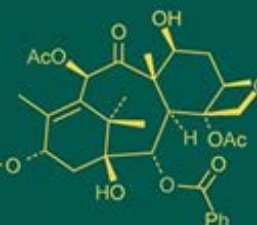
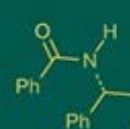
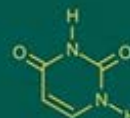
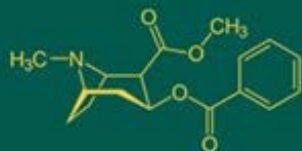


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(8): 236-239

www.biochemjournal.com

Received: 25-05-2026

Accepted: 26-06-2026

Dr. Chamaraj Pujar

Dept. of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bangalore, KVAFSU, Karnataka, India

Dr. Narasimha Murthy

Dept. of Veterinary Gynaecology and Obstetrics, Veterinary College, Bangalore, KVAFSU, Karnataka, India

Dr. BM Ravindranath

Dept. of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bangalore, KVAFSU, Karnataka, India

Dr. Sharada R

Dept. of Veterinary Microbiology, Veterinary College, Gadag, KVAFSU, Karnataka, India

Dr. Mahadevappa Gowri

Associate Professor, Department of Livestock Farm Complex, Veterinary College, Bengaluru, Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Karnataka, India

Dr. Sudha G

Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Gadag, Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Karnataka, India

Dr. Pallavi Bhatabre

Veterinary Officer, Government of Karnataka (GOK), Gadag, Karnataka, India

Dr. Pruthviraj

Veterinary Officer, Government of Karnataka (GOK), Raichur, Karnataka, India

Corresponding Author:**Dr. Chamaraj Pujar**

Dept. of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bangalore, KVAFSU, Karnataka, India

Influence of different estrus synchronization protocols on vaginal bacterial load in goats exhibiting estrus

Chamaraj Pujar, Narasimha Murthy, BM Ravindranath, Sharada R, Mahadevappa Gouri, Sudha G, Pallavi Bhatabre and Pruthviraj

DOI: <https://doi.org/10.33545/26174693.2026.v10.i8c.9506>

Abstract

The present study was undertaken to evaluate changes in vaginal bacterial load in goats subjected to different estrus synchronization protocols. A total of 36 clinically healthy, cyclic, non-pregnant does were randomly allocated into three equal groups (n=12each). Group I received a progesterone-impregnated intravaginal sponge for seven days followed by PGF_{2α} administration at sponge withdrawal, Group II received a letrozole-impregnated intravaginal sponge for seven days followed by PGF_{2α}, and Group III received oral clomiphene citrate for five days followed by PGF_{2α}. Deep vaginal secretions were collected aseptically and cultured for total bacterial count. In Group I, the bacterial load increased significantly from 0.49±0.13 CFU/mL on Day 0 to 7.02±0.12 CFU/mL at sponge removal and subsequently declined to 0.25±0.01 CFU/mL at estrus. In Group II, bacterial counts increased from 1.46± 0.10 to 6.23 ± 0.19 CFU/mL and decreased to 0.54±0.06 CFU/mL at estrus. Group III showed a non-significant reduction from 1.43±0.50 to 0.34± 0.09 CFU/mL. The findings indicate that intravaginal sponge-based synchronization protocols temporarily increase vaginal bacterial load, whereas oral clomiphene citrate exerts minimal influence on the vaginal microbial environment. The bacterial counts returned near baseline levels at estrus, suggesting that the alterations were transient and did not persist after sponge removal.

Keywords: Goat, estrus synchronization, vaginal bacterial load, progesterone sponge, letrozole, clomiphene citrate, cfu/ml

