Efficacy of anti-microbial agents on vaginal microorganisms and reproductive performance of synchronized estrus ewes

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ABSTRACT

Objective: To isolate and identify microflora and fungal species at different phases during estrus synchronization of ewes and estimate their prevalence; compare the effectiveness of antimicrobial administration to intravaginal sponge on the changes in the vaginal microorganisms and reproductive performance. Methods: Sixty Egyptian ewes were allocated into three equal groups (G: 1, 2 and 3). G1 was inserted with vaginal sponge containing medroxyprogesterone acetate and served as control; without antimicrobial additive. The other two groups were treated as G1, but sponges were previously injected with ciprofloxacin (G2), while sponges of G3 were injected with ciprofloxacin and clotrimazole. Vaginal swabs were collected from each treated ewe, prior sponge insertion, at sponge withdrawal and 48 h later for microbiological investigation and bacterial count. On the day of sponge removal, 300 IU/eCG was administered for each treated ewe. The identified bacterial strains before sponge insertion were tested for sensitivity with antimicrobial disks. Results: Bacterial isolates before sponge insertion were more sensitive to ciprofloxacin. Frequencies of ewes in estrus; the interval from sponge withdrawal to onset estrus and the duration of estrus were statistically similar among treated groups. The pregnancy rate in G2 (100.0%) was higher than G1 (66.7%) and G3 (82.4%). The total bacterial count before sponge insertion was similar between all treatments and increased significantly in all groups on the day of sponge withdrawal. The prevailing bacteria on D0, D14 and 48 h after sponge removal for all treated groups were Staphylococcus spp. followed by Escherichia coli. Regarding to fungus species, percentages of isolation increased from 5.00% (before sponge insertion) to 100.00% and 88.89% at sponge withdraw for G1 and G2, respectively. In G3, the fungus was declined from 10% (before sponge insertion) to 5% (at sponge removal). Conclusions: The concomitant treatments by antimicrobial to the vaginal sponge which used for estrus synchronization in ewes can improve reproductive performance.

1. Introduction

Intravaginal sponge impregnated with progestogens being the most commonly used for estrus synchronization in sheep[1–3]. Sponges containing progestagens are used for long periods of similar lifespan to a cyclic corpus luteum, regardless of the stage of the cycle or the follicular status of the ovary at the time of treatment[4]. The presence of these sponges in the vagina for this period acts as a foreign body and constitutes a predisposing factor for vaginitis and generates changes in the vaginal environment with accumulation of purulent mucous secretion, and foul smelling fluids. This infection is often due to proliferation of the local microflora and by secondary opportunistic Gram negative Enterobacteriaceae invaders, mainly Escherichia coli (E. coli)[1–7]. Coliforms, as well as Gram-positive cocci, mainly Staphylococcus spp. and Streptococcus spp., are the most common bacterial species present in those infections[6]. Changes in these bacteria of the vagina and its population with the abnormal vaginal flow were correlated to a high incidence of

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unfertilized ova in artificially inseminated ewes, with impaired embryo development and subsequent decrease fertility rates of estrus synchronized ewes[8]. In some laboratories and commercial products manufacturers recommend that to avoid vaginitis and decreases the perceived odour at sponge withdrawal, antibiotics should be added to sponges before insertion to the vagina. Although tetracycline is antibiotic, most widely used in association with sponge treatments, there are several studies reporting that it is the antibiotic with the greatest number of resistant colonies[7,9,10] and this may explain the decrease in conception rates after its application. Others observed the application of gentamycin to vaginal sponges is efficient in preventing vaginal infections provoked by intravaginal sponge[11]. Thus, the objectives of the study were to determine, characterize the vaginal bacterial flora in native ewes at the time of sponge insertion for estrus synchronization and subsequent in vitro bacterial susceptibility to different antibiotics. Changes in the bacteria and fungus population at different stages (sponges withdrawal and estrus) following intravaginal sponge insertion with different antimicrobial treatment; and evaluating the bacteria and fungus effects on the subsequent reproductive performance were also investigated.

2. Materials and methods

2.1. Animals management and study location

The study was conducted on a total 60 multiparous, non-pregnant native Egyptian ewes, aged 3-5 years and weighing 35-45 kg. The animals were clinically healthy, free from reproductive disorders and fed on maintenance ration containing Egyptian clover plus concentrate mixture with 16.6% crude protein. Water and mineral supplement were available ad-libitum. The present study was carried out during spring months (late March to May) at the Animal Reproduction Research Institute, Giza province (located at latitude of 30° 00’29”N, longitude of 31° 12’39”E, altitude of 30 m above sea level).

