Tropical Animal Health and Production (2025) 57:307
https://doi.org/10.1007/s11250-025-04550-0

REGULAR ARTICLES

Lactobacillus plantarum and supernatant: vaginal health and reproductive parameters of ewes synchronized with fluorogestone or medroxyprogesterone acetate

Baris Guner[1] · **Tevhide Elif Guner**[2]

Received: 25 February 2025 / Accepted: 9 July 2025 / Published online: 18 July 2025

Abstract

This study investigated the effects of Lactobacillus plantarum (LAC) and its cell-free supernatant (CFS) on vaginal health and reproductive performance in ewes. The ewes were synchronized using fluorogestone acetate (FGA) or medroxyprogesterone acetate (MPA) impregnated intravaginal sponges. A total of 196 Merino ewes were randomly assigned to four groups in a 2 × 2 factorial design. Intravaginal sponges remained for 14 days, and vaginal discharge, sponge weight change, estrus response, and pregnancy rates were evaluated. No adverse health effects were observed following intravaginal probiotic treatment. There was a tendency ($p = 0.07$) for higher sponge loss in the FGA group (13.2%) compared to the MPA group (5.7%). On the day of sponge removal, 90.8% of ewes exhibited vaginal discharge. Although not statistically significant, mean vaginal discharge scores were lower in LAC-treated groups compared to CFS-treated groups. While *L. plantarum* had no significant effect on vaginal discharge scores in the MPA group (40.7% in MPA+LAC vs. 41.7% in MPA+CFS for score 2; $p > 0.05$), it significantly reduced purulent/hemorrhagic discharge in the FGA group (29.7% in FGA+LAC vs. 47.6% in FGA+CFS; $p < 0.05$). Estrus initiation occurred significantly earlier in the FGA group (36.8 ± 1.9 h) compared to the MPA group (49.1 ± 1.2 h; $p < 0.01$), although neither the probiotic treatment nor its interaction with progestagen type significantly influenced estrus timing. Pregnancy rates were significantly higher in the FGA+LAC (71.7%) and FGA+CFS (75.6%) groups compared to the MPA+LAC (49.0%) and MPA+CFS (50.0%) groups ($p < 0.01$). Our findings suggest that FGA-based synchronization improves pregnancy rates, while treatment with *L. plantarum* may enhance vaginal health, providing a potential non-antibiotic approach for reproductive management.

Keywords Estrus synchronization · Intravaginal sponge · *Lactobacillus plantarum* · Pregnancy rate · Vaginal discharge

Introduction

Intravaginal progestagen-impregnated sponges (IVS) are widely used for estrus synchronization in ewes, mimicking the luteal phase for 12–14 days (Gonzalez-Bulnes et al. 2020; Guner et al. 2024). Unlike CIDR devices, IVS obstruct the vaginal canal, altering discharge dynamics and fostering bacterial imbalances that lead to vaginitis (Suárez et al. 2006; Gonzalez-Bulnes et al. 2020). These disruptions promote opportunistic pathogens, negatively impacting sperm quality and fertilization (Manes et al. 2014; Vasconcelos et al. 2016; Martinez-Ros et al. 2018; Gonzalez-Bulnes et al. 2020).

Antibiotics, administered systemically or locally, have been used to control bacterial overgrowth and prevent infections (Gatti et al. 2011; Vasconcelos et al. 2016; Ojeda-Hernández et al. 2019; Guner and Kisadere 2022). However, their widespread use contradicts efforts to minimize antibiotic residues in animal production (Gonzalez-Bulnes et al. 2020; Regecová et al. 2022; Výrostková et al. 2021). Probiotic-based alternatives, particularly those utilizing *Lactobacillus* spp., have emerged as a non-antibiotic approach to maintaining vaginal health. Although *Lactobacillus* spp. are

✉ Baris Guner
baris.guner@balikesir.edu.tr

[1] Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir 10050, Türkiye

[2] Department of Food Hygiene and Technology, Health Sciences Institute, Balıkesir University, Balıkesir, Türkiye

not predominant in the ovine vaginal microbiota (Swartz et al. 2014), their antibacterial properties have been explored to modulate vaginal microbiota (Serrano et al. 2020; van de Wijgert and Verwijs 2020; Barba et al. 2024; Guner et al. 2024; Toquet et al. 2025). Nevertheless, studies on the inhibitory effects of lactic acid bacteria supernatants against pathogenic bacteria remain limited (Danilova et al. 2019; Guner et al. 2022).

