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The efficacy of intravaginal antibiotic or their combination with different gonadotropins on vaginitis and fertility in ewes synchronized with progesterone impregnated intravaginal sponges

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Abstract: This study was conducted with two experiments to investigate the efficacy of intravaginal antibiotic or their combination with different gonadotropin use on vaginitis and the fertility in Berrichon ewes. All ewes (n = 106) were synchronized with intravaginal sponges containing 60 mg medroxyprogesterone acetate for 14 days. For the first experiment, ewes (n = 50) received 50 mg enrofloxacin (TRT) to sponge prior to insertion or received no treatment to sponge (CON) during nonbreeding season. For the second experiment, ewes (n = 56) were randomly assigned to one of the four groups (TRT-E; TRT-G; CON-E; CON-G) according to the presence of antibioterapy (TRT or CON) to sponge and variety of gonadotropins at sponge withdrawal (equine chorionic gonadotropin-eCG; E, 500 IU) or 24 h later after sponge withdrawal (gonadotropin-releasing hormone-GnRH; G, 10 µg) during breeding season. Vaginitis and odour were scored according to amount/aspect and odour of discharge. The addition of enrofloxacin did not change the vaginitis score (0.65 ± 0.52 vs. 0.79 ± 0.67, p = 0.25) and vaginal discharge odour score (2.07 ± 0.71 vs. 2.25 ± 0.84, p = 0.26) in both experiments. Oestrus response and pregnancy rate were 88%; 56% for TRT and 80%; 40% for CON in the first experiment (p > 0.05). These parameters were 92.3%; 69.2% for TRT-E, 86.7%; 53.3% for CON-E, 71.4%; 7.1% for TRT-G, and 64.3%; 21.4% for CON-G group. The pregnancy rate was higher (p = 0.002) in TRT-E group than TRT-G and CON-G. Additionally, oestrus response (p = 0.05) and pregnancy rate (p = 0.0001) were higher in eCG group (89.2%; 60.7%) than GnRH group (67.8%; 14.3%) in the second experiment. In conclusion, local antibiotherapy with enrofloxacin did not alter vaginitis score and fertility. However, GnRH reduced the oestrus response and pregnancy rate compared to eCG.

Key words: Enrofloxacin, ewes, fertility, GnRH, vaginitis

1. Introduction

Improving pregnancy rate is critically important for the frequency of lambing and lifetime productivity of ewes [1,2]. Oestrus synchronization is widely used to improve the pregnancy rate and also regulate lambing date [3]. The conventional approach to oestrus synchronization, by mimicking the luteal phase duration of the estrous cycle, is the use of intravaginal progesterone devices for 12–14 days followed by gonadotropins such as equine chorionic gonadotropin (eCG) [4].

Intravaginal devices containing progestagen impair the vaginal environment, quality of ram spermatozoa, sexual attractiveness of ewes and these effects reduce the pregnancy rate [5,6]. Additionally, the long duration of intravaginal sponge increased this detrimental effect as vaginitis compared to intravaginal silicon devices [7]. However, an intravaginal silicon-based device, which allows the drainage of vaginal secretion, is not commercially available in some countries. The lack of a definitive prevention protocol has led to the recommendation of using local intravaginal antibiotics to reduce the severity of vaginitis [8,9]. The local use of some antibiotics such as tetracycline and penicillin is determined to be ineffective due to the resistance of microorganisms [10,11]. The predominant gram-negative bacteria (mainly *Escherichia coli*) most frequently isolated at sponge removal in synchronization protocol [12–14]. Previous studies revealed the fluoroquinolones are sensitive to vaginal microorganisms [10,13] and recent report revealed that the use of enrofloxacin was the most effective antibiotic to decrease bacterial vaginal growth [12]. The first hypothesis was that the addition of enrofloxacin to sponge might reduce severity of vaginitis, increase oestrus response and pregnancy rate in ewes during breeding and nonbreeding season.