2.2. Experimental design and treatment schedule

The ewes were randomly allocated into three groups (G1, G2 and G3) of equal numbers (n=20 ewes/each group). G1 was inserted by the aid of an applicator with polyurethane vaginal sponge containing 30 mg medroxy-progesterone acetate; MAP (DEPO-PROVERA, Pfizer), and served as a control with no antimicrobial addition to the application of gentamycin to vaginal sponges is efficient in preventing vaginal infections provoked by intravaginal sponge[11]. The second vaginal swab was cultured using different media for isolation and purification of bacteria included: Blood agar, MacConkey agar, Nutrient agar, Edward blood agar and Mannitol salt agar. Plates were incubated at 37 °C, and examined daily for bacterial growth, for a period of 3 d for bacterial isolation[13]. All
bacterial genera isolates were identified on the basis of colony characteristics, Gram staining, and biochemical reactions, including: urease test; citrate test; indole test, motility test, nitrate test, catalase activity tests, the fermentation of carbohydrates; and triple sugar iron. Genera were classified according to Martins et al.[7] and Cruickshank et al.[14]. All media used for bacterial isolation, identification and count are produced by Oxoid company.

2.7. Antibiotic sensitivity test

Vaginal bacterial flora is seldom recovered as single isolates and often they are isolated as mixed bacteria. Therefore, to determine the most appropriate antibiotic that has the ability to eliminate the vaginal flora, the identified strains isolated (before sponge insertion) as single or mixed bacteria were tested for sensitivity in vitro with antimicrobial agents according to Derbala[15]. Panel of fourteen antimicrobial different disks of the most frequently used antimicrobials and by diffusion method with Mueller-Hinton agar (Merck, Sao Paulo, SP, Brazil) in accordance with the protocols of Clinical and Laboratory Standards Institute: AMC: Amoxicillin + Clavulanic acid; AML: Amoxicillin; A/S: Ampicillin + Sultbictam; OT: Oxxtetraycilline; C: Chloramphenicol; CIP: Ciprofloxacin; OFX: Ofloxacin; CEQ: Cefquinome; CX: Cloxacillin; E: Erythromycin; G: Gentamycin. NOR: Norfloxacin; AK: Amikacin; and NE: Neomycin.

2.8. Fungal isolation

All vaginal swabs were inoculated on plates of Sabouraud dextrose agar containing antibiotic and incubated at 25 °C for 7 d. The first examinations of the plate were done after 2 d to determine the degree of fungal growth. Representative growth was isolated on Sabouraud dextrose agar slopes for further identification according to Samson[16].

2.9. Statistical analyses

SPSS 10.0.1 software was used for all data statistical analyses. The effects of the treatments on the onset of estrus, duration of estrus and total bacterial count were statistically analyzed using analysis of variance, with the GLM General factorial procedure of SPSS and post hoc mean comparisons were performed using Duncan test. Estrus response, pregnancy, lambing and fecundity rates were compared between treatment groups using the chi-square test. Statistical significance was defined as P<0.05.

3. Results

The current work was carried out on sixty ewes, three of them (one from G1 and two from G2) were excluded as vaginal sponge fall and not present inside vagina at the time of sponge withdraw, where in the treatment G3 no sponges losses occurred. Comparing the frequency of isolation of each microorganism in the present study, the bacterial culture of the vaginal swabs before sponge insertion showed the most prevalent isolates of bacterial flora was Staphylococcus spp. (43/57; 70.43%) which recorded as a summation for both single and mixed isolates, whereas the incidence of E. coli and Streptococcus isolated as mixed with other bacteria were 24/57 (42.11%) and 19/57 (33.33%), respectively, and only 4 out 57 (7.00%) of vaginal swabs before sponge insertion shown fungus isolation (Table 1).