Variations in progestagen composition, sponge texture, size, and absorption capacity affect teh efficacy of intravaginal probiotic treatment on vaginal flora,. Structural properties of IVS significantly influence vaginal alterations, as larger sponges with higher absorption capacity may retain more secretions, promoting bacterial colonization and prolonged inflammation (Martinez-Ros et al. 2018; Gonzalez-Bulnes et al. 2020). Ewes typically exhibit estrus (75–100%) within 24–72 h after IVS removal (Olivera-Muzante et al. 2011), with fluorogestone acetate (FGA) inducing a more rapid estrus initiation than medroxyprogesterone acetate (MPA) (Romano 1996). However, the 48-hour after IVS removal is critical for vaginal healing (Quereda et al. 2020; Guner et al. 2022), suggesting that early estrus in FGA-treated ewes may increase the risk of semen deposition in an inflamed vaginal environment. Given the widespread use of different progestagens in field conditions, it is essential to assess the effects of intravaginal treatments with both progestagens. This study aimed to evaluate the impact of *Lactobacillus plantarum* or its cell-free supernatant, applied at sponge insertion, on vaginitis severity and reproductive performance in ewes synchronized with FGA or MPA in a field study.

Materials and methods

Animals management

The experimental procedures were approved by the Balıkesir University Animal Care Committee (Reference Number: 2021/3–4), ensuring compliance with ethical standards for animal research. This study was conducted on 196 clinically healthy Merino ewes, all of which had a parity of two. Ewes exhibited normal physiological and behavioral parameters, including good appetite, absence of lameness, and no signs of neurological or digestive disorders. Their alertness to environmental stimuli was demonstrated through responsive ear movements.

Throughout the study, the ewes were maintained on a nutritionally balanced total mixed ration and were provided with daily grazing access to natural pasture for four hours. Additionally, they had unrestricted access to fresh water and shaded areas to ensure optimal welfare conditions.

Experimental design

The estrus synchronization protocol was conducted during the breeding season, spanning from May 25 to June 24, in Balıkesir, Türkiye. A total of 196 Merino ewes were randomly assigned to one of four experimental groups following a 2 × 2 factorial design. The grouping was based on two factors: (1) the type of intravaginal treatment administered prior to sponge insertion either *Lactobacillus plantarum* (LAC) or its cell-free supernatant (CFS), and (2) the type of intravaginal sponge used—either fluorogestone acetate (FGA) or medroxyprogesterone acetate (MPA).

All ewes received intravaginal sponges impregnated with either 60 mg of medroxyprogesterone acetate (Esponjavet®, HIPRA, Istanbul, Türkiye) or 20 mg of fluorogestone acetate (Chronogest CR, MSD, Istanbul, Türkiye) for a duration of 14 days. The sponges differed in their physical characteristics, with FGA sponges measuring 4 × 4 cm and weighing 0.96 g, while MPA sponges measured 4 × 3 cm and weighed 1.17 g.

Lactobacillus plantarum and its cell-free supernatant were prepared following the protocol described by Guner et al. 2024). The bacterial culture and supernatant were produced two days prior to application and stored in sterile tubes at 4–6 °C until use. Prior to sponge insertion, 1 mL of either *L. plantarum* suspension or its cell-free supernatant was injected into each intravaginal sponge to ensure uniform distribution of the treatment. To maintain aseptic conditions, the vulvar area of each ewe was disinfected with 1% benzalkonium chloride (Zefirol, Dermosept, Istanbul, Türkiye) before sponge insertion. Additionally, all instruments and materials used for sponge application were thoroughly disinfected to prevent contamination. At the time of sponge removal, all ewes received a single intramuscular injection of 500 IU equine chorionic gonadotropin (eCG; Gonaser®, HIPRA, Türkiye, or Folligon®, MSD, Türkiye).

Vaginal discharge scores were recorded at the time of sponge removal, and each sponge was weighed after removal. The difference in weight was calculated as sponge weight change, serving as an indirect measure of vaginal discharge accumulation throughout the application period. Vaginal discharge was assessed based on its volume and composition, specifically whether it was clear, purulent, or hemorrhagic. The scoring system, adapted from (Martinez-Ros et al. 2018), categorized discharge as follows: score 0: for negligible or no discharge, score 1: for a small amount of clear discharge, and score 2: for an abundant amount of purulent or hemorrhagic discharge at the time of sponge removal.

