tetracycline were resistance; Ofloxacin, Erythromycin, Chloramphenicol and Gentamycin were intermedier resistance than other antibiotics.

CONCLUSION

The results of the study revealed that the use of progestagen impregnated and blank sponge (non-progestagen) treatments stimulated inflammation of the vagina with increase of bacterial counts. The bacterial counts increased at sponge withdrawal and then drastically decreased at 48 h after sponge removal in progestagen treatment group. However, these decreases were not recorded at 48 h after sponge removal in blank sponge group. From sponge withdrawal to estrous, a drastic decrease in bacterial count was recorded in progestagen sponge treatment group. The reduction of number of bacteria in the vagina from removal of sponge being considered to result from disappearance of immunosuppressive effect of progestagens and high oestrogen level, which contribute to the very fast clearance of the bacterial load. This reduction in bacterial counts appears normal after 48 h removal of sponge in progestagen-impregnated group, because of ewes applied intravaginal progestagen sponge for estrus induction. Nevertheless, since ewes treated without progestagen sponge did not come into estrus, reductions of bacterial counts were not appearance naturally. The number of bacteria in the vagina increases during the first times of sponge treatments and decreases to the normal level at the time of estrus. At this time, fertility was not effected with changes happened in vagina. Finally, results of the

study indicate that the important role of progesterone was not happen on the increasing of the number of vaginal bacteria during sponge treatment.

ACKNOWLEDGEMENT

This study was supported by Dicle University Research Fund (Project 07-01-17).

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