| Bacterial isolates                  | no. | %     |
|-------------------------------------|-----|-------|
| Single isolates                     |     |       |
| Staphylococcus spp.                 | 10/57 | 17.54 |
| Streptococcus spp.                  | 0/57  | 0.00  |
| E. coli                             | 0/57  | 0.00  |
| Pseudomonas spp.                    | 0/57  | 0.00  |
| Klebsiella spp.                     | 0/57  | 0.00  |
| Mixed isolates                      |     |       |
| Staphylococcus spp. + Streptococcus spp. | 9/57 | 15.79 |
| Staphylococcus spp. + E. coli       | 14/57 | 24.56 |
| Staphylococcus spp. + Streptococcus spp. + E. coli | 10/57 | 17.54 |
| No bacterial growth                 | 14/57 | 24.56 |
| Fungal isolates                     |     |       |
| Aspergillus fumigatus               | 0/57  | 0.00  |
| Penicilium spp.                     | 2/57  | 3.51  |
| Alternaria spp.                     | 0/57  | 0.00  |
| Cladosporium spp.                   | 2/57  | 3.51  |

The most vaginal isolated bacteria showed sensitivity to Ciprofloxacin (13/43; 30.23%), Cefquinome (7/43; 16.28%), Ofloxacin (6/43; 13.95%), Amoxicillin/Clavulanic acid (5/43; 11.63%) and Gentamycin (4/43; 9.30%), whereas less than 7.00% of bacterial isolates were sensitive to Amikacin, Norfloxacin, Oxytetracycillin and Ampicillin/Sulbictam. Indeed, only one drug (Ciprofloxacin) was highly susceptible and effective against the majority of isolates (Table 2). However, the vaginal bacteria isolates were resistant to Amoxicillin, Chloramphenicol, Cloxacillin, Erythromycin, and Neomycin (Table 2).

3.2. Fungal isolation

All vaginal swabs were inoculated on plates of Sabouraud dextrose agar containing antibiotic and incubated at 25 °C for 7 d. The first examinations of the plate were done after 2 d to determine the degree of fungal growth. Representative growth was isolated on Sabouraud dextrose agar slopes for further identification according to Samson[16].
Efficacy of antibiotics on single and mixed bacterial isolates from vagina of ewes before application of vaginal sponge for estrus synchronization.

Table 2

| Antimicrobials (concentration) (µg) | Single isolates (%) | Mixed isolates (%) | Overall (%) |
|-----------------------------------|---------------------|--------------------|-------------|
|                                   | Staphylococcus spp. | Streptococcus spp. + E. coli | Staphylococcus spp. + E. coli | Staphylococcus spp. + E. coli |
| AMC (20/10)                       | 1/10 (10.00)        | 0/14 (0.00)        | 3/10 (30.00) | 5/43 (11.63) |
| AML (25)                          | 0/10 (0.00)         | 0/14 (0.00)        | 0/10 (0.00)  | 0/43 (0.00)  |
| A/S (10/10)                       | 1/10 (10.00)        | 0/14 (0.00)        | 0/10 (0.00)  | 1/43 (2.33)  |
| OT (30)                           | 0/10 (0.00)         | 2/14 (14.29)       | 13/43 (30.23)| 6/43 (13.95) |
| C (30)                            | 0/10 (0.00)         | 0/14 (0.00)        | 0/10 (0.00)  | 2/43 (4.65)  |
| CIP (5)                           | 2/10 (20.00)        | 5/14 (35.71)       | 10/43 (23.26)| 7/43 (16.28) |
| OFX (5)                           | 2/10 (20.00)        | 4/14 (28.57)       | 13/43 (30.23)| 6/43 (13.95) |
| CEQ (30)                          | 1/10 (10.00)        | 3/14 (21.43)       | 3/43 (7.03)  | 7/43 (16.28) |
| CX (5)                            | 0/10 (0.00)         | 0/14 (0.00)        | 0/10 (0.00)  | 0/43 (0.00)  |
| E (15)                            | 0/10 (0.00)         | 0/14 (0.00)        | 0/10 (0.00)  | 0/43 (0.00)  |
| G (10)                            | 2/10 (20.00)        | 2/14 (14.29)       | 4/43 (9.30)  | 2/43 (4.65)  |
| NOR (5)                           | 0/10 (0.00)         | 0/14 (0.00)        | 0/10 (0.00)  | 0/43 (0.00)  |
| AK (30)                           | 1/10 (10.00)        | 2/14 (14.29)       | 3/43 (6.98)  | 2/43 (4.65)  |
| NE (30)                           | 0/10 (0.00)         | 0/14 (0.00)        | 0/10 (0.00)  | 0/43 (0.00)  |

Table 3

Incidence of bacterial and fungal isolates from vagina of ewes at different days of sponge’s treatment.

