RESEARCH ARTICLE

Behavioural and Physiochemical Dynamics of the Canine Estrous Cycle: A Tool for Enhancing Breeding Efficiency

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Abstract

Efficient canine breeding relies on accurate detection of the fertile window, particularly during proestrus and estrus. Over the last decade, the interest in high-quality pedigree dogs as pets has surged, driven by smaller family structures and increased awareness. As dog breeding has transformed into a blend of passion, artistry, and science, finding the optimal times for conception and breeding requires constant effort. The present study was conducted during July to December 2024 at Jaipur (Rajasthan) to evaluate the behavioural and physiochemical changes occurring throughout the estrous cycle in bitches to enhance their breeding efficiency. Eighteen healthy bitches, aged 2-4 years, were assessed for behavioural changes (like aggression, tendency to run away from home, frequent urination and flagging of tail, lordosis reflex) and physiochemical parameters (vulvar turgidity, edema, mucous membrane color, vaginal discharge, and vaginal pH). Tail deflection and lordosis emerged as the most consistent behavioural signs of estrus. Vulvar turgidity and edema peaked mid-cycle, while vaginal discharge and pH showed a decreasing trend toward ovulation. Though these external indicators offered valuable guidance, individual variability limited their reliability. Therefore, combining behavioural and physiochemical assessments provided a practical, low-cost recommendation for breeders and veterinarians to improve estrus detection and breeding efficiency, especially where hormonal assays are not accessible. This integrated method could significantly improve timing for natural mating or artificial insemination in domestic dogs.

Key words: Behavioural changes, Bitches, Estrous cycle, Physiochemical changes

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Introduction

The successful breeding of domestic dogs (*Canis familiaris*) is dependent on precise identification of the fertile window within the estrous cycle. Despite advancements in reproductive technologies and diagnostic tools, many breeding failures can still be attributed to misjudgment of the optimal time for mating. The knowledge on canine ovarian physiology and duration of the canine estrous cycle is considerably longer than one in the most of the other animals (Nagashima and Songsasen, 2021). Canine ovulation occurs approximately 44 h after LH surge. Moreover, the oocytes ovulated in canines are primary oocytes and undergo further development in the distal portion of the oviduct to form secondary oocytes before fertilization. Fertilization occurs 2 to 3 days after ovulation when the ova, which are ovulated, have completed meiotic divisions (Groppetti *et al.,* 2015; Sridevi, 2015; Kudalkar *et al.,* 2020). Understanding both the behaviour and physiochemical changes in the external genitalia including its secretions during the estrous cycles specially during proestrus and estrus phases become essential for breeders and veterinarians seeking to improve conception rates, reduce the need for repeated mating and optimize the use of assisted reproductive techniques (Romagnoli, 2006; Kustritz, 2012; Nagashima and Songsasen, 2021).

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The estrous cycle in the bitch is divided into four stages: proestrus, estrus, diestrus, and anestrus. Among these, proestrus and estrus are the most critical from a reproductive standpoint, as they encompass the period leading up to and including ovulation. During these stages, a complex interplay of endocrine signals, anatomical changes, and behaviour patterns occurs (Sharma *et al.,* 2022). The behavioural signs exhibited by female dogs during these phases include tail

flagging, lordosis reflex, standing for the male, increased urination and restlessness (Oluwatoyin *et al.,* 2012; Sharma *et al.,* 2022; Reckers *et al.,* 2022). However, the connection between hormonal changes and behaviours is often inconsistent and signs may not always align perfectly with internal hormonal changes, which can lead to mistimed mating. Physiochemical signs, on the other hand, such as vulvar swelling, vulvar turgidity, colour of vulvar mucus membrane, vaginal discharge and changes in vaginal pH, offer more reliable indicators of the dog's reproductive stage. During proestrus, vaginal bleeding, vulvar turgidity, swelling of the vulva, and serosanguinous discharge are commonly observed due to increased estrogen levels (Concannon, 2011; Kustritz, 2012; Jaller *et al.,* 2017; Maksimović *et al.,* 2025; McRae *et al.,* 2025). As the dog transitions into estrus, the vulva becomes softer, discharge becomes lighter in color, vulvar mucous membrane changes from deep pink in proestrus to very pale in anoestrus and standing behaviour typically occurs (Noakes *et al.,* 2009; Ese *et al.,* 2018; Skliarov *et al.,* 2022).