Introduction

Goat production contributes substantially to rural livelihoods, particularly among small and marginal farmers, owing to the species' adaptability, low input requirements, and high reproductive potential. According to the 20th Livestock Census (DAHD, 2019) ^[1], India possesses 148.88 million goats, highlighting the growing importance of goat farming in the national livestock sector. Reproductive efficiency is a major determinant of productivity and profitability in goat farming systems. Estrus synchronization protocols employing intravaginal progesterone devices, prostaglandin F_{2α} analogues, and other hormonal approaches are widely used to facilitate planned breeding and improve reproductive management. Abecia *et al.* (2012) ^[2] reported that intravaginal progesterone devices provide predictable control of ovarian activity and are among the most commonly adopted synchronization methods in small ruminants. Similarly, Rubianes and Menchaca (2002) ^[3] demonstrated that prostaglandin-based protocols effectively induce luteolysis and synchronize estrus when a functional corpus luteum is present. However, intravaginal devices may alter the vaginal microenvironment. Oliveira *et al.* (2013) ^[4] observed progressive increases in vaginal bacterial load during sponge retention in goats, whereas Manes *et al.* (2013) ^[5] reported that intravaginal synchronization devices significantly increased aerobic bacterial populations at the time of device removal. Opportunistic microorganisms such as *Escherichia coli* and *Staphylococcus spp.* have frequently been isolated following sponge use in small ruminants ^[6]Penna *et al.*, 2013 ; ^[7]Ababneh and Degefa, 2006 ^[6, 7]. Nevertheless, bacterial counts generally return to near-baseline levels after sponge withdrawal and fertility is not consistently compromised ^[8]Suárez *et al.*, 2006; ^[9]Martins *et al.*, 2009 ^[8, 9].

Understanding the dynamics of vaginal bacterial populations during estrus synchronization is therefore important for evaluating vaginal health and the safety of synchronization protocols. The present study was undertaken to evaluate changes in vaginal bacterial load in was undertaken to evaluate changes in vaginal bacterial load in goats exhibiting estrus under different estrus synchronization protocols, including progesterone sponge, letrozole sponge, and oral clomiphene citrate treatments.

Materials and Methods

The study was conducted at the Department of Livestock Farm Complex and the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bengaluru, from March 2025 to August 2026 under uniform management conditions. The experimental design was based on short-term intravaginal synchronization protocols commonly employed in goats, which have been reported to provide synchronization efficiency comparable to conventional long-term treatments (^[10]Karaca *et al.*, 2010; Vilarino *et al.*, 2011^[11]) ^[10].

Experimental animals

Thirty-six clinically healthy, cyclic, non-pregnant female goats aged between one and four years were selected. Pregnancy was ruled out by transabdominal ultrasonography. The animals were randomly allocated into three equal groups ($n = 12$ per group).

Synchronization protocols

Group I: Progesterone sponge +operatorname{PGF}2a

Does received a 350 mg natural progesterone-impregnated intravaginal sponge (Avikesil-S®) for seven days. At sponge withdrawal, 125 µg cloprostenol sodium was administered intramuscularly.

Group II: Letrozole sponge +operatorname{PGF}2a

Does received a 7.5 mg letrozole-impregnated intravaginal sponge for seven days followed by 125 µg cloprostenol sodium at sponge withdrawal.

Group III: Clomiphene citrate +PGF2a

Does received clomiphene citrate at 150 mg/day orally for five consecutive days after copper sulphate administration, followed by 125 µg cloprostenol sodium intramuscularly.

Bacteriological analysis

Deep vaginal secretions were collected aseptically using sterile cotton-tipped swabs. The swabs were introduced into the cranial vagina, rotated gently to absorb vaginal secretions adequately, and immediately transferred into sterile Brain Heart Infusion (BHI) broth for transport to the microbiology laboratory. For determination of total bacterial count, the samples were vortexed thoroughly and 100 µL of the broth suspension was

inoculated onto Plate Count Agar (PCA) plates using the spread plate technique under aseptic conditions. The inoculated plates were incubated aerobically at 37 °C for 24 hours. Following incubation, visible bacterial colonies were counted, and the results were expressed as colony-forming units per milliliter (CFU/mL) of vaginal secretion.

Statistical analysis

The total vaginal bacterial count (CFU/mL) recorded at different sampling points during estrus synchronization in Group I and Group II was analyzed using one-way repeated measures analysis of variance (ANOVA), followed by Bonferroni-adjusted multiple pairwise comparisons to identify differences between observation periods. In Group III, where bacterial counts were measured at only two time points (before treatment and at exhibited estrus), comparisons were performed using the paired Student's *t*-test. Data are presented as mean $\pm$ standard error of the mean (SEM), and differences were considered statistically significant at $P < 0.05$.