| Sponge antibiotics contents | G1: without antimicrobial treatment (%) | G2: Ciprofloxacin (%) | G3: Ciprofloxacin+ Clotrimazole (%) |
|-----------------------------|---------------------------------------|-----------------------|-------------------------------------|
| Gram positive cocci         |                                       |                       |                                     |
| Staphylococcus spp.         | 14/19 (73.68)                         | 5/18 (27.78)          | 7/20 (35.00)                        |
| Streptococcus spp.          | 10/19 (52.63)                         | 3/18 (16.67)          | 3/20 (15.00)                        |
| Gram negative bacilli       |                                       |                       |                                     |
| E. coli                     | 10/19 (52.63)                         | 1/18 (5.56)           | 1/20 (5.00)                         |
| klebsiella spp.             | 0/18 (0.00)                           | 0/18 (0.00)           | 0/20 (0.00)                         |
| Pseudomonas aeruginosa      | 1/19 (5.26)                           | 1/18 (5.56)           | 1/20 (5.00)                         |
| Fungus                      |                                       |                       |                                     |
| Aspergillus fumigatus       | 5/19 (26.32)                          | 0/18 (0.00)           | 0/20 (0.00)                         |
| Penicillium spp.            | 3/19 (15.79)                          | 1/18 (5.56)           | 1/20 (5.00)                         |
| Alternaria spp.             | 0/19 (0.00)                           | 0/18 (0.00)           | 0/20 (0.00)                         |
| Cladosporium spp.           | 11/19 (57.89)                         | 5/18 (27.78)          | 7/20 (35.00)                        |

D0: Day of sponge insertion; D14: Day of sponge withdraw; DE: 48 h after sponge withdraw.

Incidence of bacterial count (x104) isolates from vagina of ewes at different days of sponge treatment (mean±SE).

Table 4

Incidence of bacterial count (x104) isolates from vagina of ewes at different days of sponge treatment (mean±SE).

Treatment groups | Before sponge insertion | Day of sponge withdraw | 48 h after sponge withdraw |
|-----------------|-------------------------|------------------------|---------------------------|
| G1 (Control)    | 18.14±3.20a            | 68.57±16.08b           | 17.41±3.63c               |
| G2 (Ciprofloxacin) | 18.06±2.44b         | 33.51±8.98c            | 13.63±2.34c               |
| G3 (Ciprofloxacin/ Clotrimazole) | 19.94±1.17b    | 39.40±2.70b            | 15.76±1.26c               |

Means in the same raw followed by different superscripts (a, b, c) are significantly different (P<0.05).

The overall mean percentage of ewes observed in estrus was 80.7% and the frequencies of ewes came into estrus were similar among the ewe groups (Table 5). The interval from sponge withdrawal to onset
of estrus, duration of estrus, lambing and fecundity rates were not different among treated groups (Table 5). However, the pregnancy rate for ewes received sponge injected with ciprofloxacin (G2) was higher (100.0%) than control; G1 (66.7%) and that received sponges containing combination of antimiycotic plus ciprofloxacin; G3 (82.4%). In the same manner, as shown in (Table 5), the lambing rate was higher in ciprofloxacin treated ewes group; G2 (92.9%) than control without antimicrobial; G1 (80.0%) and G3 (78.6%).

| Parameters                  | Sponge antimicrobial contents | Overall      |
|-----------------------------|-------------------------------|--------------|
|                             | G1: Without antimicrobial     | G2: Ciprofloxacin | G3: Ciprofloxin/Clotrimazole |
| Estrus response (%)         | 15/19 (78.9)                 | 14/18 (77.8)  | 17/20 (85.0)  | 46/57 (80.7)   |
| Time to onset (mean±SE)      | 39.7±1.56                    | 39.8±1.61     | 40.6±1.46     | 40.0±0.89      |
| Duration of estrus/h (mean±SE) | 30.8±0.90                | 32.5±1.97     | 28.1±2.79     | 30.5±1.09      |
| Pregnancy rate (%)          | 10/15 (66.7)                 | 11/14 (82.4)  | 38/46 (82.6)  |
| Lambing rate (%)            | 8/10 (80.0)                  | 11/14 (78.6)  | 32/38 (84.2)  |
| Fecundity rate (%)          | 9/10 (90.0)                  | 13/14 (92.9)  | 36/38 (94.7)  |

**Table 5** Efficacy of vaginal sponge antimicrobial treatments used for estrus synchronization on reproductive performance of treated ewes.

