REVIEW ARTICLE

THE ESTROUS CYCLE IN BITCHES: MECHANISMS, INFLUENCING FACTORS, AND CLINICAL RELEVANCE

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ABSTRACT

The estrous cycle of female dogs is a complex, hormonally regulated process comprising proestrus, estrus, diestrus, and anestrus. Its variability is influenced by breed-specific genetics, environmental factors, and microbial dynamics within the reproductive tract. Methods of estrus determination, including vaginal cytology, hormonal assays, ultrasonography, and thermography, remain integral to reproductive management, while emerging artificial intelligence-based approaches offer novel advancements in cycle prediction and monitoring. Despite extensive research on hormonal mechanisms, significant gaps remain in understanding the interplay between endocrine regulation, microbial colonization, and genetic predisposition in cycle variability. The use of hormonal contraceptives and gonadectomy introduces long-term physiological alterations, including chronic luteinizing hormone elevation, which has been linked to metabolic disorders, orthopedic complications, and neoplasia. Additionally, microbial influences on reproductive health, particularly the role of vaginal and uterine microbiota in fertility and disease susceptibility, require further investigation. The prolonged anestrus phase in canines poses a significant challenge for assisted reproductive technologies, limiting the efficacy of artificial insemination and embryo transfer. This review critically examines current methodologies for estrus cycle determination, explores factors contributing to interindividual variability, and evaluates the long-term implications of reproductive interventions on fertility and overall canine health.

Keywords: Female dogs, reproductive phases, reproductive health

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INTRODUCTION

The estrous cycle of female dogs, often interchangeably used with the term reproductive cycle, consists of four well-defined phases: proestrus, estrus, diestrus, and anestrus. Unlike the menstrual cycle in humans, the canine estrous cycle is characterized by distinct physiological and hormonal changes that regulate fertility and reproductive potential. Most bitches are monoestrous, physiologically entering estrus once or twice per year, with cycle length and frequency varying across breeds (Concannon, 2011). Small and toy breeds (e.g., Chihuahuas, Toy Poodles) cycle every 4–6 months, whereas large breeds (e.g., Mastiffs, Great Danes) cycle annually. Certain breeds, such as Basenjis and Tibetan Mastiffs, have ability to display seasonal estrus, typically in spring or autumn (Root Kustritz, 2012). Regulated by the hypothalamic-pituitary-gonadal axis, estrous transitions involve estrogen, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), influencing ovulation timing and fertility (as discussed in Kutzler, 2007). In addition to hormonal dynamics, vaginal and uterine microbiomes are important for reproductive health. It is well documented that the vagina harbors many bacterial species in contrast to the uterus. Dominant vaginal commensals include Staphylococcus spp., Streptococcus spp., and Escherichia coli (Maksimović et al., 2012). Vaginal mycoplasmas (Mycoplasma spp., Ureaplasma spp.) are also frequently detected and may contribute to subclinical infections, infertility, or pregnancy loss (Maksimović et al., 2018). The uterus of healthy bitches remains a sterile environment despite the consistent presence of bacteria in the vagina (Maksimović et al., 2012). However, hormonal fluctuations can predispose bitches to infections such as pyometra and vaginitis. Another major consideration regarding reproductive system health status is the decision to spay a female dog. Spaying has direct implications on the estrous cycle. Removing the ovaries eliminates the source of estrogen and progesterone, thereby halting the cycle entirely. Veterinarians often recommend early spaying of pet dogs that are not intended for breeding to prevent the inconvenience of heat/estrus periods and avoid reproductive health risks. Nevertheless, a recent study (Kutzler, 2020) highlights concerns regarding gonadectomy (spay/neuter) and its impact on luteinizing hormone (LH) and prolactin regulation. After removal of the gonads, the pituitary continues to secrete LH at high levels due to the loss of negative feedback. This chronic LH elevation may contribute to several long-term health effects in the bitch. Despite the primary hormonal mechanisms governing estrus are well understood, significant variability exists in the timing and duration of each phase across breeds. Further research is needed to elucidate the influence of environmental, genetic, and nutritional factors on these fluctuations (Yaeger and Fales-Williams, 2025). Reproductive technologies such as artificial insemination, embryo transfer, and superovulation remain less effective in dogs than in livestock due to challenges in estrus synchronization, largely attributed to the prolonged anestrus phase (McRae et al., 2025). It remains unclear how fluctuations in vaginal microbiota throughout the estrous cycle may impact fertility and reproductive health, though the extent of their influence on conception rates and pregnancy outcomes (Maksimović et al., 2012; Maksimović et al., 2018; Egyptien et al., 2024). The long-term effects of hormonal contraceptive-induced estrus suppression and gonadectomy warrant further investigation, as some evidence suggests potential impacts on ovarian function and fertility (Bawaskar, Lakde and Raghuwanshi, 2024). In this review, we address these gaps, their influence on the canine estrus cycle, breeding outcomes and long-term reproductive health.