A total of eighteen rams were introduced to the flock for natural mating. Estrus detection was conducted using crayon marks from harnesses worn by the rams, which

Table 1 Effect of treatment with *Lactobacillus plantarum* (LAC) or cell-free supernatant (CFS) on retention rates and vaginal Dsicharge scores in Ewes synchronized with fluorogestone acetate (FGA) or Medroxyprogesterone acetate (MPA)

Variables	MPA-LAC	MPA-CFS	FGA-LAC	FGA-CFS	Over-all
Number of ewes	51	54	46	45	196
Retention rate	50	49	40	39	178
Score 0; Negligible	5	2	2	3	12
Score 1; Mild	21	21	22	14	78
Score 2; Purulent or Hemor-ragic	24	26	16	22	88

Values in rows labeled with different letters (a, b) exhibit statistically tendency ($p<0.05$). (MPA, medroxyprogesterone acetate; FGA, fluorogestone acetate; LAC, *Lactobacillus plantarum*; CFS, *Lactobacillus plantarum cell-free supernatant*)

Table 2 Effect of treatment with *Lactobacillus plantarum* (LAC) or cell-free supernatant (CFS) on vaginal discharge score and sponge weight change in Ewes synchronized with fluorogestone acetate (FGA) or Medroxyprogesterone acetate (MPA)

Variables	MPA+LAC	MPA+CFS	FGA+LAC	FGA+CFS
Vaginal discharge score	1.38±0.09	1.49±0.08	1.35±0.08	1.49±0.10
Sponge weight change	6.03±0.35^{a}	6.05±0.25^{a}	7.25±0.35^{b}	7.13±0.24^{b}

Values in rows labeled with different letters (a, b) exhibit statistically tendency ($p<0.05$). (MPA, medroxyprogesterone acetate; FGA, fluorogestone acetate; LAC, *Lactobacillus plantarum*; CFS, *Lactobacillus plantarum cell-free supernatant*)

were transferred onto the rumps of receptive ewes. Ewes exhibiting estrus behavior were identified twice daily over a 96-hour observation period, following the methodology described by (Ungerfeld and Rubianes 2002). Pregnancy diagnosis was performed 35 days post-mating using transrectal ultrasonography with a linear probe (7.5 MHz) to confirm pregnancy status (Hasvet 838, Hasvet, Antalya, Türkiye).

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics (version 27, IBM Corp., Armonk, NY, USA). A General Linear Model (GLM) was employed to evaluate the effects of different intravaginal sponge applications on vaginitis scores and sponge weight change. In this model, progestagen type was included as a fixed factor, while treatment groups were incorporated as a covariate to account for potential interactions. The chi-square (χ^2) test was used to compare estrus detection and pregnancy rates among groups. Additionally, Spearman's rank correlation analysis was performed to examine the relationship between vaginitis scores and both pregnancy and estrus response rates. Results for continuous variables were expressed as mean ± standard error of the mean (SEM), while categorical variables were presented as percentages. Statistical significance was defined as $p<0.05$, and statistical tendencies were considered within the range of $0.05<p<0.10$.

Results

Throughout the experiment, no adverse effects on the health status of ewes receiving intravaginal treatment were observed. The overall sponge loss rate was 9.2%. There was a tendency ($p=0.07$) for a higher sponge loss rate in the MPA group (5.7%, 6/105) compared to the FGA group (13.2%, 12/91). Among ewes that experienced sponge loss before the scheduled removal day, four out of six ewes in the MPA group and eight out of twelve ewes in the FGA group exhibited estrus. Furthermore, pregnancy was confirmed in one ewe from the MPA group and seven ewes from the FGA group.

On the day of sponge removal, vaginal discharge was observed in 178 ewes (90.8%), while 18 ewes (9.2%) experienced sponge loss without any signs of vaginal discharge (Table 1). Mild to severe discharge was observed in over 80% of ewes in both groups, with purulent or hemorrhagic discharge being slightly more frequent in the FGA group (47.6% vs. 41.7%, Table 1).

There was no significant difference in vaginal discharge scores between ewes treated with LAC or CFS in the MPA group ($p>0.05$). However, FGA+LAC group had a statistically lower rate of purulent/hemorrhagic vaginal discharge than FGA+CFS group ($p<0.05$). The type of progestagen (MPA or FGA) did not have a significant effect on vaginal discharge scores ($p>0.05$). LAC treatment resulted in a slight, yet statistically insignificant, reduction in overall vaginal discharge scores within the FGA group (Table 2). Additionally, sponge weight change was significantly higher in the FGA group compared to the MPA group ($p<0.05$). However, intravaginal treatment had no significant effect on sponge weight change. Furthermore, no significant interaction was detected between progestagen type and treatment concerning vaginal discharge scores or sponge weight change ($p>0.05$; Table 2).