**4. Discussion**

The most prevalent bacterial isolates before sponge insertion were *Staphylococcus* spp. (70.43%) followed by *E.coli* (42.11%) and *Streptococcus* spp. (3.33%). The fungus was isolated from 7% of vaginal swabs before sponge insertion. In the same pattern, Oliveira et al.[10] found the predominant bacteria isolated before sponge insertion was Gram positive which constitute 63.6% (21/33 isolates), while the Gram negative bacteria showed 33.4% (12/33 isolates). Moreover, Majeed et al.[17] found the most prevalent bacterium belonged to the genus *Staphylococcus* spp. before sponge insertion was 57.9% (22/38 isolates), while the Gram negative bacteria showed 42.1% (16/38 isolates). However, Manes et al.[1] reported more than 90% (19/21 isolates) of the bacterial flora present at device insertion was mainly Gram positive, contrary the Gram negative bacteria was 0%.

With regards to antimicrobial sensitivity, results herein and other studies[10] revealed that, *Staphylococcus* spp. were members of the normal vaginal microbes of small ruminants, so it is necessary to prevent the growth of opportunistic microorganisms such as *E. coli*. The concomitant use of antimicrobial is often suggested to reduce the undesirable effects caused by the sponges[5,7]. The identified strains of vaginal bacterial flora obtained in this study either as single isolate or mixed (more than one bacterial colony type) were tested for sensitivity *in vitro* with fourteen different antibiotics. Ciprofloxacin was effective against the majority of bacterial flora isolates before sponge insertion followed by Cefoxinome and Ofloxacin, while gentamycin is the third rank. This finding is consistent with previous reports by Carson et al.[18], Gabriel Martins et al.[19] and Bruno Penna et al.[20]. On the other side, Suarez et al.[5] recorded Gentamycin and cefazolin were the most effective compounds to prevent bacterial growth following the use of progestin impregnated intravaginal sponges in ewes. However, the concomitant use of antibiotics is often suggested to reduce the undesirable effects caused by the sponges, but this practice may induce antibiotic resistance[5,7]. It is well known that antimicrobial drugs are frequently overused for other indications, including diarrhea and respiratory diseases, which could have contributed to selection of resistant strains in the microbiota. Subsequently, there is a paucity of studies regarding antimicrobial susceptibility of bacteria isolated from the vagina of ewes may vary not only due to the primary incriminating factor, but also with the region where the study was conducted. Similar observations have been observed by Majeed et al.[17] and Oliveira et al.[10]. However, the flora present after the local use of antibiotics is completely different to that observed in the vagina of ewes with spontaneous estrus[1]. These changes in the vaginal flora patterns which associated with estrus synchronization may be affected by many factors. Suarez et al.[5] reported that the hormonal changes status, such as estrus cycle could effect on vaginal bacterial population, especially when progesterone levels were high, as progesterone suppresses specific components of the immune system and natural killer cell activity[21]. Ultimately, Lewis[22] and Pineda[23] suggested that progestogens analogues have immune suppressive effects and may alter the vaginal bacterial flora. All vaginal sponges used herein containing MAP as progestagens for estrus synchronization so we can assume that MAP sponge content induced the same effects to all ewe groups, so the variation in the result findings are attributed to antimicrobial sponge content and sponge itself rather than MAP.