Canine Estrous Cycle

Based on the annual frequency of estrous cycles, mammals are categorized as polyestrous, seasonally polyestrous, or monoestrous. Polyestrous species exhibit a continuous and evenly distributed estrous cycle throughout the year, while seasonally polyestrous species undergo estrous cycles only during specific times of the year (mostly spring, summer or autumn). Domestic dogs (*Canis lupus* *familiaris*) are classified as monoestrous, as they typically experience a single estrus per breeding season, which may occur at any point during the year. The canine estrous cycle is a hormonally regulated process comprising four distinct phases: proestrus, estrus, diestrus, and anestrus, each characterized by specific endocrine, physiological and behavioral changes (Feldman and Nelson, 2004; Concannon, 2011). Determining the phase of the estrus cycle is critical for breeding management, diagnosing reproductive disorders, and optimizing artificial insemination timing. Various techniques, including vaginal cytology, hormone assays, ultrasonography, and thermography, are employed to identify the specific cycle stage. Each method for determining the estrus cycle in dogs has its own advantages and limitations. Vaginal cytology is commonly used due to its affordability and accessibility, while hormonal assays provide more precise ovulation detection. Ultrasonography is useful for confirming ovulation and assessing ovarian health, and infrared thermography offers a non-invasive alternative. Artificial intelligence-based detection uses deep learning to analyze cytology images and accurately predict estrus cycles. It minimizes observer bias and can be integrated into mobile apps for field use, though it requires extensive data training and is still experimental (Rajan et al., 2024).

Vaginal cytology is a widely used method that evaluates the morphology of epithelial cells to determine the estrus cycle stage. Proestrus is characterized by intermediate and superficial cells, while estrus shows a predominance of cornified cells. Diestrus features a sharp decline in superficial cells with increased parabasal cells, and anestrus consists mostly of basal and parabasal cells (Kutzler, 2018). This method is cost-effective but requires expert interpretation. Progesterone and LH testing are gold standards for ovulation timing. A progesterone rise above 2 ng/mL signals ovulation, while the LH peak occurs 24-48 hours before ovulation (Meyers-Wallen, 2007). Though highly accurate, hormonal assays are costly and require laboratory analysis. Ultrasonography allows real-time assessment of ovarian structures. Proestrus presents with the small follicles, estrus shows fluid-filled follicles, and diestrus is marked by corpora lutea formation (Miller et al., 1992). It is a reliable but equipment-dependent method requiring trained personnel. Infrared thermography is a non-invasive method that detects temperature changes in the perineal region, correlating with estrus due to hormonal changes (Olğaç et al., 2017). However, environmental factors can impact accuracy, making it less reliable than hormonal assays.

Phases of Canine Estrus Cycle

Proestrus

Duration: 7–10 days (range: 3–17 days)

Key Characteristics

- Physical/clinical signs: Vulvar swelling, serosanguinous vaginal discharge, and increased attractiveness to males, although females refuse mating (Concannon, 2011).
- Hormonal changes: Rising estrogen levels from developing ovarian follicles stimulate epithelial proliferation in the uterus and vagina (as outlined by Feldman and Nelson, 2004). Estrogen peaks at the end of proestrus, triggering the preovulatory luteinizing hormone (LH) surge (as previously described by Concannon, 2009).
- Ovarian activity: Follicles growth, mature and reach a diameter of 6–10 mm before ovulation (Johnston et al., 2001).
- Vaginal cytology: Predominantly non-cornified epithelial cells and red blood cells (Kutzler, 2018; Root Kustritz, 2006).

Estrus

Duration: 5–9 days (range: 3–21 days)

Key Characteristics

- Behavioral receptivity: The female allows mating, indicated by a posture with an elevated pelvis and deviated tail (as outlined by Feldman and Nelson, 2004).
- Time of ovulation: Occurs 2–3 days after

the LH surge. Oocytes are immature at ovulation and require 48–72 hours for meiosis completion (postovulatory maturation) before fertilization (as previously described by Concannon, 2009).

- Hormonal changes: Estrogen declines sharply after the LH surge, while progesterone rapidly increases, reaching a peak of 15–90 ng/mL during diestrus (Feldman & Nelson, 2004).
- Vaginal cytology: The predominance of superficial cornified epithelial cells (>90%) anuclear or with picnotic nuclei indicates optimal timing for mating (Root Kustritz, 2006; Kutzler, 2007; Kutzler, 2018).