In addition to undesirable effects as vaginitis following the use of intravaginal sponge [12], the repeated oestrus

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synchronization with eCG leads to the formation of eCG neutralizing antibodies in ewes [15,16]. Additionally, the production of eCG from the blood of pregnant mares is considered to be a serious concern by animal welfare organizations [17]. The use of gonadotropins in conjunction with an intravaginal sponge is required for synchronizing estrus and ovulation in ewes [18]. There have been inconsistency fertility outcomes after the use of gonadotropin releasing hormone (GnRH) as alternative gonadotropin instead of eCG [19–22]. The second hypothesis was that use of GnRH could result in a similar oestrus response and pregnancy rate compared to eCG after reduced vaginitis with enrofloxacin in ewes during the breeding season. The objectives of the present study were to determine the efficacy of enrofloxacin supplementation to sponge prior to insertion on vaginitis and pregnancy rate during the breeding season and nonbreeding season and the efficacy of GnRH in combination with sponge containing enrofloxacin on oestrus response and pregnancy rate during the breeding season.

2. Materials and methods

2.1. Animals and management

The experimental procedures were approved by the University of Balıkesir Animal Care Committee (Reference Number: 2021-3/4). Berrichon ewes (n = 106) with an average of 3–5 years age were used on a sheep farm located in Balıkesir (39° 33' N, 26° 58' E) in Turkey. Ewes were fed a totally mixed ratio according to NRC recommendation for ewes in addition to grazing on natural pasture during the experiment. Ewes had a free access to water and shade.

2.2. Experimental design

The first experiment was carried out involved multiparous 50 Berrichon ewes during the nonbreeding season (April–May) and the second experiment was carried out involved multiparous 56 Berrichon ewes during the breeding season (July–August). Body condition score (BCS) was evaluated by the palpation of the spinous and transverse processes of lumbar vertebrae at the beginning of the study in both experiments. It is based on a scale of 1–5, score 1 was considered very skinny and score 5 was very fat [23]. All ewes (n = 106) were synchronized with intravaginal sponges containing 60 mg medroxyprogesterone acetate (MAP; Esponjavet, HIPRA, Turkey) for 14 days.

2.2.1. The first experiment

Ewes (n = 50) were randomly divided into two equal groups (n = 25 for each group) involved treatment group (TRT) and control group (CON) according to BCS of ewes. Ewes in the CON did not receive any treatment prior to sponge insertion. Ewes in the TRT received 50 mg injectable enrofloxacin solution (Baytril-K, 5%, Bayer, USA) to sponge with six different points to prevent overflow of solutions before sponge insertion. Antibiotic solution was injected to sponges after they are placed into the applicator. Sponge applicators were disinfected with 1% benzalkonium chloride (Zefirol, Dermosept, Turkey) before each application. All ewes received one intramuscular injection of 500 IU equine chorionic gonadotropin (eCG; Gonaser, HIPRA, Turkey) at the time of sponge withdrawal.

2.2.1. The second experiment

Ewes (n = 56) were randomly assigned to one of the four groups in a 2 × 2 factorial design according to the presence of antibiotherapy (CON: no treatment or TRT: enrofloxacin) before sponge insertion and variety of gonadotropins (eCG; E or GnRH; G) at sponge withdrawal. Ewes in the TRT (n = 27) received 50 mg injectable enrofloxacin to sponge before insertion. Additionally, ewes were subjected to the 500 IU eCG at the time of sponge withdrawal (TRT-E; n = 13) or 10 µg GnRH 24 h after sponge withdrawal (TRT-G; n = 14). Ewes in the CON (n = 29) did not receive any treatment prior to sponge insertion and ewes were subjected to the 500 IU eCG at the time of sponge withdrawal (CON-E; n = 15) or 10µg GnRH (Buserelin, Oviren®, Topkim, Turkey) 24 h after sponge withdrawal (CON-G; n = 14).