Fungus isolations generally showed dramatically decreases in G1, 2 and 3, two days after sponge removal. This may be by the exerting eect of estrogen, which increased at these phases (proestrus and estrus) inducing surprising increases in local immune response for all treated groups, and this effects promoted by antimiycotic containing vaginal sponge in treating ewes of G3. It is noticeable the application of ciprofloxacin alone increases the incidence of fungus isolation, as totally 61.11% at estrus phase compared to control group (42.11%) and ciprofloxacin/ clotrimazole (5%). Likewise, Glover and Larsen[24] concluded that the extensive antibiotic use posed little risk for the development of fungus infection. In the same line, Jinping et al.[25] hypothesized that baseline fungus culture obtained from vagina requiring antibiotic therapy may develop symptomatic vulvovaginal mycotic infection after antibiotic therapy. Similarly, Bluestein et al.[26] reported 35% fungus colonization at baseline increased to 50% after 10 d of antibiotic therapy. To our knowledge, this is the first prospective cohort study applied antimicrobial [(ciprofloxacin and antimiotic) contents vaginal sponge for estrus synchronization in ewes in Egypt and being monitored for fungus isolation and compared results to a control group of ewes that not exposed to antibiotics. The total bacterial count was significantly decreased (P<0.05) at the time of estrus (mating) compared to the time before sponge insertion in ciprofloxacin ewes (G2) and antimycotic/ ciprofloxacin ewes (G3), whereas, in the control group (G1; without antimicrobial) this counts were relatively similar. In another study related to ewes, it was observed that bacterial populations returned to numbers similar to those observed before sponge insertion 2 d after
sponge withdrawal[27,28]. Furthermore, Manes et al.[1] recorded that, the pH of the vaginal flow on day of sponge insertion and before insemination showed no significant differences between treatments. The total bacteria count obtained from samples collected on D0 was similar between all treatments and increased significantly in all groups at the end of the period (D14; day of sponge withdraw). However, two d after sponge removal the vaginal bacterial population returned to levels similar to those observed prior to their insertion in the control group. These results are in agreement with those observed by Martins et al.[7], Amin[27] and Martins et al.[29]. This reduction in the CFU may be due to the removal of the irritating agent (sponge, or vaginal device) and promoted by physiologically surprising through the increase in local immune response caused by the estrogenic effects during proestrus and estrus phases. Actually, groups treated with antimicrobial agents used in this study showed a significantly decrease (P<0.05) in CFU counts 48 h after sponge withdraw than those before sponge insertion, and this may be due to the effects of the antimicrobial agents (ciprofloxacin with/without antimycotic) to additionally other previously mentioned factors. The frequencies of ewes came into estrus were relatively similar among ewe groups. Moreover, the interval from sponge withdrawal to onset estrus and the duration of estrus were not different among groups. Similarly, Nihat Ozyurtlu et al.[30] recorded that the estrus response was 90% vs 80%, whereas, the interval to estrus was 44 h vs. 45 h for Awassi ewes treated with intravaginal sponges injected with and without antibiotic, respectively. In this study, the pregnancy rate for ewes received sponge impregnated with ciprofloxacin (G2) was significant higher than control (G1) and that received sponges containing combination of antimycotic and ciprofloxacin (G3). Indeed, there is a trend of improving the pregnancy rate by 33.33% (G2) and 17.65% (G3) higher than control (G1). In the same manner, the fecundity rate in G2 improved by 9.08% than G1 and by 7.14% than G3. Furthermore, the lambing rate was higher in G2 than G1 (control) and G3. These achieving results indicate that, antimicrobial containing vaginal sponge affects the reproductive performance in sheep. These results are in agreement with Nihat Ozyurtlu et al.[30].

The declining in the pregnancy, lambing and fecundity rates in ewes treated by vaginal sponge without antimicrobial (control; G1) may be due to the sponge act as a foreign body that induces changes in the normal vaginal environment which favors bacterial growth causing vaginitis and could have a direct effect on sperm fertilization ability[1]. However, Vinoles et al.[31] claimed that the vaginitis had no effect on sperm survival and conception rate. Although, the number of vaginal bacteria returns to basal values at time of mating in this study and in others[32], changes in the normal vaginal flora composition such as the presence of opportunistic Enterobacteriaceae family were incriminated[1,7]. In the same line, Manes et al.[11] and Martins et al.[7] reported that E. coli was the most prevalent bacteria in ewes after device removal that reduced sperm motility through sperm adhesion and agglutination and caused important morphological changes that altered its function in spermatozoa[33]. The infiltration of biologically active substances (as reactive oxygen species) released by leukocytes in the vaginal epithelium as the course of the inflammatory response may act against the spermatozoa dysfunction contributing to fertility decrease, affecting the reproductive response and the fertility from the estrus synchronization treatments[5]. High levels of reactive oxygen species have been reported as responsible to structural, metabolic and functional alterations of spermatozoa[34]. Additionally, the action of lipopolysaccharides which produced as components of Gram negative bacteria[35] have been linked to infertility and pregnancy losses and could also have a negative effect on the viability and motility of spermatozoa[36]. In this sense of possible explanation, the antibiotic treatment only prevented the increase in the bacterial counts, but not the changes in the flora composition observed after the use of vaginal sponge. All these factors may explain the lower conception and fecundity rates obtained in control ewes.