Diestrus

Duration: ~60 days (regardless of pregnancy status)

Key Characteristics

- End of sexual receptivity: The female no longer accepts the male. Vulvar swelling subsides, and serosanguinous discharge ceases. Diestrus is defined as the first day after estrus when the female refuses the male (Feldman and Nelson, 2004; Maksimović, 2010).
- Luteal phase: The corpus luteum secretes progesterone, maintaining levels >2 ng/mL for 30–60 days to support pregnancy (Feldman and Nelson, 2004).
- Progesterone decline: Levels drop to <1 ng/mL by day 60, triggering parturition in pregnant dogs or terminating pseudopregnancy (Concannon, 2009, 2011).
- Pseudopregnancy: Non-pregnant females exhibit a hormonal profile very similar to that of pregnant individuals and may display maternal behaviors (e.g., nest-building, mammary gland development, lactation) (Johnston et al., 2001).
- Health risks: Elevated progesterone increases the risk of pyometra, particularly in older females (Root Kustritz, 2006).
- Vaginal cytology: This phase is characterized by a marked decrease in cornified superficial cells and an increase in non-cornified parabasal and intermediate cells. Additionally, there is a notable influx of neutrophils during beginning of this stage. The abrupt decline in superficial cells, accompanied by the reappearance of parabasal cells and neutrophils, is a hallmark of the diestrual shift (Root Kustritz, 2006; Kutzler, 2018).

Anestrus

Duration: 3–4 months (longer in large and giant breeds)

Key Characteristics

- Reproductive inactivity: Anestrus is a phase of reproductive dormancy with no clinically observable changes or behavioral alterations. Although considered a resting phase, some studies suggest that the ovaries and pituitary gland remain active during this period (Maksimović, 2010).
- Hormonal quiescence: Ovarian inactivity, low estrogen levels (<15 pg/mL), and low progesterone levels (<1 ng/mL) (Johnston et al., 2001).
- Uterine recovery: The endometrium undergoes repair and involution (requires 85-90 days due to the type of placenta) during this phase (Feldman and Nelson, 2004).
- Breed variability: Smaller breeds (e.g., Chihuahuas) may cycle every 4–6 months, while larger breeds (e.g., Great Danes) often have intervals of 8–12 months (Root Kustritz, 2006).
- Vaginal cytology: Anestrus consists mostly of basal and parabasal cells (Root Kustritz, 2006; Kutzler, 2007; Kutzler, 2018).

Machine Learning and Artificial Intelligence-Based Estrous Cycle Prediction in Bitches

The application of machine learning (ML) and artificial intelligence in veterinary reproductive science is rapidly evolving. These advanced computational techniques aim to improve the accuracy and efficiency of estrous cycle detection in bitches, reducing human error and enabling real-time monitoring. Machine learning models can analyze vaginoscopic images, hormonal levels, and physiological indicators to predict different stages of the estrous cycle. The fundamental principle of ML-based estrous prediction is the ability of artificial intelligence systems to detect subtle reproductive changes that may not be easily identified by human observation of clinical signs (Rajan et al., 2024). Artificial intelligence and machine learning hold tremendous potential for revolutionizing estrous cycle prediction in bitches. By incorporating vaginoscopic imaging, hormonal analysis, and cytological evaluation, AI-driven models provide rapid, precise, and objective monitoring of reproductive cycles (Rajan et al., 2024). Although challenges such as data availability and computational costs persist, advancements in deep learning and mobile integration are poised to make artificial intelligence-assisted evaluation or detection, not limited only to estrus cycles, but also to become a standard tool in veterinary practice in the near future.

Algorithms and Methodologies

AI algorithms used for estrus detection often rely on a combination of sensor data, computer vision, and machine learning models. The primary approaches include Computer Vision and Deep Learning, Wearable and Sensor-Based Systems and Time-Series and Predictive Models.

Convolutional Neural Networks (CNNs) and machine vision systems are widely applied for image and video analysis to detect behavioral changes associated with estrus (Neethirajan, 2022). For instance, CNNs can process video feeds to identify increased mounting behavior in cattle or restlessness in dogs, which are common estrus indicators.

Wearable and Sensor-Based Systems - Wearable sensors, such as accelerometers and temperature monitors, are commonly integrated with AI algorithms to track physical activity, body temperature, and posture changes (Sharifuzzaman et al., 2024). These systems can provide continuous, real-time data, improving estrus detection accuracy.