2.2.3. Scoring of vaginal discharge

The characteristics of vaginal discharge were evaluated as vaginitis score at the time of sponge removal according to the amount and aspect of discharge (score 0; negligible, score 1; clear and moderate amount of discharge, score 2; abundant purulent or hemorrhagic discharge) [7]. The odour of vaginal discharge (score 0; none, score 1; moderate, and score 2; abundant) was determined at sponge removal according to Viñoles et al. [11].

2.2.4. Oestrus detection, mating and ultrasound examination

Fertile rams (n = 10) with mating crayon marks were used for natural mating following the sponge withdrawal. For both experiments, oestrus detection was performed by observing mated ewes twice a day for 5 days after sponge withdrawal. Pregnancy diagnosis was performed via transrectal ultrasound (Hasvet 838, Hasvet, Turkey) at 30 days following the removal of intravaginal sponge. The presence of a clear anechoic embryonic vesicle with a viable embryo (heartbeat) was evaluated as a positive pregnancy diagnosis.

2.3. Statistical analysis

The SPSS 23.0 software (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses. Distribution of vaginal discharge score, vaginal discharge odour score, onset of oestrus, and BCS between groups were evaluated using Shapiro–Wilk test and were analyzed using independent samples t-test in the first experiment. These parameters among groups were analyzed using one-way ANOVA in the second experiment. Differences among groups were determined by posthoc Games–Howell test based on homogeneity test. All results are expressed as mean ± SEM. Estrous response and pregnancy rate were analyzed using chi-square test. Statistical significance level was considered at $p \leq 0.05$ and statistical tendencies were defined at $0.05 < p < 0.10$.

3. Results

There was no significant difference between the body condition score of ewes in both experiments at the beginning of the study. However, ewes had higher BCS (3.62 ± 0.09 vs. 3.20 ± 0.05) during the breeding season than ewes during the nonbreeding season (p = 0.001). Season also altered the mean vaginitis score which was statistically higher (p = 0.001) in the nonbreeding season (1.05 ± 0.07) than breeding season (0.36 ± 0.07). Except for the two-loss sponge, irrespective of the variation according to antibiotic and gonadotropins, the distribution of vaginitis score was 35.6% (37/104), 56.7% (59/104), and 7.7% (8/104) for scores 0, 1, and 2, respectively. The percentage of vaginitis score 1 was statistically higher (p = 0.002) than that of score 0 and score 2.

3.1. The first experiment

The percentage of vaginitis score 0 and 1 were 56%; 44% in CON and 72%; 28% in TRT in the first experiment. Abundant purulent or hemorrhagic discharge (score: 2) was not detected after sponge withdrawal in both groups. The mean vaginitis score (p = 0.25) and vaginal discharge odour score (p = 0.17) were not significantly different between TRT (0.28 ± 0.09; 1.92 ± 0.16) and CON (0.44 ± 0.10; 2.24 ± 0.17), respectively. In addition, treatment with enrofloxacin did not significantly change (p = 0.45) oestrus response in TRT (88%) compared to CON (80%). The time to onset of oestrus was not different between groups (p = 0.24). Numerically increased pregnancy rate (56% vs. 40%) was not statistically significant (p = 0.27) in TRT compared to CON (Table 1).

3.1. The second experiment

The percentage of vaginal score 0, 1 and 2 were 8.9%, 73.2% and 14.3%. Unlike the first experiment, abundant purulent or hemorrhagic discharge (score: 2) was detected in eight ewes and two sponge were loss before sponge withdrawal in the second experiment. Seven of these ewes belonged to CON-E and CON-G. The mean vaginitis score (p = 0.84) and vaginal discharge odour score (p = 0.86) were not different among groups. Oestrus response was 92.3% for TRT-E, 86.7% for CON-E, 71.4% for TRT-G, and 64.3% for CON-G . This variation was not significantly different among groups (p = 0.26). Time to onset of oestrus was also not different among groups (p = 0.75). However, pregnancy rate was higher (p = 0.002) in TRT-E group (69.2%) than TRT-G (7.1%) and CON-G (21.4%, Table 2).