The mean onset of estrus was significantly earlier ($p<0.05$) in the FGA groups (36.8 ± 1.9 h) compared to the MPA group (49.1 ± 1.2 h, Table 3). The onset of estrus (mean ± SEM) for each treatment group was as follows: MPA+LAC (50.4 ± 1.8 h), MPA+CFS (47.8 ± 1.6 h),

Table 3 Effect of treatment with *Lactobacillus plantarum* (LAC) or cell-free supernatant (CFS) on reproductive parameters in Ewes synchronized with fluorogestone acetate (FGA) or Medroxyprogesterone acetate (MPA)

	MPA+LAC	MPA+CFS	FGA+LAC	FGA+CFS
Number of ewes	51	54	46	45
Onset of estrus (h)	50.4±1.8[a]	47.8±1.6[a]	38.0±2.9[b]	35.7±2.5[b]
Estrus response	90.2 (46)	83.3 (45)	80.4 (37)	77.8 (35)
Conception rate	49.0[a] (25)	50.0[a] (27)	71.7[b] (33)	75.6[b] (34)
Pregnancy rate	54.3[a] (25)	60.0[a] (27)	89.1[b] (33)	97.1[b] (34)

Values in rows labeled with different letters (a, b) exhibit statistically tendency ($p<0.05$). (MPA, medroxyprogesterone acetate; FGA, fluorogestone acetate; LAC, *Lactobacillus plantarum*; CFS, *Lactobacillus plantarum cell-free supernatant*)

FGA+LAC (38.0±2.9 h), and FGA+CFS (35.7±2.5 h). The estrus response rates in the FGA+LAC, FGA+CFS, MPA+LAC, and MPA+CFS groups were 80.4% (37/46), 77.8% (35/45), 90.2% (46/51), and 83.3% (45/54), respectively, with no statistically significant differences among groups ($p>0.05$). However, pregnancy rates were significantly higher in the FGA+LAC (71.7%, 33/46) and FGA+CFS (75.6%, 34/45) groups compared to the MPA+LAC (49.0%, 25/51) and MPA+CFS (50.0%, 27/54) groups ($p<0.01$). Similar to estrus onset, a significant difference in both conception and pregnancy rates was observed between the two progestagen types ($p<0.01$; Table 3).

Discussion

Our study evaluated the effects of intravaginal treatment (LAC, *Lactobacillus plantarum* or CFS, *Lactobacillus plantarum* cell-free supernatant) and two different intravaginal sponge composition (fluorogestone acetate and medroxyprogesterone acetate) on vaginitis and reproductive parameters in ewes. Vaginal discharges are considered a physiological response of the vaginal mucosa to the presence of a foreign body, and the characteristics of discharge or sponge loss are influenced by the size and texture of the material used in sponge manufacturing (Swelum et al. 2015). In this study, the overall sponge loss rate was 9.2%, which falls within the previously reported range of 1–10% in ewes (Viñoles et al. 2001; Swelum et al. 2015; Guner and Kisadere 2022; Guner et al. 2024). While intravaginal devices generally have lower retention rates compared to sponges (Swelum et al. 2015), direct comparisons between FGA and MPA remain limited (Boland et al. 1979; Ainsworth and Shrestha 1983). In our findings, sponge loss was more frequent in the FGA group than in the MPA group, contrasting with Boland et al. (1979), who reported higher loss rates with MPA. The larger size, higher absorption capacity, and greater mechanical pressure of FGA sponges may contribute to retention difficulties, particularly in young ewes with narrower vaginal canals. Consistent with Kilboz and Karaca (2010), who observed a 37.5% sponge loss rate in young goats, anatomical limitations should be considered in future applications. Despite sponge loss, estrus expression and conception still occurred in both groups, suggesting that hormonal absorption was sufficient before sponge expulsion in some animals (Guner et al. 2024; Kilboz and Karaca 2010). Therefore, allowing ram introduction upon sponge loss may be a beneficial management strategy under field conditions.

