Journal of Animal and Veterinary Advances 7 (6): 723-727, 2008
ISSN: 1680-5593

The Effectiveness of Using Antibiotic with Intravaginal Sponge and Duration of Sponge Treatments on the Vaginal Flora and Fertility in Anestrous Ewes

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Abstract: The aim of this study was to compare effectiveness of antibiotic administration to intravaginal sponge before sponge insertion and investigate duration of sponge treatment for determining changes in the vaginal bacterial flora and fertility parameters. Intravaginal sponges impregnated with 30 mg FGA were inserted in 30 Awassi ewes for long-term (14 days; LT), long-term with antibiotic added sponges (LT-A) and short-term (7 days; ST) during the non-breeding season. All ewes received 400 IU PMSG at sponge withdrawal. Bacterial counts were performed on the vaginal flora samples obtained before the introduction of the sponges, at sponge withdrawal and day of estrous in the treatment groups. The mean value for the colony forming units (x 10^3 mL^{-1}) were 5.31, 2.92 and 4.91 on the day of intravaginal sponge insertion and increased to 163.97, 68.34 and 147.0 ($p<0.05$) at sponge withdrawal, decreased on the day of estrous to 6.97, 4.53 and 5.88 in group LT, LT-A and ST, respectively ($p<0.05$). According to the antibiotics susceptibility test, clindamycin, erythromycin, penicillin and vancomycin were more resistance than the other antibiotics. The frequency of ewes in estrous, pregnancy rates and the interval to onset of estrous were similar among groups in the study ($p>0.05$). It was concluded that intravaginal sponge treatments increased bacterial counts, but this increase returned normal values at estrous time. Changes in the number of vaginal flora were not different statistically in the antibiotic added and not added sponge treatment groups at sponge withdrawal and estrous time. Antibiotic administrations to sponge prevented bacterial growth by fist days of sponge treatment. However, this did not affect bacterial count and reproductive response on the day of estrous.

Key words: Antibiotics, ewes, fertility, intravaginal sponge, vaginal flora

INTRODUCTION

Estrous synchronization in ewes is achieved by control of the luteal phase of the estrous cycle (Wildeus, 1999). Progestagen treatments have been widely used to synchronise estrous in ewes, traditionally by the use of intravaginal devices for long periods (12-14 days). However, recent studies showed that progesterone priming as short as 5-7 days is as effective as the traditional long-term primings to induce estrous (Menchaca and Rubianes, 2001; Ungerfeld and Rubianes, 2002; Vinoles *et al*., 2001).

Intravaginal sponges have been the traditional treatment of choice for estrous synchronization (Menchaca and Rubianes, 2001; Wildeus, 1999), but an abnormal hemorrhagic and putrid vaginal discharge can be seen at sponge withdrawal (Scudamore, 1988; Hashemi *et al*., 2006) and increases in the vaginal flora numbers can be observed (Amin, 1996). 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). Intravaginal releasing device gives rise to problem such as vaginitis. In addition to this mentioned above, Scudamore (1988) had postulated that 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. Therefore, some researcher reported that use of antibiotics in the sponge prior to insertion was to be efficient in preventing vaginal infections, provoked by intravaginal sponge treatment in goat and ewes (Ungerfeld and Rubianes, 2002; Vinoles *et al*., 2001). It was observed that bacterial populations returned to numbers similar to those observed before sponge insertion at estrous time (Amin, 1996; Suarez *et al*., 2006).

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Differently previous studies, the aim of this study were to compare the effectiveness of antibiotic administration to intravaginal sponge before sponge insertion and the changes in the number of bacterial populations at long term and short-term sponge treatment following intravaginal sponge insertion. Also, bacterial susceptibility of vaginal flora and fertility parameters of all treatment groups were recorded in anestrous ewes.