The use of enrofloxacin (TRT, TRT-E, CON-E) to sponge did not alter the vaginitis score (0.65 ± 0.52 vs. 0.79 ± 0.67, p = 0.25) and vaginal discharge odour score (2.07 ± 0.71 vs. 2.25 ± 0.84, p = 0.26) compared to control (CON, CON-G, TRT-G). Irrespective of the treatment groups with/without enrofloxacin, the onset of oestrus after sponge withdrawal was not different between eCG (44.11 ± 3.36 h) and GnRH (40.25 ± 5.59 h) groups (p = 0.56). However, eCG group (89.2%; 25/28) had greater (p = 0.05) oestrus response in compared to GnRH group (67.8%; 19/28). Additionally, pregnancy rate was statistically higher (p = 0.0001) in eCG (60.7%; 17/28) than GnRH (14.3%; 4/28).

4. Discussion

Progestagen-impregnated intravaginal sponges provoke vaginal infections caused by opportunistic secondary invaders vaginitis and lead to accumulation of abnormal hemorrhagic and putrid vaginal discharge. In ewes, a significant increase in the vaginal flora number and disruption of the bacterial composition has also been observed [24]. Although the number of vaginal bacteria returns to basal values by the day of estrus, the normal vaginal flora composition is still altered [12]. These

Table 1. Comparison of vaginitis scores and fertility parameters following with (TRT) or without (CON) intravaginal enrofloxacin in Berrichon ewes during the nonbreeding season.

Parameters	TRT (n = 25)	CON (n = 25)	p
BCS	3.62 ± 0.11	3.62 ± 0.15	1.00
Vaginitis score	0.28 ± 0.09	0.44 ± 0.10	0.25
Discharge odour score	1.92 ± 0.16	2.24 ± 0.17	0.17
Oestrus response (%)	88 (22)	80 (20)	0.45
Onset of oestrus (h)	41.6 ± 3.75	35.0 ± 4.04	0.24
Pregnancy rate (%)	56 (14)	40 (10)	0.27

Table 2. Comparison of vaginitis scores and fertility parameters after combination of different gonadotropins and intravaginal enrofloxacin in Berrichon ewes during the breeding season.

Parameters	TRT-E (n = 13)	TRT-G (n = 14)	CON-E (n = 15)	CON-G (n = 14)	p
BCS	3.19 ± 0.09	3.36 ± 0.06	3.13 ± 0.12	3.11 ± 0.09	0.27
Vaginitis score	1.00 ± 0.11	1.00 ± 0.00	1.07 ± 0.16	1.15 ± 0.19	0.84
Discharge odour score	2.15 ± 0.15	2.28 ± 0.16	2.35 ± 0.20	2.15 ± 0.27	0.86
Oestrus response (%)	92.3 (12)	71.4 (10)	86.7 (13)	64.3 (9)	0.26
Onset of oestrus (h)	46.38 ± 4.86	44.07 ± 8.07	42.13 ± 4.74	36.42 ± 7.89	0.75
Pregnancy rate (%)	69.2 (9)^{A}	7.1 (1)^{C}	53.3 (8)^{AB}	21.4 (3)^{BC}	0.002

^{A,B,C}: Values with different superscripts within a row differ at $p \leq 0.05$.