Results

The vaginal bacterial load varied significantly among different stages of synchronization in the intravaginal sponge-treated groups. In Group I, bacterial load increased markedly from Day 0 to sponge removal and declined significantly at estrus. A similar pattern was observed in Group II. In contrast, Group III showed a reduction in bacterial count from before treatment to estrus, but the difference was statistically non-significant. Both intravaginal protocols showed a pronounced increase in bacterial count at sponge removal followed by a rapid decline at estrus.

In Group I, the bacterial load increased significantly from 0.49 ± 0.13 CFU/mL on Day 0 to 7.02 ± 0.12 CFU / mL at sponge removal and subsequently declined to 0.25 ± 0.01 CFU/mL at estrus. A similar pattern was observed in Group II, where bacterial counts increased from 1.46 ± 0.10 to 6.23 ± 0.19 CFU/mL and decreased to 0.54 ± 0.06 CFU/mL at estrus. The temporal pattern observed in both sponge-treated groups closely resembles the trends reported by Suárez *et al.* (2006) ^[8] and Oliveira *et al.* (2013) ^[4], who described maximal bacterial proliferation during sponge retention followed by rapid restoration of normal vaginal microbiota after device withdrawal.

Table 1: Mean ($\pm$ SEM) vaginal bacterial count (CFU/mL) in goats under different synchronization protocols Figure 1. Comparative changes in vaginal bacterial count during synchronization and estrus

Group	Day 0	Sponge removal	Estrus	P-value
Group I (Progesterone sponge)	0.49 ± 0.13	7.02 ± 0.12	0.25 ± 0.01	<0.01
Group II (Letrozole sponge)	1.46 ± 0.10	6.23 ± 0.19	0.54 ± 0.06	<0.01
Group III (Clomiphene citrate)	1.43 ± 0.50	—	0.34 ± 0.09	0.065