Consistent with previous studies (Quereda et al. 2020; Guner et al. 2024), all ewes (except those with sponge loss) exhibited vaginal discharge. Prolonged progestagen treatments contribute to the absorption of vaginal secretions by polyurethane sponges, leading to abnormal discharge after sponge removal (Al-Hamedawi et al. 2003; Martinez-Ros et al. 2018). The extended presence of the sponge may promote microbial proliferation and inflammation, exacerbating vaginal discharge (Manes et al. 2010, 2018; Guner et al. 2022). Young ewes are more prone to severe vaginal discharge than mature ewes (Martinez-Ros et al. 2018; Guner and Saat 2021). FGA sponges may absorb more fluid due to their material properties. Hence, it would be expected that the FGA sponge has more adverse effects on vaginal discharge than MPA sponge. Our findings indicate that sponge composition did not significantly affect vaginal discharge scores.

The use of antibiotics has traditionally been a common approach to managing vaginal inflammation associated with intravaginal sponges (Berruga et al. 2008; Serrano et al. 2020). However, their application has been linked to disruptions in the vaginal microbiota, leading to a decline in specific beneficial bacterial populations (Serrano et al. 2020). Additionally, concerns regarding antibiotic residues in milk and meat highlight the necessity of alternative strategies to mitigate these risks (Berruga et al. 2008). Given these challenges, recent studies have emphasized the potential of intravaginal probiotics as a preventive measure against sponge-induced vaginitis (Quereda et al. 2020; Serrano et al. 2020; Guner et al. 2022; Toquet et al. 2025).

Lactobacillus spp. (Quereda et al. 2020; Toquet et al. 2025), and their supernatant (Guner et al. 2022) have shown promising results in supporting vaginal health and reducing vaginitis in vivo studies. Probiotic applications help restore microbial balance by reducing neutrophilic infiltration and alleviating microbiota disruptions caused by intravaginal devices (Quereda et al. 2020; Toquet et al. 2025). As in our study, previous studies have also reported the advantage of not freezing the bacteria for a long time (Toquet et al. 2025).

There has been limited study (Danilova et al. 2019; Guner et al. 2022) in the literature evaluating the effectiveness of bacterial supernatant on vaginitis in ewes. Danilova et al. (2019) demonstrated the inhibitory effect of *Lactobacillus plantarum* supernatant on pathogenic bacteria in vitro study. Guner et al. (2022) reported the administration of *Lactobacillus plantarum* supernatant reduce *Enterobacteriaceae* count at the time of sponge removal in ewes.

In our hypothesis, the larger sponge size of FGA could have promoted more severe vaginal discharge than MPA. Using different progestagens did not affected vaginal discharge in this study. The mean vaginal discharge score was insignificantly lower in groups that were treated *Lactobacillus plantarum* compared to supernatant groups. The absence of a negative control group in this field study limits the ability to fully evaluate the specific impact of the treatments on vaginal discharge severity; future studies incorporating untreated controls would help clarify these effects. The farmer, who applied estrus synchronization using an intravaginal sponge, had concerns that severity of vaginal discharge would reduce fertility (Guner et al. 2024). At this point, the farmer's concerns about the control group without any intravaginal treatment limited our study. Further histological analyses of vaginal tissue are needed to better understand the efficacy of lactic acid bacteria or supernatant in ewes that synchronized with FGA and MPA.

There was no difference in the proportion of ewes expressed estrus among groups, however ewes in FGA group (36.8 ± 1.9 h) expressed estrus in earlier than ewes in MPA group (49.1 ± 1.2 h). More ewes expressed the first 24 h after sponge removal in FGA group compared to MPA group. Our findings mean that most ewes synchronized with FGA exhibit standing heat at earlier times than MPA ewes. Likely our findings, earlier estrus initiation in FGA grup than MPA group were demonstrated in previous studies in ewes (Robinson et al. 1967), in does (Romano 1996, 2004). The earlier onset of estrus in FGA ewes probably due to the differences in the rate of absorption and metabolization of each progestagen. Abrupt decrease in progesterone concentration after sponge removal stimulates GnRH release and FSH increase (Swelum et al. 2015). The difference in estrus initiation between MPA and FGA could be resulted from higher potential to suppress estrus during insertion, quick elimination time and short half-life of FGA compared to MPA. Moreover, estrus was observed more precise and concentrated in ewes FGA group than MPA group (Robinson et al. 1967; Romano 1996).