bacterial vaginal changes might affect the subsequent fertility [9,12]. Additionally, prevalent bacterial load in the vaginal environment after device removal compromises the viability of the ram spermatozoa [6]. Vaginitis, caused by progesterone impregnated intravaginal sponge, is a significant factor for decreased fertility due to altered vaginal environment and damaged sperm viability in ewes [11,24]. The vaginal discharge rate was 100% after long-term synchronization in multiparous Berrichon ewes in both experiment. Previous studies have reported vaginal discharge rates ranging from 98.5% to 100% after the use of progesterone impregnated intravaginal sponge [7,25]. Although the percentage of vaginal discharge was similar according to synchronization protocol, duration of synchronization with sponge affects the characteristics of vaginal discharge [7,26]. Martinez-Ros et al. [7] reported that the majority of vaginal discharge belonged to score 2 (83.3%) for long-term synchronization (14 days) and score 1 (89.5%) for short-term synchronization (7 days). The majority of vaginal discharge (56.7%) was score 1 after long-term synchronization in the present study. To reduce the severity of vaginitis leading to the reduction on pregnancy rate, previous studies recommend the local use of effective antibiotics to microorganisms [9,24,26,27]. The first hypothesis was the reduced vaginitis and improved pregnancy rate after adding enrofloxacin which is reported as belonging to the susceptible group (fluoroquinolones) in recent studies [12,13].

The major concern of most previous studies about some antibiotics such as penicillin and tetracycline is resistance to vaginal microorganisms [9,10]. However, Viñoles et al. [11] reported that adding chlortetracycline reduced the amount of vaginal discharge and odour of sponges but pregnancy rate did not increase after adding antibiotic to sponge [11]. Previous studies reported that ciprofloxacin [10,13] and enrofloxacin [12] was the most susceptible and effective antibiotics to control vaginitis in ewes. Mohammed et al. [13] determined that vaginitis reduced after addition of ciprofloxacin to sponge. However, the local use of enrofloxacin with vaginal sponges did not statistically reduce the vaginitis score and vaginal odour score in this study. In the first experiment, ewes had low vaginitis scores in both treatment (0.28) and control group (0.44) after progesterone impregnated intravaginal sponge in this study. Although treatment with enrofloxacin decreased the vaginitis score numerically compared to the control group, the efficacy of the treatment was not statistically different in ewes had low vaginitis scores in this study.

In the second hypothesis, GnRH was used as alternative gonadotropin to obtain similar fertility instead of eCG in the breeding season. Increased sexual attractiveness by controlling vaginitis with intravaginal enrofloxacin can provide accurate results for comparing oestrus response and pregnancy rate among different gonadotropins. The most commonly used gonadotropin is eCG which promotes final follicular growth, shortens the estrous interval, and synchronizes ovulation in ewes [3]. Although a single injection of GnRH results in ovulation in seasonally anestrous ewes, the normal luteal function of most ewes is absent due to inadequate follicle development before the induction of ovulation so the use of GnRH is recommended during the breeding season [28]. GnRH treatment induces the LH surge within 1–4 h postadministration in ewes [19]. The application of GnRH commonly used is to regulate the time of ovulation within 24 to 44 h after sponge withdrawal [18,21,29]. However, there has been a limited study comparing the efficacy of GnRH with eCG on the onset of oestrus, oestrus response, and pregnancy rate after natural mating in ewes [19,20,22,30,31]. The onset of oestrus after sponge withdrawal varied from 36.4 h to 46.4 h and it was not different among groups in the second experiment. In addition, the use of GnRH (40.2 h) did not change the onset of oestrus compared to eCG

(44.1 h) in this study. Previous studies reported that GnRH delayed the onset of oestrus [19] or GnRH did not change the onset of estrus compared to eCG [20,31].