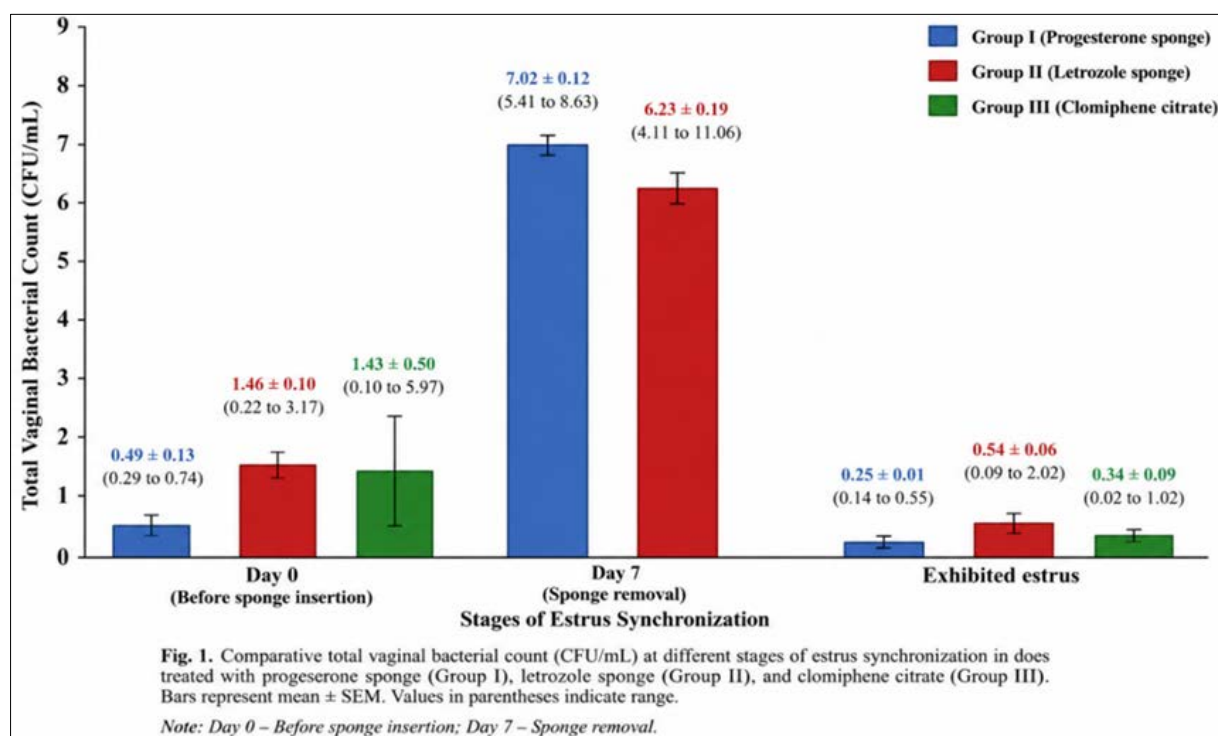


Fig 1: Comparative total vaginal bacterial count (CFU/mL) at different stages of estrus synchronization in does treated with progesterone sponge (Group I), letrozole sponge (Group II), and clomiphene citrate (Group III). Bars represent mean ± SEM. Values in parentheses indicate range

Discussion

The present study demonstrated that intravaginal sponge-based synchronization protocols produced significant alterations in vaginal bacterial load, whereas the oral clomiphene citrate protocol produced minimal microbial changes. The marked increase in bacterial count observed at sponge removal may be attributed to the physical presence of the intravaginal device, which acts as a foreign body and promotes retention of vaginal secretions, reduced aeration, increased moisture, and bacterial proliferation, as previously suggested by Penna *et al.* (2013) [6]. The findings are in close agreement with earlier reports in small ruminants. Suárez *et al.* (2006) [8] reported a substantial increase in vaginal bacterial load at sponge withdrawal in ewes treated with medroxyprogesterone acetate sponges, followed by restoration of bacterial counts to near-baseline levels at estrus. Likewise, Gatti *et al.* (2011) [12] documented a significant rise in aerobic vaginal bacterial populations at sponge removal with rapid decline within 24 hours after sponge withdrawal. Oliveira *et al.* (2013) [4] further demonstrated that bacterial proliferation increased progressively during sponge retention in goats, with the highest counts recorded on the day of device removal.

The decline in bacterial load at estrus observed in the present study suggests restoration of normal vaginal drainage and aeration following sponge withdrawal. Similar observations were reported by Ozyurtlu *et al.* (2008) [13] and Mohammed *et al.* (2017) [14], who found that bacterial counts returned close to baseline levels after removal of intravaginal synchronization devices.

The comparatively stable bacterial profile observed in the clomiphene-treated group indicates that the major factor influencing vaginal bacterial dynamics was the intravaginal device rather than hormonal treatment alone. This observation supports the conclusions of Manes *et al.*

(2013) [5], who reported that bacterial proliferation was primarily associated with device retention irrespective of the hormonal agent used.

Although bacterial species were not identified in the present study, previous investigators have frequently isolated opportunistic organisms following synchronization treatments. Penna *et al.* (2013) [6] reported predominance of *Staphylococcus* and *Streptococcus* species during spongebased protocols, while Sargison *et al.* (2007) [15] identified coliform bacteria, particularly *Escherichia coli*, as common organisms associated with post-synchronization vaginal infections in small ruminants.

Importantly, despite the temporary increase in bacterial load, conception and kidding rates were not adversely affected in the present investigation. Similar conclusions were reached by Martins *et al.* (2009) [9][15] and Suárez *et al.* (2006) [8], who observed that transient microbial alterations associated with intravaginal sponges did not consistently compromise fertility. The use of a seven-day short-term synchronization protocol may also have contributed to the favorable outcome. Menchaca and Rubianes (2004) [16] proposed that short-term protocols reduce prolonged exposure of the vaginal mucosa to exogenous hormones while maintaining synchronization efficiency. Comparable reproductive responses between short- and long-term protocols have also been demonstrated by Karaca *et al.* (2010) [10] and Vilarino *et al.* (2011) [11].