Furthermore, conception rate and pregnancy rate were significantly higher in the FGA group than MPA group in our study. Our findings align with previous research, where pregnancy rates with MPA-based sponges ranged from 41.4 to 79.0% (Ungerfeld and Rubianes 2002; Manes et al. 2014; Garoussi et al. 2020; Pérez-Clariget et al. 2021; Guner et al. 2024), whereas those with FGA-based sponges varied between 45% and 90% (Altincekic et al. 2020; Quereda et al. 2020; Al-Dahal et al. 2022; Di Giorgio et al. 2022). Swelum et al. (2015) determined higher FSH levels after sponge removal in FGA group. Although there was no difference in estrus response, estrus initiation is earlier and serum concentration of estradiol (E2) and progesterone (P4) was higher in CIDR group than FGA group in previous study. This difference in serum hormone levels resulted in a higher pregnancy rate in CIDR group than FGA group. Like the previous results in CIDR group compared to FGA, changes in estrus initiation time and pregnancy rate were similar in our FGA groups compared to MPA groups. Thus, earlier initiation of estrus expression and higher pregnancy rate could be time-dependent early decreases in progesterone and early increases in estradiol-17beta levels in FGA group compared to MPA group in our study.

Conclusion

The administration of LAC or CFS did not induce any adverse effects in ewes. Notably, LAC treatment at the time of sponge insertion resulted in a lower vaginal discharge score in both progestagen groups. Moreover, ewes in FGA group exhibited an earlier onset of estrus and had a higher pregnancy rate than ewes in MAP group. This study provides practical insights that are highly relevant for field applications, as it was conducted under commercial-like conditions using commonly available progestagen-based intravaginal sponges. Evaluating the effects of two distinct progestagen types (differing in size and formulation) on vaginal health outcomes is particularly important, as these variables may influence the incidence and severity of vaginitis. Understanding how the physical and hormonal characteristics of synchronization materials affect reproductive parameters supports more informed decision-making in veterinary practice.

Author contributions All authors contributed to conceptualization, methodology, investigation and writing the final manuscript. All authors read and approved the fnal manuscript.

Funding No funding was received for this research.

Data availability The data that support the fndings of this study are available from the corresponding author upon reasonable request.

Declarations

Declarations Statement of animal rights All procedures performed on ewes had been approved by Balikesir University. The experimental procedures were approved by the Balıkesir University Animal Care

Committee (Reference Number: 2021/3–4).

Conflict of interest All authors declare that they have no confict of interest.

References

Ainsworth L, Shrestha JNB (1983) Effect of type of intravaginal progestagen treatment of estrous response and reproductive performance of Ewes theriogenology. 19:869–875 (Elsevier)

Al-Dahal EM, Al-Dahal YEM, Mahmood MK, Saleem JN (2022) Comparing some reproductive characters of Awassi Ewes ınserted with Spanish (ovejero comp.) and Holland (ıntervet comp.) made vaginal sponges. J Curr Res Eng Sci Technol 8:79–86

Al-Hamedawi TM, Khammas DJ, Al-Ubaidi AS (2003) Effect of estrus synchronization on vaginal flora and subsequent fertility in Ewes Iraqi. J Veterinary Sci 16:73–79

Altincekic SO, Cilo BD, Duru S, Koyuncu M (2020) Investigation of the effect of ıntravaginal device-type and treatment-duration on vaginal features in Ewes and determination of antibiotic susceptibility. Indian J Anim Res 55:924–929 (Agricultural Research Communication Centre)

Barba M, Toquet M, García-Roselló E, Gomis J, Quereda JJ, González-Torres P, Carbonetto B, Gómez-Martín Á (2024) Description of the vaginal microbiota in nulliparous Ewes during natural mating and pregnancy: preliminary signs of the male preputial microbiota modulation frontiers in microbiology. 14:1224910 (Frontiers Media SA)

Berruga MI, Rodriguez A, Rubio R, Gallego R, Molina A (2008) Short communication: antibiotic residues in milk following the use of ıntravaginal sponges for estrus synchronization in dairy Ewes. J Dairy Sci 91:3917–3921 (Elsevier)

Boland MP, Kelleher D, Gordon I (1979) Comparison of control of oestrus and ovulation in sheep by an ear implant (SC-21009) or by intravaginal sponge (cronolone or map). Anim Reprod Sci 1:275–281 (Elsevier)

Danilova TA, Adzhieva AA, Danilina GA, Polyakov NB, Soloviev AI, Zhukhovitsky VG (2019) Antimicrobial activity of supernatant of lactobacillus plantarum against pathogenic microorganisms. Bull Exp Biol Med 167:751–754 (Springer New York LLC)

Di Giorgio S, Vullo C, Monti S, Giambrone G, Catone G, Marino G, Panzera M (2022) Effect of 20 mg fluorogestone acetate (FGA) sponges on reproductive parameters of dairy ovine breeds in Southern. Italy Acta Sci Veterinariae 50:1887 (Universidade Federal do Rio Grande do Sul)

