

Diagnostic utility of exfoliative vaginal cytology for reproductive management in bitches

F.S. Raju^{1*}, K.A. Raju², A.U. Kiran², S. Paidi¹, N. Likitha¹, T.P. Balagopalan¹

¹Department of Veterinary Clinical Complex, ²Department of Veterinary Gynaecology and Obstetrics, School of Veterinary and Animal Sciences, Centurion University of Technology and Management, Gajapati district, Odisha-761211, India.

*Corresponding author Email: sebastin.raju@cutm.ac.in

Journal of Livestock Science (ISSN online 2277-6214) 17: 639-642

Received on 8/9/25; Accepted on 16/8/26; Published on 19/8/26

doi. 10.33259/JLivestSci.2026.639-642

Abstract

The study was conducted on 50 randomly selected bitches of different breeds presented to the Veterinary Clinical Complex, School of Veterinary and Animal Sciences, Centurion University of Technology and Management, Odisha, to evaluate the utility of exfoliative vaginal cytology (EVC) for estrous-cycle staging and identification of the optimal breeding period, and to assess pregnancy outcome following cytology-guided breeding. The animals were divided into two groups: Group I (n=25) comprised bitches presented during proestrus, while Group II (n=25) comprised bitches presented for pregnancy verification. Vaginal smears were collected from the cranial vagina using cotton-tipped swabs, fixed in methanol, stained with Giemsa, and examined at 40× magnification. In Group I, smears were collected on alternate days until cytological evidence of diestrus was observed. Breeding was advised when ≥80% of epithelial cells were cornified, and pregnancy was subsequently confirmed one month later. In Group II, pregnancy was verified by ultrasonography based on the breeding history reported by the owners. The cytological findings in Group I showed a progressive transition from parabasal and intermediate cells during proestrus to predominantly superficial cornified cells during estrus. Pregnancy was confirmed in 21 of 25 (84%) bitches in Group I, whereas 13 of 25 (52%) bitches in Group II were pregnancy positive. The findings support the practical value of EVC as a simple, minimally invasive, and economical method for estrous-cycle staging and reproductive management in bitches.

Keywords: exfoliative vaginal cytology; estrus detection; breeding time; pregnancy diagnosis; bitch

Introduction

Fertility management in the bitch is clinically important because the timing of mating or insemination strongly influences reproductive outcome. Studies on semen evaluation of dog semen has been reported with objective of artificial insemination (Kumar et al., 2025). Proper diagnosis of heat and accurate time of insemination is key for success in artificial insemination. Accurate identification of the fertile period is therefore an essential component of canine reproductive management. Mistimed breeding may occur because the onset of vulval discharge, behavioural receptivity, and the actual peri-ovulatory period do not always coincide closely enough to permit reliable breeding decisions based only on owner observation. Recent clinical work has emphasized the importance of objective cycle-staging methods for improving the timing of breeding and pregnancy success (Martínez-Mojica et al., 2023; Famakinde et al., 2025).

Exfoliative vaginal cytology is a simple, inexpensive, and minimally invasive technique that provides information on the cellular changes associated with the canine estrous cycle. The progressive change from parabasal and intermediate epithelial cells during proestrus to predominantly superficial and cornified cells during estrus provides a practical cytological basis for cycle staging. However, interpretation of vaginal cytology can vary because definitions of epithelial-cell characteristics and the thresholds used to identify cycle stages are not completely uniform (Reckers et al., 2022). Consequently, objective clinical evaluation of cytology remains relevant, particularly where hormonal assays are not readily accessible.

The need for the present study arose from the practical requirement for a readily available in-clinic method to identify the appropriate breeding period in bitches and thereby reduce the likelihood of breeding at an unsuitable stage of the cycle. Vaginal cytology has also been used as one of the parameters for determining the stage of estrus and timing of artificial insemination in clinical populations of bitches (Martínez-Mojica et al., 2023). Therefore, the present study was taken with aim to evaluate the diagnostic utility of exfoliative vaginal cytology for staging the estrous cycle and identifying the optimal breeding period in bitches, and to assess pregnancy outcome following breeding guided by cytological evidence of estrus.