MATERIALS AND METHODS

Animals and treatment: The experiment was carried out under natural conditions during anestrous season in the southeast of Turkey. Total 30 Awassi ewes (2 and 5 years old) divided into 3 groups (n = 10). This region is situated at 37°55′01″N latitude and 40°16′46″E longitude and at an altitude of 660m. The yearly temperature in the area is on average 15.7°C. Ewes were selected randomly for this study. The ewes grazed on natural pasture all day and water was offered ad libitum. Vaginal sponges (30 mg FGA; Chronogest, Intervet International B.V., Boxmeer, Netherlands) were inserted for different periods of time. Intravaginal sponge remained in situ for 14 days in Group I (long-term; LT), sponge with antibiotic added (0.2 mL Deposilin®, Intervet) remained 14 days in Group II (long-term with antibiotic; LT-A) and sponge remained only 7 days in Group III (short-term; ST). All ewes received an intramuscular injection of 400 IU PMSG (Chorono-Gest/PMSG, Intervet International B.V., Boxmeer, Netherlands). All ewes were joined with fertile rams with marking harnesses following sponge withdrawal. Sexual receptivity was observed by the marks on the rump of the ewes at 12 h observation intervals for 4-day period. Estrous responses (%), interval to onset of estrous and pregnancy rates were recorded according to groups.

Collection of vaginal samples: 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 all treated ewes immediately prior to the introduction of sponges, at sponge withdrawal and on day of estrous.

Bacterial count and antibiotic susceptibility: Samples for bacterial count were taken from three groups. The samples were collected from the vagina, at approximately 2 cm from the vulvae lips, using sterile hyssops by direct contact, without rubbing. For total bacterial counts, the first of the hyssops was vigorously vortexed 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. The 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 have been used in the sensitivity tests of the isolated bacteria.

BD Diagnostic Instrument Systems, Sparks, MD, USA were used in accordance with the instructions of the producer firm in the sensitivity tests of antibiotic. BD Phoenix™ 100 automatic Microbiological Identification System is a device designed for the rapid determination of the bacteria and antimicrobial sensitivity tests. In this system, 100 identification tests and antimicrobial sensitivity tests can be held. In order to determine the type of the bacteria the ID part of the system contains a serial conventional, kromantojenic and florojenic biochemical substances.

Statistical analysis: The colony units (CFU mL^{-1}) were Analysed by Variance (ANOVA) for repeated measures of logarithmic normalized data distribution. The statistical model was consisted with the effects of group, day, the interaction between group and time and the random effect of ewe within a group. Comparison of bacterial counts among the treated groups was done by ANOVA. Reproductive response such as frequencies of estrous and pregnancy rates were compared x^2 –test. The interval from device withdrawal to estrous onset was compared by analysis of variance.

RESULTS

All ewes in the study showed the production of vaginal flow with different quantity at sponge withdrawal. Characteristic view of vaginal flow seemed as purulent mucous collection. At any instance of the time, the number of total vaginal bacteria was not significantly different between groups ($p>0.05$). Also in each group, vaginal bacterial counts were not significantly different at the sponge insertion and estrous time. However, the bacterial counts of these instances were significantly different that of at sponge withdrawal. The mean values for the colony forming units (CFU) ($\times 10^3$ mL^{-1}) were 5.31, 2.92 and 4.91 on the day of intravaginal sponge insertion and increased to 163.97, 68.34 and 147.0 at sponge withdrawal in group LT, LT-A and ST, respectively ($p<0.05$). Then, the mean value decreased drastically ($p<0.05$) on the day of estrous to 6.97, 4.53 and 5.88 in

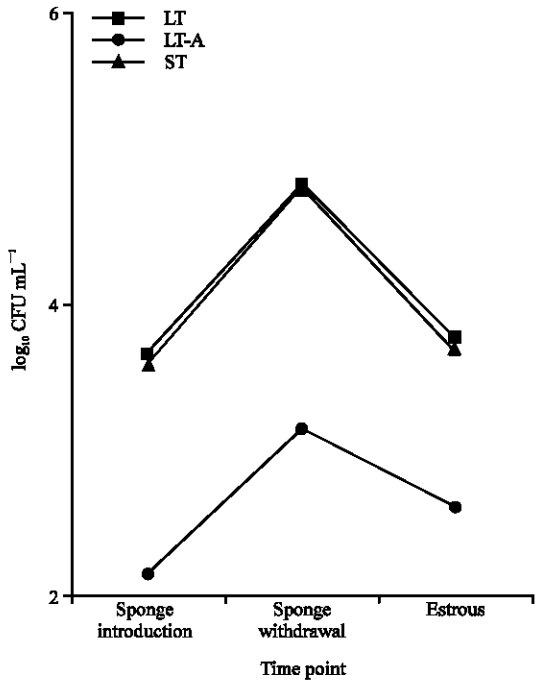

Fig. 1: Vaginal bacterial counts expressed as $\log_{10}$ colony forming units mL^{-1} ($\log_{10}$ CFU mL^{-1}). Sponge was remained in vagina for 14 days (LT); 14 days with antibiotic (LT-A) and 7 days (ST)