Time-Series and Predictive Models - Recurrent Neural Networks (RNNs) and Long Short-Term Memory (LSTM) networks are also employed to analyze time-series data from sensors, capturing subtle patterns in movement and physiological changes over extended periods (Gulzar and Hussain, 2023).

Natural Language Processing (NLP) for Vocalization Analysis - AI models can analyze vocalizations in animals, such as dogs, which tend to increase during estrus. This less common but emerging approach leverages speech recognition algorithms adapted for animal vocal patterns (Burrai et al., 2024).

Multimodal Data Fusion - Recent studies focus on integrating multiple data sources, including video, sound, and biometric data, to enhance predictive accuracy. This approach reduces false positives and negatives by cross-referencing different estrus indicators (Mohebbi et al., 2025).

Limitations and Challenges

Despite their promise, AI-based estrus detection systems face several challenges. The setup for AI systems, including sensors, cameras, and computational infrastructure, can be prohibitively expensive for small-scale farms (Amer et al., 2024). AI models rely on vast amounts of high-quality data for training. Inconsistent or insufficient data can lead to poor model performance and unreliable predictions (Nair, 2024). Maintaining AI systems requires technical expertise, regular updates, and robust cybersecurity to protect sensitive animal data (Zhang et al., 2024). There are ongoing debates about animal privacy, the ethical implications of continuous monitoring, and the potential stress on animals due to wearable devices (Gupta, 2024).

AI systems can be sensitive to environmental conditions, which may affect their reliability. For instance, weather, barn design, and herd size can all impact sensor performance (Cho and Kim, 2023).

Hormonal Factors Influencing the Estrous Cycle

The regulation of the estrous cycle in dogs is controlled by a complex interplay of endocrine signals involving the hypothalamic-pituitary-gonadal (HPG) axis. The major hormones responsible for coordinating estrous transitions include gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen, progesterone, and prolactin (as previously described by Concannon, 2009). Hormones regulating estrus cycle in dogs are presented in Table 1.

Hypothalamic Control and Gonadotropin Regulation

The cycle begins with GnRH release from the hypothalamus, which stimulates the anterior pituitary to secrete LH and FSH. These gonadotropins promote follicular development and maturation as well as estrogen synthesis within the ovaries. LH surge is the key trigger for ovulation, occurring approximately 48 hours before follicular rupture and oocytes release. Studies have shown that LH concentrations exhibit pulsatile release during anestrus, but increase in frequency as proestrus begins, reaching peak values just before ovulation. FSH, while also involved in follicular recruitment, appears to play a more limited role in ovulation in dogs compared to other species. The FSH profile during the cycle remains relatively stable except for a minor peak at the beginning of proestrus (Concannon, 2009).

Estrogen and Progesterone Dynamics

Estrogen, primarily estradiol-17β, is synthesized by the granulosa cells of ovarian follicles in response to FSH and plays a crucial role in stimulating the behavioral and physiological signs of estrus. Estrogen levels rise throughout proestrus, peaking just before the LH surge, then decline sharply as ovulation occurs. High estrogen levels induce the characteristic cornification of vaginal epithelial cells, which is used clinically to determine estrous status through cytology (Concannon, 2009; Kutzler, 2007). Progesterone, produced by the corpora lutea after ovulation, maintains pregnancy and regulates cycle length. In the dog, unlike most mammals, progesterone secretion begins before ovulation, concurrent with the LH surge (Concannon, 2009). Serum progesterone concentrations increase significantly after ovulation, reaching peak levels during diestrus, and decline gradually unless pregnancy occurs (Hinderer et al., 2021). Progesterone's early rise makes it a valuable marker for ovulation timing in breeding management (Concannon, 2009).

Prolactin and Luteal Function

Prolactin, a pituitary hormone, plays an essential role in luteal maintenance in the dog. Unlike many species where luteal support depends on LH, prolactin becomes the primary luteotropic hormone after 20 days of estrus (Concannon, 2009). Experimental suppression of prolactin via dopamine agonists (e.g., cabergoline) leads to premature luteolysis and shortened diestrus, which is why prolactin inhibitors are commonly used for estrus induction in bitches with prolonged anestrus (as discussed in Kutzler, 2007).

Influence of Thyroid Hormones on Estrous Regulation

There is increasing evidence that thyroid dysfunction affects estrous cycling in bitches. Hypothyroidism, a common endocrine disorder in dogs, has been associated with prolonged anestrus, irregular cycles, and reduced fertility. The mechanism is likely linked to thyroid hormones' regulatory role in metabolic and reproductive function, including ovarian steroidogenesis (Hinderer et al., 2021).