Materials and methods

This study utilized 50 randomly selected bitches of various breeds presented to the Veterinary Clinical Complex, School of Veterinary and Animal Sciences, Centurion University of Technology and Management, Odisha. Of these, 25 were presented with observed proestrus (Group I) and 25 were presented for pregnancy verification (Group II).

Vaginal discharge was collected from the cranial vagina. A cotton-tipped swab, moistened with distilled water when required, was introduced through the vulva in a dorsal direction and advanced horizontally above the clitoral fossa and urethral papilla into the vagina. After gently rubbing or rolling the swab against the vaginal wall, it was removed and rolled onto a glass slide. The smears were fixed in methanol, air-dried, and stained with Giemsa. Smears were examined under 40× magnification, and multiple fields containing more than 200 epithelial cells were evaluated.

In Group I, vaginal smears were collected on alternate days from presentation until cytological evidence of diestrus was observed. Bitches were advised for breeding when approximately $\geq 80\%$ of the epithelial cells were cornified. The breeding protocol consisted of three matings at one-day intervals. Owners were instructed to return after one month for pregnancy confirmation.

In Group II, pregnancy was verified by ultrasonography on the day of presentation, based on the breeding history provided by the owners. The pregnancy status was recorded as positive or negative.

Results and discussion

The vaginal smears collected from Group I showed a progressive increase in superficial epithelial cells from proestrus to estrus, with a marked predominance of cornified superficial cells at the stage selected for breeding. Pregnancy confirmation one month after cytology-guided breeding identified 21 positive cases out of 25 bitches (84%). In Group II, ultrasonography performed at presentation identified 13 pregnancy-positive cases out of 25 (52%).

The observed cytological transition is consistent with the established pattern of increasing epithelial-cell cornification as the bitch progresses towards estrus. Recent work has also emphasized that vaginal cytology remains clinically useful while highlighting the importance of standardized definitions of exfoliated cell types (Reckers et al., 2022). In a clinical study of bitches undergoing artificial insemination, vaginal cytology was used together with serum progesterone concentration to determine estrous-cycle stage and timing of insemination (Martínez-Mojica et al., 2023). More recently, comparison of pre-breeding cycle-staging techniques has continued to support the clinical relevance of vaginal cytology for breeding management (Famakinde et al., 2025).

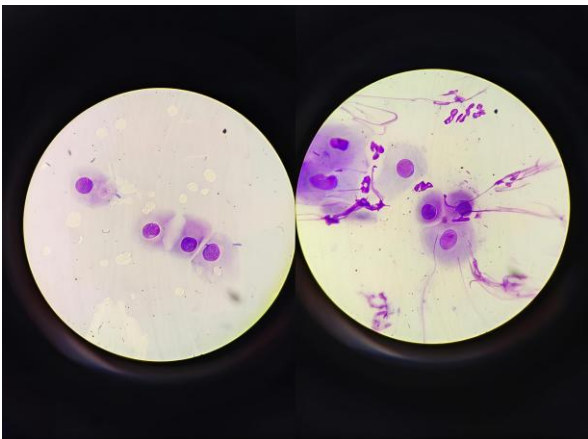


Fig 1. Representative Giemsa-stained exfoliative vaginal cytology smears showing the predominant epithelial-cell patterns during proestrus and estrus (40×).

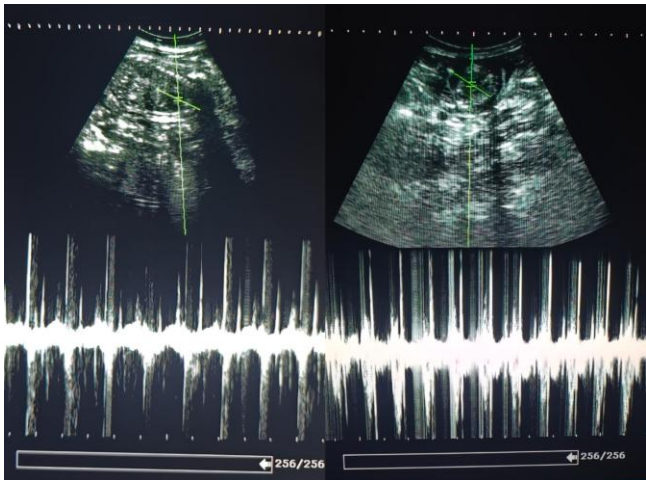


Fig 2. Representative ultrasonographic image demonstrating pregnancy in a bitch.