In conclusion, the employ of intravaginal sponges containing progesterone analogue during estrus synchronization protocols in ewes caused increases in the presence of total bacterial count, changing the vaginal microflora and opportunistic pathogens, as well as vaginal fungus were stimulated inflammation of the vagina. Staphylococcus spp. was the most frequently recovered from the vaginal samples cultured, and all isolates had a high sensitivity to ciprofloxacin antimicrobials that measured control of vaginitis. The concomitant use of antibiotics is often suggested to reduce the undesirable effects caused by sponges, so if the choice is made to incorporate antibiotics, an antibiotic sensitivity test should be performed to select the appropriate compound(s). Vaginal sponges when injected with antimicrobial agents (ciprofloxacin with/without antimycotic) can improve reproductive performance (pregnancy and fecundity rates).

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Manes J, Fiorentino MA, Kaiser G, Hobar F, Alberio R, Sanchez E, et al. Changes in the aerobic vaginal flora after treatment with different intravaginal devices in ewes. Small Rum Res 2010; 94: 201-204.
[2] Muna NF. Oestrus synchronization in indigenous ewes using cloprostenol. Master thesis. Bangladesh Agricultural University; 2012.
[3] Abecia JA, Forcada F, Gonzalez-bulnes A. Hormonal control of reproduction in small ruminants. Anim Reprod Sci 2012; 130: 173-179.
[4] Menchaca A, Rubianes E. New treatments associated with timed artificial insemination in small ruminants. Reprod Fert Develop 2004; 16: 403-413.
[5] Suarez G, Zunino P, Carol H, Ungerfeld R. Changes in the aerobic
vaginal bacterial mucous load and assessment of the susceptibility to antibiotics after treatment with intravaginal sponges in anoestrous ewes. Small Rumin Res 2006; 63: 39-43.

[6] Sargison ND, Howie F, Mearns R, Penny CD, Foster G. Shiga toxin-producing Escherichia coli as a perinatal cause of abortion in a closed flock of Suffolk ewes. Vet Rec 2007; 160: 875-876.

[7] Martins G, Figueira L, Penna B, Brando F, Vargès R, Vasconcelos C, et al. Prevalence and antimicrobial susceptibility if vaginal bacteria from ewes treated with progesterone-impregnated intravaginal sponges. Small Rumin Res 2009; 81: 182-184.

[8] Scudamore CL. Intravaginal sponge insertion technique. Vet Rec 1988; 123: 554.

[9] Manes J, Fiorentino MA, Hozbor F, Paolocchi F, Alberio R, Ungerfeld R. Changes in the aerobic vaginal bacterial load and antimicrobial susceptibility after different oestrus synchronization treatments in goats. Anim Prod Sci 2013; 53: 555-559.

[10] Oliveira JK, Martins G, Esteves LV, Penna B, Hamond C, Fonseca JF, et al. Changes in the vaginal flora of goats following a short term protocol of estrus induction and synchronization with intravaginal sponges as well as their antimicrobial sensitivity. Small Rumin Res 2013; 113: 162-166.

[11] Guerra MMP, Mota RA, Mergulhao FCC, Lima RF, Souza AF, Melo EH, et al. Study of the microbial flora and evaluation of the effectiveness of Gentocin (R) 40 mg in the prevention of vaginal infection in dairy goats submitted to estrus synchronization. Hora Vet 2002; 22: 13-17.

[12] Koneman EW, Allen SD, Dowell VR, Sommers HM. Colour atlas and text book of diagnostic microbiology. 2nd ed. London: Lippincott Co.; 1983.

[13] Quinn PJ, Carter ME, Markay BK, Carter GR. Clinical veterinary microbiology. London: Mosby; 2011, p. 22-91.

[14] Cruickshank R, Daguid JP, Marmion BP, Swain RHA. The practice of medical microbiology. 12nd ed. London: Longman Group Ltd; 1975.

[15] Derbala MK. Diagnosis and treatment of endometritis in mare. Ph.D. Thesis. Beni-Suef Univ; 2013.

[16] Samson RA. A compilation of the aspergilli des cribed since 1965. Stud Mycol 1979; 18: 1-38.

[17] Majeed AF, Al-Rawi HM, Al-Kubaisi SMA, Al-Jumaily TMN. Vaginal bacteria flora concurred with vaginal sponges in black Iraqi goats. Iraqi J Veter Sci 2012; 26 (Supplement 4): 123-124.