Conclusion

The present investigation demonstrated that intravaginal progesterone and letrozole sponge-based estrus synchronization protocols temporarily increased vaginal bacterial load during sponge retention, with the highest bacterial counts recorded at the time of sponge withdrawal. However, bacterial counts declined markedly at exhibited estrus, indicating rapid restoration of the normal vaginal microenvironment after device removal. This pattern of transient bacterial proliferation followed by post-withdrawal normalization is in agreement with the observations of Suárez *et al.* (2006) [8], Oliveira *et al.* (2013) [4], and Manes *et al.* (2013) [5]. In contrast, the oral

clomiphene citrate protocol did not cause significant alterations in vaginal bacterial load, suggesting minimal influence on vaginal microbial ecology. Despite temporary increases in bacterial population, reproductive performance remained unaffected, as evidenced by satisfactory estrus induction, conception, and kidding rates. Collectively, the findings indicate that short-term intravaginal synchronization protocols are effective reproductive management tools that do not induce persistent vaginal dysbiosis or compromise fertility in goats.

References

1. 20th Livestock Census. Department of Animal Husbandry, Dairying and Fisheries Ministry of Fisheries, Animal Husbandry and Dairying, New Delhi, Government of India. 2019.
2. Abecia JA, Forcada F, Gonzalez-Bulnes A. Hormonal control of reproduction in small ruminants. *Anim. Reprod. Sci.* 2012;130:173–179.
3. Rubianes E, Menchaca A. Relation between progesterone concentrations during the early luteal phase and follicular dynamics in goats. *Theriogenology*. 2002;57:1411–1419.
4. 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 oestrus induction and synchronisation with intravaginal sponges as well as their antimicrobial sensitivity. *Small Rumin. Res.* 2013;113(1):162–166.
5. Manes J, Fiorentino MA, Hozbor F, Paolicchi F, Alberio R, Ungerfeld R. Changes in the aerobic vaginal bacteria load and antimicrobial susceptibility after different oestrous synchronisation treatments in goats. *Anim. Prod. Sci.* 2013;53(6):555–559.
6. Penna B, Libonati H, Director A, Sarzedas AC, Martins G, Brandão FZ, *et al.* Progestin-impregnated intravaginal sponges for estrus induction and synchronization influences on goats vaginal flora and antimicrobial susceptibility. *Anim. Reprod. Sci.* 2013;142(1–2):71–74.
7. Ababneh MM, Degefa T. Bacteriological findings and hormonal profiles in the postpartum Balady goats. *Reprod. Domest. Anim.* 2006;41(1):12–16.
8. Suárez 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 anestrus ewes. *Small Rumin. Res.* 2006;63(1–2):39–43.
9. Martins G, Figueira L, Penna B, Brandão F, Varges R, Vasconcelos C, *et al.* Prevalence and antimicrobial susceptibility of vaginal bacteria from ewes treated with progestin-impregnated intravaginal sponges. *Small Rumin. Res.* 2009;81:182–184.
10. Karaca F, Doğruer G, Sarıbay MK, Ates CT. Oestrus synchronization with short-term and long-term progestagen treatments in goats: the use of GnRH prior to short-term progestagen treatment. *Ital. J. Anim. Sci.* 2010;9:22–23.
11. Vilariño M, Rubianes E, Menchaca A. Re-use of intravaginal progesterone devices associated with the Short-term Protocol for timed artificial insemination in goats. *Theriogenology*. 2011;75(7):1195–1200.
12. Gatti M, Zunino P, Ungerfeld R. Changes in the aerobic vaginal bacterial mucous load after treatment with intravaginal sponges in anoestrous ewes: Effect of medroxyprogesterone acetate and antibiotic treatment use. *Reprod. Domest. Anim.* 2011;46(2):205–208.
13. Ozyurtlu N, Yeyilmis S, Kucukaslan Y. The effectiveness of using antibiotic with intravaginal sponge and duration of sponge treatments on the vaginal flora and fertility in anestrus ewes. *J. Anim. Vet. Adv.* 2008;7(6):723–727.
14. Mohammed KM, Nabih AM, Darwish GM. Efficacy of anti-microbial agents on vaginal microorganisms and reproductive performance of synchronized estrus ewes. *Asian Pac. J. Reprod.* 2017;6(3):121–127.
15. Sargison ND, Howie F, Mearns R, Penny CD, Foster G. Shiga toxin-producing *Escherichia coli* as a perennial cause of abortion in a closed flock of Suffolk ewes. *Veterinary Record*. 2007;160(25):875. *Sci.* 77:1–14.
16. Menchaca A, Rubianes E. New treatments associated with timed artificial insemination in small ruminants. *Reprod. Fertil. Dev.* 2004;16(4):403–413.