Measurement of vaginal pH has proven useful in tracking ovulation, as studies report a gradual pH decrease from proestrus to ovulation (Antonov *et al.,* 2014; Utomo *et al.,* 2023). Jayasudha *et al*. (2020) recorded significantly ($p<0.05$) lower pH (acidic) at the time of ovulation. Similar studies on vaginal pH were also recorded by Labib *et al.* (2018) in bitches, who attributed it to a progressive decrease in estrogen and gradual increase of progesterone levels. Given the individual variability among bitches, combining behavioural observation, vaginal cytology, and pH testing offers a more accurate and affordable method for timing mating or insemination-especially where hormonal tests are not feasible (Fay *et al.,* 2003; Romagnoli, 2006; Kustritz, 2012; Haji *et al.,* 2018). This study was focused on the behavioural and physiochemical changes in the canine estrous cycle and their practical implications, aiming to enhance breeding efficiency in domestic dogs.

Materials and Methods

The present study was conducted during July to December 2024 at Veterinary Clinical Complex and the Department of Veterinary Gynaecology and Obstetrics, PGIVER Jaipur (Rajasthan, India). The institute is located at 26°:9" North latitude and 75°: 46" East longitude at an average altitude of 1417 feet from sea level. The study was conducted on 18 healthy estrous bitches of different breeds, aged 2-4 years, with the history of pro-estrus bleeding which were presented to VCC, PGIVER Jaipur. History for onset of proestrual bleeding, behavioural signs exhibited by bitches like aggression, tendency to run away from home, frequent urination and flagging of tail, lordosis reflex, were documented. While performing physical examination of bitches, vagina was explored with gloved finger to rule out any vaginal abnormalities and evaluated the turgidity (Fig. 1) or flaccidity of the vulva, measurement of vulvar edema, the appearance of the mucous membrane, the nature of vaginal discharge (Fig. 2) and vaginal pH.

The degree of vulvar edema during the different stages of proestrus was assessed using a Vernier calipers for measurement (Fig. 3). The pH of vaginal discharge was measured using pH strip (HiMedia Laboratories Pvt. Ltd, Mumbai) within a range from 1 to 14 (Fig. 4). The pH paper was left in contact with the vaginal wall and the vaginal secretion for at least 30 seconds. The readings were recorded by comparing the changes in the color of pH strip with the appropriate table strip. The numerical data generated was analysed using statistical tools to derive mean and standard errors, and one-way ANOVA for meaningful differentiation of the results.

Results and Discussion

Behavioural Changes

Bitches in estrus exhibited characteristic behaviours, such as increased urination (61.11%, 11/18), tail deflection (77.77%, 14/18), lordosis reflex (66.66%, 12/18), restlessness (44.44%, 8/18), and, in some cases, aggression (72.22%, 13/18) or attempts to seek out males by escaping from their home (77.77%, 14/18). These signs were also reported by Romagnoli (2006), Oluwatoyin *et al.* (2012) and Ese *et al.* (2018).

Tail deflection and the lordosis reflex were among the most prominent signs, aligning with finding by Kudalkar *et al.* (2020), who recorded 91.66% and 83.33% occurrence, respectively. These findings supported the use of behavioural signs as practical indicators for determining the optimal breeding time, as previously emphasized by Fay *et al*. (2003) and Kustritz (2012). Nevertheless, due to occasional inconsistencies between behaviour and hormonal status, it remains advisable to complement behavioural observation with other diagnostic methods for precise breeding management.

Physiochemical Changes

Physiochemical changes observed during the proestrus and estrus phases of the estrous cycle in bitches during the current study were as under.

Colour of vulvar mucous membrane

The pink vulvar mucous membrane was found in 14 bitches (77.77%) and the congested in 4 bitches (22.22%) out of 18 bitches. The finding of the colour of vulvar mucous membrane in the present study agreed with the Ese *et al.* (2018), who noted that deep pink colour during the proestrus phase prepares the female dog's reproductive system for mating and pregnancy. An almost similar finding for the pink vulvar mucous membrane in 75% and congested mucous membrane in 25% of estrus bitches was observed by Kudalkar *et al.* (2020).