Table 1: The resistance of vaginal flora to antibiotics before sponge insertion

Antibiotics	Group LT	Group LT-A	Group ST
Amoxicillin/Clavunate	0/10	0/10	0/10
Ampicillin	0/10	0/10	0/10
Cefazolin	1/10	0/10	0/10
Ciprofloxacin	1/10	0/10	0/10
Clindamycin	4/10	0/10	2/10
Erythromycin	5/10	0/10	5/10
Gentamycin	0/10	0/10	0/10
Nitrofurantoin	0/10	0/10	0/10
Ofloxacin	0/10	0/10	0/10
Oxacillin	0/10	0/10	0/10
Penicillin G	0/10	6/10	0/10
Trimethoprim/Sulfamethoxazole	1/10	0/10	0/10
Vancomycin	3/10	0/10	0/10

Table 2: Estrous response, the interval from sponge withdrawal to estrous and pregnancy rates in anoestrous ewes[a]

Groups	Ewes	Ewes in estrous (%)	Interval to onset of estrous (h)	Pregnancy rate (%)
LT	10	8/10 (80)	45.88±4.42	5/8 (62.5)
LT-A	10	9/10 (90)	44.56±4.39	6/9 (66.67)
ST	10	7/10 (70)	44.43±4.31	4/7 (57.14)

a No differences ($p>0.05$) were observed between groups in the studied parameters

groups LT, LT-A and ST respectively. After logarithmic transformation of the data, the values (CFU mL^{-1}) for all groups from the day of sponge insertion until the day of estrous are presents in Fig. 1.

The sensitivity of bacterial flora is presented in Table 1. No bacteria resistant to amoxicillin/clavunate, ampicillin, gentamycin, nitrofurantoin, ofloxacin and oxacillin were recorded at any point in time for the different groups. According to antibiotic susceptibility test, clindamycin, erythromycin, penicillin and vancomycin were more resistance in all samples. Each of three samples in group LT was resistance cefazolin, ciprofloxacin and trimethoprim/sulfamethoxazole and this could be seen easily in Table 1.

The frequencies of ewes in estrous and pregnancy rates were similar statistically among groups in the study ($p>0.05$). The interval from device withdrawal to estrous onset was not different among treatment groups ($p>0.05$). Percentage of ewes that came into estrous and pregnancy rate for groups are presented in Table 2.

DISCUSSION

Suarez *et al.* (2006) reported that hormonal changes status such as estrous cycle could be effect on vaginal bacterial population. Thus, 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 influences such as estrous cycle status.

Using intravaginal sponges generated a localized inflammation concurrent with the accumulation of a less foul-smelling fluid and a significant increase in bacterial load (Suarez *et al.*, 2006; Romano, 2004). The presence of a foreign body such as sponge in the vagina increased the local mucous secretion. Thus, some researchers reported that administration of antibiotic to the sponges before insertion to vagina prevented vaginitis (Vinoles *et al.*, 2001; Ungerfeld and Rubianes, 2002). Suarez *et al.*, (2006) hypothesized that the bacteria present at moment of intravaginal sponge insertion and its by-products could later promote further inflammation. The Controlled Internal Drug Release (CIDR) devices do not absorb nor impede drainage of vaginal secretions and accumulation of vaginal mucous secretions were not noted, as is often the case in intravaginal sponges (Romano, 2004; Carlson *et al.*, 1989; Greyling and Brink, 1987; Welch, 1984).