Table 1. Pregnancy rate in group I and group II

Group	No. of animals	Method of breeding/diagnosis	Pregnancy positive	Pregnancy rate (%)
Group I (proestrus; bred as per EVC)	25	Breeding guided by EVC (≥80% cornified cells)	21	84
Group II (presented for pregnancy verification)	25	Based on owner-reported breeding; verified by USG	13	52

In the present study, breeding was guided by the cytological finding of ≥80% cornified epithelial cells. The pregnancy-positive proportion was 84% in this cytology-guided group. In contrast, 52% of the bitches presented for pregnancy verification after owner-reported breeding were pregnancy positive. Because the two groups were managed under different clinical circumstances and were not randomized, these values should be interpreted as an observational clinical comparison rather than as a controlled estimate of treatment effect. Nevertheless, the findings indicate the potential practical value of incorporating EVC into routine breeding management.

Ultrasonography remains an important method for confirming pregnancy after breeding, while vaginal cytology provides information primarily on the stage of the estrous cycle. Thus, the two methods serve complementary clinical purposes: EVC assists in reproductive timing, whereas ultrasonography confirms pregnancy. The combined use of appropriate cycle staging and pregnancy diagnosis may improve the efficiency of reproductive consultations in clinical practice.

Conclusion

The present study demonstrated that exfoliative vaginal cytology is a practical and minimally invasive method for staging the estrous cycle and identifying the breeding period in bitches. The progressive change from parabasal and intermediate cells during proestrus to predominantly superficial cornified cells during estrus provided a useful cytological indicator for reproductive management. Pregnancy was confirmed in 84% of bitches bred following cytological identification of estrus, compared with 52% pregnancy positivity among bitches presented for verification after owner-reported breeding. Although these observations are not from a randomized controlled comparison, they support the clinical usefulness of EVC as an accessible adjunct for optimizing breeding management.

Acknowledgement

The authors wish to express special thanks to the Dean, School of Veterinary and Animal Sciences, and Centurion University of Technology and Management for providing the facilities to conduct the study and permission to publish the article.

Conflict of interest

The authors declare no conflict of interest in the conduct of this study.

References

- 1) Famakinde SA, Obafemi OM, Ajayi TA, Akinbote OR, Olanrewaju AO, Ola DB, Fakorede OO, Leigh OO, Salaudeen BA, Rabiou MB. 2025. Performance of pre-breeding cycle-staging techniques in bitches: Clinical comparability of vaginal cytology and progesterone assay. *Ovozoa: Journal of Animal Reproduction* 14(1): 1-7. doi: 10.20473/ovz.v14i1.2025.1-7.
- 2) Kumar P., Kumar A., Azad C.S., Sheetal S.K. 2023. Studies on different physical characteristics of mongrel dog semen. *Journal of Livestock Science* 14: 104-108 doi. 10.33259/JLivestSci.2023.104-108
- 3) Martínez-Mojica M, Guzmán A, Rosales-Torres AM, Herrera-Barragán JA, Castillo-Juárez H, Hernández-Coronado CG. 2023. Pregnancy success in bitches – evaluation of interactions between artificial insemination method, serum progesterone concentration and vaginal cytology parameters. *Acta Scientiae Veterinariae* 51: 1902. doi: 10.22456/1679-9216.127693.
- 4) Mason SJ. 2018. Current review of artificial insemination in dogs. *Veterinary Clinics of North America: Small Animal Practice* 48(4): 567-580. doi: 10.1016/j.cvsm.2018.02.005.
- 5) Nesa NLMS, Trilaksana IGNB, Puja IK. 2022. Vaginal cytology and receptivity to mating behaviour of Kintamani bitch during the proestrus to estrus phase. *International Journal of Veterinary Sciences and Animal Husbandry* 7(3): 38-41. doi: 10.22271/veterinary.2022.v7.i3a.420.
- 6) Reckers F, Klopffleisch R, Belik V, Arlt S. 2022. Canine vaginal cytology: A revised definition of exfoliated vaginal cells. *Frontiers in Veterinary Science* 9: 834031. doi: 10.3389/fvets.2022.834031.