Garoussi MT, Mavadati O, Bahonar M, Ragh MJ (2020) The effect of Medroxyprogesterone acetate with or without eCG on conception rate of fat-tail Ewes in out of breeding season. Trop Anim Health Prod 52:1617–1622 (Springer)

Gatti M, Zunino P, Ungerfeld R (2011) Changes in the aerobic vaginal bacterial mucous load after treatment with ıntravaginal sponges in anoestrous ewes: effect of Medroxiprogesterone acetate and antibiotic treatment use reproduction in domestic animals, vol 46. John Wiley & Sons, Ltd), pp 205–208

Gonzalez-Bulnes A, Menchaca A, Martin GB, Martinez-Ros P (2020) Seventy years of progestagen treatments for management of the sheep oestrous cycle: where we are and where we should go reproduction. Fertility Dev 32:441 (CSIRO PUBLISHING)

Guner B, Kisadere I (2022) The efficacy of intravaginal antibiotic or their combination with different gonadotropins on vaginitis and fertility in Ewes synchronized with progesterone impregnated intravaginal sponges Turkish. J Veterinary Anim Sci 46:575–581 (TUBITAK)

Guner B, Saat N (2021) Comparison of pregnancy rates after Short - Term and long -Term synchronization protocol in Ewes- pilot study Erciyes Üniversitesi. Veteriner Fakültesi Dergisi 18:69–74 (Erciyes University)

Guner B, Kisadere İ, Tavsanli H, Demirbilek SK, Keskin A (2022) Comparison of the efficacy of Enrofloxacin and lactobacillus plantarum cell-free supernatant treatments on vaginitis in Ewes. J Res Veterinary Med 41:105–111 (Bursa Uludağ University)

Guner B, Ayalp Erkan A, Ozturk B, Guner TE, Kisadere I, Demirbilek SK, Keskin A, František Z (2024) Efficacy of intravaginal lactic acid bacteria, cell-free supernatant, or Enrofloxacin on vaginitis and fertility in Ewes synchronized with progesterone-based protocol agriculture. 14:604 (Multidisciplinary Digital Publishing Institute)

Kilboz Eİ, Karaca F (2010) Üreme Mevsimi Dışında Genç Keçilerde flugeston Asetat vaginal Sünger ve Norgestomet Kulak Implantı Uygulamalarıyla Östrüslerin Uyarılması Yüzüncü Yıl. Üniversitesi Veteriner Fakültesi Dergisi 21:1–6 (Van Yuzuncu Yıl University)

Manes J, Fiorentino MA, Kaiser G, Hozbor F, Alberio R, Sanchez E, Paolicchi F (2010) Changes in the aerobic vaginal flora after treatment with different intravaginal devices in Ewes. Small Ruminant Res 94:201–204 (Elsevier)

Manes J, Hozbor F, Alberio R, Ungerfeld R (2014) Intravaginal placebo sponges affect negatively the conception rate in sheep small ruminant research. 120:108–111 (Elsevier)

Manes J, Fiorentino MA, Martino SS, Ungerfeld R (2018) Changes in the vaginal microbiota in Ewes after insertion of intravaginal sponges at different stages of the oestrous cycle livestock science. 208:55–59 (Elsevier)

Martinez-Ros P, Lozano M, Hernandez F, Tirado A, Rios-Abellan A, López-Mendoza M, Gonzalez-Bulnes A (2018) Intravaginal device-type and treatment-length for ovine estrus synchronization modify vaginal mucus and microbiota and affect fertility animals. 8:226 (Multidisciplinary Digital Publishing Institute)

Ojeda-Hernández F, del Moral-Ventura S, Capataz-Tafur J, Peña-Castro J, Abad-Zavaleta J, Chay-Canul A, Ramon-Ugalde J, Ungerfeld R, Meza-Villalvazo V (2019) Vaginal microbiota in pelibuey sheep treated with antimicrobials at the removal of intravaginal sponges impregnated with flurogestone acetate small ruminant research. 170:116–119 (Elsevier)

Olivera-Muzante J, Fierro S, López V, Gil J (2011) Comparison of prostaglandin- and progesterone-based protocols for timed artificial insemination in sheep theriogenology. 75:1232–1238 (Elsevier)

Pérez-Clariget R, López-Pérez Á, Ungerfeld R (2021) Treatments with intravaginal sponges for estrous synchronization in ewes: length of the treatment, amount of medroxyprogesterone, and administration of a long-acting progesterone Tropical Animal Health and Production, 53, 345 (Springer Science and Business Media B.V.)