Fig. 1: Showing turgid vulva

Fig. 2: Showing serosanguinous discharge from the vagina

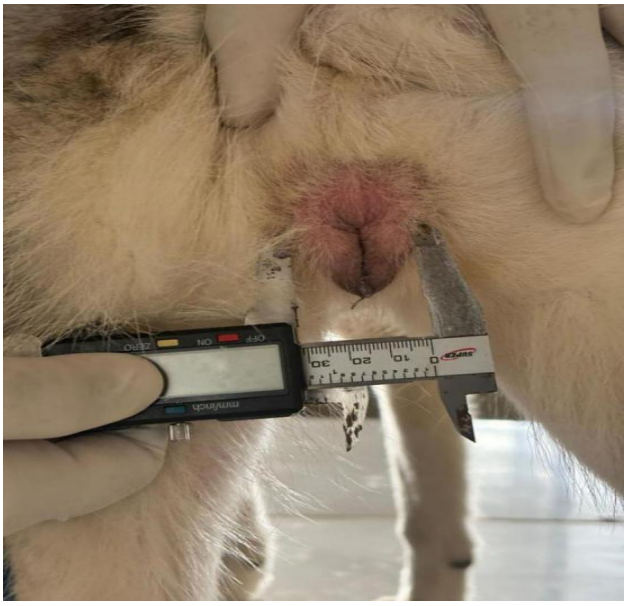

Fig. 3: Measurement of vulvar edema using Vernier caliper

Fig. 4: Estimation of vaginal pH

Evaluation of vulvar turgidity and edema

In this study, vulvar turgidity was scored as 1 (present) and 0 (absent). Mean scores on the 3rd, 6th, 9th day and on predicted ovulation day were 0.66 ± 0.096, 1.00 ± 0.00, 0.61 ± 0.055, and 0.22 ± 0.055, respectively, with the highest on day 6. Differences however were not statistically significant (p>0.05). These findings aligned with Concannon (2011) and Jaller *et al.* (2017), who found vulvar turgidity during estrus in 87.5% and 90% of bitches, respectively. A decrease in vulvar turgidity observed near ovulation agreed with Skliarov *et al.* (2022), who also noted vulvar softening during estrus. Kudalkar *et al.* (2020) reported less prominent signs, possibly due to breed variations.

The overall mean values of vulvar edema as measured by the vulva's size in inches during the proestrus phase on the 3rd, 6th, 9th day and on predicted day of ovulation were 0.88±0.074, 0.96±0.102, 1.29±0.050, and 1.18±0.052, respectively. The highest mean value was recorded on the 9th day. The edema did not differ significantly (p>0.05) between these days. Fay *et al.* (2003) and Kustritz (2012) explained the notable increase in the vulvar swelling during the proestrus

phase, However, we could not find such differences in the measurement of vulvar edema with regards to the increase in the vulvar swelling during the proestrus days.

Evaluation of vaginal discharge

In the present study, the presence and absence of serosanguinous discharge were graded as 1 and 0, respectively. The mean scores of serosanguinous discharge on the 3rd, 6th, 9th day of proestrus and predicted day of ovulation were 1.00±0.00, 0.67±0.00, 0.33±0.00, and 0.27±0.055, respectively. The highest mean score was observed on the 3rd day. A continuous decrease in serosanguinous discharge was noted from the 3rd day of proestrus to the day of ovulation. In the present finding, during the beginning of the proestrus phase escape of red blood cells from the inflamed endometrium through diapedesis, which is triggered by estrogen, was observed as a serosanguinous discharge, which corroborated with the finding of Goodman (2001), Feldman and Nelson (2004) and Maksimović *et al.* (2025). The same finding was also recorded by Jaller *et al.* (2017). The presence of serosanguinous discharge in this study was graded as 1 and 0 based on the presence and absence of discharge, whereas other workers (Oluwatoyin *et al.,* 2012; Fay *et al.,* 2003) rated the actual amount of vaginal discharge.

Vaginal pH

The mean vaginal pH values on the 3rd, 6th, 9th day of proestrus and day of ovulation were 7.48±0.087, 7.43±0.0455, 6.94±0.052, and 6.81±0.040, respectively. The highest mean value was recorded on the 3rd day. The vaginal pH did not differ significantly ($p>0.05$) across these days. The result was in line with the findings of Labib *et al.* (2018), who also noted the continuous drop in pH during the different days of the proestrus phase. This might be due to the changes in the concentration of reproductive hormones. However in cow, vaginal pH levels decreased during estrus and tended to be constant or stable during the diestrus phase (Fesseha and Degu, 2020). The pH level decreased from 7.0 to 6.72 the day before entering the estrus phase. At the beginning of the estrus phase, the pH level reached 6.54. Research of Layek *et al.* (2011) in cows showed the lowest pH level before ovulation, which was 6.45, whereas, Antonov *et al.* (2014) and Utomo *et al.* (2023) observed higher side pH value of vaginal fluids during the initial days of the proestrus. In the present study, vaginal pH did not differ significantly ($p>0.05$) in various stages of proestrus phase in bitches. On the contrary, Utomo *et al.* (2023) found that there were significant differences in pH at various stages such as proestrus, estrus, LH surge and ovulation. This may be presumed to be due to individual breed variations.

Conclusion

The findings of the present study showed distinct patterns across the estrous cycle in bitches for behavioural and physiochemical parameters like tail deflection, lordosis reflex, vulvar turgidity, edema, discharge, and vaginal pH. Tail deflection and lordosis were the most reliable behavioural signs of estrus. Vulvar turgidity and edema peaked mid-cycle, while discharge and pH declined as ovulation approached. Despite their usefulness in estrus detection, individual variation in expression highlighted the importance of combining these indicators with hormonal or diagnostic methods for precise breeding management.

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