In this study, bacterial counts increased significantly ($p<0.05$) at sponge withdrawal; a drastic decrease was recorded on the day of estrous time, similar to those observed before intravaginal sponge insertion. Suarez *et al.* (2006) observed relatively similar results in their study during short-term and long-term sponge treatments. From sponge withdrawal to estrous, a drastic decrease in bacterial count was recorded, similar to those

observed before sponge insertion. 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 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 5-13 after intravaginal sponge treatment. Since the changes of the vaginal flora remained constant from 5th day to sponge withdrawal and the bacterial counts were returned normal values on the day of estrous, no differences in estrous response and interval to onset of estrous or pregnancy rate were observed for LT, LT-A and ST sponge treatment groups. Treatment of anestrous ewes with short-term progestagens were sufficient to induce fertile estrous (Knights *et al*., 2001; Ungerfeld and Rubianes, 2002). Dogan and Nur (2006) recorded an 88.8% estrus response rate with intravaginal sponge and PMSG treatments during anestrous season. The estrus responses in this study were 80, 90 and 70% following LT, LT-A and ST sponge treatment. These parameters were not significantly different between all groups. Confirming those observations, the reproductive responses of the ewes (i.e., frequency of ewes in estrous; interval to onset of estrous and pregnancy rates) were similar between groups. Any differences were not seen in fertility parameters in LT, LT-A and ST groups.

Changes of vaginal environment, including the presence of phagocytes engulfing spermatozoa, could have a detrimental effect on the reproductive performance in the synchronization of ewes with sponge (Robinson *et al*., 1970). Abnormal vaginal flow or purulent mucous collection effect fertilization of ova in superovulated and artificially inseminated ewes, with impaired embryo development and low pregnancy rates (Scudamore, 1988). Thus, some researchers reported that use of antibiotics in the sponge prior to insertion to be efficient in preventing vaginal infections provoked by intravaginal sponge treatment in goat and ewes (Ungerfeld and Rubianes 2002; Vinoles *et al*., 2001). In this study, no statistical differences were recorded from the viewpoint of fertility parameters in the treatments groups whether antibiotic was added or not to sponges before insertion. It was thought that these mentioned above was depending on returning of vaginal flora to previous statement, before sponge treatment, on the day of estrous.

In this study, local application of penicillin to sponge (Deposilin®) was used to avoid vaginitis before sponge insertion. The mean values for the Colony Forming Units (CFU) ($\times 10^3 mL^{-1}$) were not statistically different between antibiotic added sponge and sponge without antibiotic addition. However, CFU values in antibiotic added group (LT-A) were lower than short-term (ST; 7 days) and long-term (LT; 14 days) sponge treatment especially at sponge withdrawal. The mean values of colony forming units returned to normal values in both antibiotic added group and groups without antibiotic administration on the day of estrous. No differences were recorded between those groups according to antibiotic administration of the sponges. Although some researchers (Ungerfeld and Rubianes, 2002; Vinoles *et al*., 2001) postulated that using antibiotic injection to sponge before sponge insertion was to be useful, changes in the number of vaginal flora was not different statistically in the antibiotic added and not added sponge treatment groups at sponge withdrawal and estrous time. It is considered that antibiotic administration to sponge is to be effective to prevent vaginitis especially in the first days of sponge treatment.

Several antibiotic resistant bacteria colonies were recorded in all treatment groups. According to antibiotic test, clindamycin, erythromycin, penicillin and vancomycin were more resistance than other antibiotics. The sensitivity of vaginal flora to different antibiotics is set out in Table 2. Ahern (1976) reported that the vaginal flora not differ significantly following the administration of oleandomycin and tetracycline in the sponge. Guerra *et al*. (2002) observed intravaginal local application of gentamycin to be efficient in preventing vaginal infections provoked by intravaginal sponge use in goat.

CONCLUSION

It is concluded that the use of different methods of intravaginal sponge treatments (LT, LT-A and ST) stimulated inflammation of the vagina with increase of bacterial counts. These changes were observed in all treatments groups. The bacterial counts increased at sponge withdrawal in all groups and then drastically decreased on the day of estrous. Despite of changes in the vaginal flora, reproductive performance of the different treatment groups such as short-term, long–term and long-term with antibiotic added sponge, were observed similarly. According to these obtained results, using antibiotic before sponge insertion is to be useful to prevent vaginitis at first days of sponge insertion, but effect of antibiotic has continuously decreased after 5 days sponge insertion. Thus, using antibiotic injection to sponge may not be necessary as it failed to demonstrate

a significant beneficial effect on the reproductive response. On the other hand, it can be used to prevent vaginitis before sponge insertion.

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