Quereda JJ, García-Roselló E, Barba M, Mocé ML, Gomis J, Jiménez-Trigos E, Bataller E, Martínez-Boví R, García-Muñoz Á, Gómez-Martín Á (2020) Use of probiotics in ıntravaginal sponges in sheep: A pilot study animals. 10:719 (Multidisciplinary Digital Publishing Institute)

Regecová I, Výrostková J, Zigo F, Gregová G, Pipová M, Jevinová P, Becová J (2022) Detection of resistant and enterotoxigenic strains of *staphylococcus warneri* isolated from food of animal origin. Foods 11(10):1496

Robinson TJ, Moore NW, Holst PJ, Smith JF (1967) The evaluation of several progestins administered in intravaginal sponges for synchronization of estrus in entire Cyclic Merino Ewe. In: Robinson TJ (ed) The control of the ovarian cycle in the sheep. Springer US, Boston, MA, pp 76–101

Romano JE (1996) Comparison of fluorgestone and Medroxyprogesterone intravaginal pessaries for estrus synchronization in dairy goats. Small Ruminant Res 22:219–223 (Elsevier)
Romano JE (2004) Synchronization of estrus using CIDR, FGA or MAP intravaginal pessaries during the breeding season in Nubian goats small ruminant research. 55:15–19 (Elsevier)
Serrano M, Climent E, Freire F, Martínez-Blanch JF, González C, Reyes L, Solaz-Fuster MC, Calvo JH, Jiménez MÁ, Codoñer FM (2020) Influence of the ovine genital tract microbiota on the species artificial ınsemination outcome. A pilot study in commercial sheep farms High-Throughput. 9:16 (Multidisciplinary Digital Publishing Institute)
Suárez G, Zunino P, Carol H, Ungerfeld R (2006) Changes in the aerobic vaginal bacterial mucous load and assessment of the susceptibility to antibiotics after treatment with intravaginal sponges in anestrous ewes. Small Rumin Res 63(1–2):39–43
Swartz JD, Lachman M, Westveer K, O'Neill T, Geary T, Kott RW, Berardinelli JG, Hatfield PG, Thomson JM, Roberts A, Yeoman CJ (2014) Characterization of the vaginal microbiota of Ewes and cows reveals a unique microbiota with low levels of lactobacilli and near-neutral ph. Front Veterinary Sci 1:116383 (Frontiers Media S.A.)
Swelum AA-A, Alowaimer AN, Abouheif MA (2015) Use of fluorogestone acetate sponges or controlled internal drug release for estrus synchronization in ewes: effects of hormonal profiles and reproductive performance theriogenology. 84:498–503 (Elsevier)
Toquet M, Gomis J, Jiménez-Trigos E, Bataller E, Barba M, Sánchez A, González-Torres P, Gómez-Martín Á (2025) Development of an innovative intravaginal model of probiotic inoculation in dairy ovine flocks: positive effects on the vaginal microbiota, vaginitis and fertility animal. 19:101417 (Elsevier)
Ungerfeld R, Rubianes E (2002) Short term primings with different progestogen intravaginal devices (MAP, FGA and CIDR) for eCG-estrous induction in anestrus Ewes. Small Ruminant Res 46:63–66 (Elsevier)
van de Wijgert J, Verwijs M (2020) Lactobacilli-containing vaginal probiotics to cure or prevent bacterial or fungal vaginal dysbiosis: a systematic review and recommendations for future trial designs. BJOG: Int J Obstet Gynecol 127:287–299 (John Wiley & Sons, Ltd)
Vasconcelos CO, de Brandão P, Martins FZ, Penna G, Souza-Fabjan B (2016) Qualitative and quantitative analysis of bacteria from vaginitis associated with intravaginal implants in Ewes following estrus synchronization Ciência rural. 46:632–636 (Universidade Federal de Santa Maria). deLilenbaumW.
Viñoles C, Forsberg M, Banchero G, Rubianes E (2001) Effect of long-term and short-term progestagen treatment on follicular development and pregnancy rate in Cyclic Ewes theriogenology. 55:993–1004 (Elsevier)
Výrostková J, Regecová I, Zigo F, Semjon B, Gregová G (2021) Antimicrobial resistance of Staphylococcus spp. Isolated from cheeses. Animals 12(1):36

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.