[18] Carson CA, Reid-Smith R, Irwin RJ, Martin WS, McEwen SA. Antimicrobial resistance in generic fecal Escherichia coli from 29 beef farms in Ontario. Can J Vet Res 2008; 72: 119-128.

[19] Martins G, Figueira L, Penna B, Brand F, Vargés R, Vasconcelos C, et al. Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progesterone-impregnated intravaginal sponges. Small Ruminant Res 2009; 10: 1016.

[20] Penn B, Libonati H, Director A, Sarzedas AC, Martins G, Felipe Z, et al. Progestin-impregnated intravaginal sponges for estrus induction and synchronization influences on goats vaginal flora and antimicrobial susceptibility. Anim Reprod Sci 2013; 142: 71-74.

[21] Scheibl P, Zerbe H. Effect of progesterone on the immune system in consideration of bovine placental retention. Deut Tierarz Chir 2000; 107: 221-227.

[22] Lewis GS. Steroidal regulation of uterine resistance to bacterial infection in livestock. Reprod Biol Endocrinol 2003; 1: 117.

[23] Pineda MH. Female reproductive system. In: Pineda MH, editor. Veterinary endocrinology and reproduction. Iowa: Iowa State Press; 2003. p. 293-341.

[24] Glover DD, Larsen B. Longitudinal investigation of candida vaginitis in pregnancy: Role of superimposed antibiotic use. Obstet Gynecol 1998; 91:115-118.

[25] Xu J, Schwartz K, Bartoces M, Monsur J, Severson RK, Jack D, et al. Effect of antibiotics on vulvovaginal candidiasis: A metro net study. J Am Board Fam Med 2008; 21: 261-268.

[26] Bluestein D, Rutledge C, Lumsden L. Predicting the occurrence of antibiotic-induced candidal vaginitis (AICV), J Fam Pract 1991; 11: 319-326.

[27] Amin JD. Effect of fluorogesterone acetate impregnated intravaginal sponges on vaginal bacterial flora of ewes. Nigerian J Anim Prod 1996; 23: 98-100.

[28] Hayat HM, EL-Nour, Yassin MH, Amal AM, Ghoniem, Esmail ME. Bacteriological and cytological vaginal findings and biochemical changes associated with different methods of synchronization in ewes. J Egypt Vet Med Assoc 2004; 64: 177-192.

[29] Martins LT, Neto PCS, Neto SG, Rauber LP, Bertolini M, Vieira AD, et al. Microbiological and functional evaluation of an alternative device for estrous synchronization in ewes. Ciencia–Rural, Santa Maria 2010; 40: 389-395.

[30] Ozyurtlu N, Yepyilmaz S, Kucukaslan Y. The effectiveness of using antibiotic with intravaginal sponge and duration of sponge treatments on the vaginal flora and fertility in anoestrous ewes. J Anim Vet Adv 2008; 7: 723-727.

[31] Vinoles C, Paganoni AC, Milton BA, Drioncourt AM, Martin GB. Pregnancy rate and prolificacy after artificial insemination in ewes following synchronisation with prostaglandin, sponges, or sponges with bactericide. Anim Prod Sci 2011; 51: 565-569.

[32] Gatti M, Zunino P, Ungerfeld R. Changes in the aerobic vaginal bacterial mucous load after treatment with intravaginal sponges in anoestrous ewes: Effect of medroxiprogesterone acetate and antibiotic treatment use. Reprod Dom Anim 2011; 46: 205-208.

[33] Schulz M, Sánchez R, Soto L, Risopatrón J, Villegas J. Effect of Escherichia coli and its soluble factors on mitochondrial membrane potential, phosphatidylserine translocation, viability, and motility of human spermatozoa. Fert Steril 2010; 94: 619-623.

[34] Fraczek M, Kupisz M. Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. J Androl 2007; 28: 325-333.

[35] Yaniz JL, Marco Aguado MA, Mateos JA, Santolaria P. Bacterial contamination of ram semen, antibiotic sensitivities, and effect on sperm quality during storage at 15 °C. Anim Reprod Sci 2010; 122: 142-149.

[36] Gorga F, Galdiero M, Buommino E, Galdiero E. Porins and lipopolysaccharide induce apoptosis in human spermatozoa. Clín Diagn Lab Immunol 2001; 8: 206-208.