Oestrus response varied from 64.3% to 92.3% and this difference did not result in significance among groups in this study. Although the onset of oestrus was not different between groups (eCG or GnRH), the administration of GnRH at 24 h after sponge withdrawal reduced the oestrus response from 89.2% to 67.8% compared to eCG in the second experiment. Martinez-Ros and Gonzalez-Bulnes [19] applied the single GnRH at 56 h after CIDR removal. The authors reported an equal oestrus response (89.5%) after short-term CIDR-eCG and CIDR-GnRH protocol. Similar to previous report, Cavalcanti et al. [31] reported that GnRH administration at 24 h after short-term protocol did not change (95.2% vs. 100%) the oestrus response compared to the control group [31]. Lone et al. [30] determined that oestrus response did not change with GnRH at 24 h after sponge withdrawal (87.5%) or eCG at sponge withdrawal (66.6%) [30]. Contrary to previous reports [19,30,31], some studies revealed the GnRH administration reduced the oestrus response in ewes [20,22]. Silva et al. [20] applied the single GnRH at 27 h after sponge removal. The authors determined that GnRH administration decreased the oestrus response from 80% to 36.4% in the long-term protocol, and from 90% to 30% in the short-term protocol. Martemucci and D'Alessandro [22] determined that use of GnRH at 30 h after sponge withdrawal decreased the oestrus response (66.6%) compared to use of eCG (92.3%) at sponge withdrawal in ewes synchronized with short-term (5-days) progesterone-based synchronization protocol. The use of eCG increases oestradiol concentration by improving size of antral follicle and simulates the final stages of follicular growth [32,33]. Consistent with the previous reports [20,22,32], administration of only GnRH at 24 h after sponge withdrawal may not allow the dominant follicle to secrete enough oestradiol for oestrus expression due to acceleration of LH surge in this study.

Consistent with the reduced oestrus response, the use of GnRH also drastically reduced the pregnancy rate from 60.7% to 14.3% compared to eCG in this study. Similar to our results, the administration of GnRH at 30 h after sponge withdrawal significantly reduced the pregnancy rate following natural mating (92.3% vs. 33.3%) compared to that of eCG in crossbreed Altamurana ewes [22]. Conversely, Martinez-Ros and Gonzalez-Bulnes [19] reported pregnancy rate was not different (68.4% vs. 57.9%, respectively) after short-term CIDR-eCG and CIDR-56h-GnRH protocol in Segureña ewes. In previous studies, breed effect may have influenced the fertility results after using progesterone-based synchronization protocol with different gonadotropins (GnRH or eCG) in ewes [31]. Additionally, early use of GnRH (at 24 h after sponge removal) may have resulted in accelerated the time of ovulation in this study. Furthermore, lack of prostaglandin $f_{2\alpha}$ ($PGF_{2\alpha}$) administration at the end of long-term protocol may have decreased the oestrus response due to altered follicular development in higher progesterone concentration [22]. Although there are limited studies evaluating the efficacy of GnRH without eCG as an alternative gonadotropin on fertility [19,22], most of the studies have evaluated the effect of GnRH after eCG compared to use of eCG alone on fertility [21,31,34,35]. Cavalcanti et al. [31] stated that conception rate after natural mating was not different (from 65% to 45%) in ewes subjected to GnRH 24 h later after eCG compared to control group received only eCG. The administration of GnRH at 42 or 48 h after sponge withdrawal did not alter (11%; 32%; 20%; and 26%, respectively) the pregnancy rate after insemination compared to control group receiving only eCG [21]. The addition of GnRH at oestrus (approximately 48 h after long-term protocol for 14 days) also did not change the pregnancy rate (83.3% vs. 72.7%, respectively) after natural mating compared to that of ewes only received eCG [34]. Another study revealed that the addition of GnRH at 48 h after vaginal progestagen (for 9 days) plus eCG significantly increased the pregnancy rate (87.5%) compared to ewes that did not receive GnRH (14.3%) in repeat breeders ewes [35]. The use of GnRH at 24 h after sponge withdrawal did not result in a similar pregnancy rate compared to eCG in this study so reduced pregnancy rate may prevent the use of GnRH as an alternative gonadotropin instead of eCG in Berrichon ewes.

In conclusion, adding the enrofloxacin to sponges did not change the vaginitis score, vaginal odour score, and fertility after long-term synchronization protocol. The use of eCG at the end of sponge withdrawal provided more control the oestrus response and the pregnancy rate compared to GnRH in Berrichon ewes during the breeding season.

Conflict of interest
The authors have no conflicts of interest in this study.

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