Role of Cortisol and Stress in Cycle Disruptions

Elevated cortisol, a marker of stress, has been shown to interfere with pulse frequency of GnRH and LH secretion, leading to suppressed estrous cycles. Chronic stress and high glucocorticoid levels may prolong anestrus or prevent normal follicular development, highlighting the importance of minimizing stressors in breeding animals (Calabrò et al., 2021).

Estrous Cycle Variability - Hormonal, Microbial, Genetic, and Environmental Factors

The domestic female canine is a non-seasonal, monoestrous species with an interestrus interval averaging about 7 months (31 weeks), but with high individual variability (range ~4–13 months) (Concannon, 2009). This variation arises largely from differences in the length of anestrus, which have a genetic basis (Kutzler, 2007). Some bitches have consistent cycle intervals (e.g. every 6–7 months), whereas others exhibit irregular timing over their lifetime. At least one breed (the Basenji) has retained a seasonal, photoperiod-regulated cycle, typically coming into heat only once annually in the autumn (Concannon, 2009). In most breeds, however, photoperiod is not overtly entrained to the cycle, though an intrinsic "free-running" circannual rhythm is suspected to influence cycling. Environmental cues can still play a role – for example, housing intact females together can sometimes lead to synchronized or altered cycle timing (towards dominate alpha female), suggesting pheromonal stimulation among them (Concannon, 2009). Extreme stress or poor body condition may also delay or disrupt estrous cycling, while optimal nutrition supports regular cycles (Calabrò et al., 2021). Notably, the uterine lining requires ~135 days post-estrus for complete histologic repair, so attempting to induce a new cycle too soon can reduce fertility (Kutzler, 2007). Thus, both innate hormonal rhythms and external factors (season for certain breeds, pheromones, stress, nutrition) contribute to estrous cycle variability in dogs.

Microbial Factors

In healthy bitches, the reproductive tract harbors a resident microbiome throughout the cycle (Maksimović et al, 2012; Lyman et al., 2019). Disruptions or infections in this microbial ecosystem can impair fertility. Certain pathogens are recognised as significant contributors to infertility in dogs. Among these, Brucella canis, a zoonotic bacterium, is particularly notable for causing abortions and persistent reproductive failure (Graham and Taylor, 2012). In contrast to opportunistic bacteria, *B. canis* stands out as a notable primary pathogen of canine infertility (Graham and Taylor, 2012). Routine screening for *B. canis* and prompt treatment of uterine/vaginal infections are, therefore, essential in breeding animals (Pretzer, 2008). While *Neospora caninum* is a well-known pathogen in cattle, its impact on canine reproductive health should not be overlooked. Vertical transmission, pregnancy complications, and the risk of congenital infections highlight the importance of monitoring and preventive measures in breeding dogs (Dubey 2003; Dubey et al., 2007). Maksimović et al. (2012) investigated the vaginal and uterine bacterial flora in clinically healthy bitches during different stages of their reproductive cycle. The most identified bacteria were *Streptococcus spp*., coagulase-negative *Staphylococcus spp*., and *Escherichia coli*. The study found that bacteria were isolated from 77.5% of vaginal swabs, with isolates more frequently detected in pure culture than in mixed cultures. It was previously proposed that vaginal microbiome is usually mixed in clinically healthy bitches, and the isolation of bacteria in pure culture suggests pathogens have overgrown normal flora (Olson et al., 1986; van Duijkeren, 1992). Maksimović et al. (2012) observed that bacterial isolates were more frequently obtained in pure culture during proestrus and anestrus, whereas mixed bacterial cultures were predominantly detected during estrus and diestrus. These findings suggest that the isolation of a pure bacterial culture does not inherently indicate an infectious process but may instead reflect normal variations in the vaginal microbiota across different stages of the estrous cycle. Notably, all uterine samples tested in the study were negative for bacterial growth, reinforcing the notion that the uterus of healthy bitches remains a sterile despite the consistent presence of bacteria in the vagina. In contrast, Paudel et al. (2023) focused on bitches diagnosed with pyometra, a common reproductive disorder characterized by uterine infection. The study revealed that *E. coli* was the most prevalent pathogen, isolated in 35.55% of cases, followed by *Pseudomonas spp*. (26.66%). More recently, Xavier et al. (2024) conducted a study analyzing clinical data, histopathological alterations, and microbiological findings in dogs with pyometra. The study revealed that *E. coli*, especially strains belonging to phylogroup B2, were the most common isolates. These strains harbored virulence genes associated with adhesion (fimH, focG, and papC) and serum resistance (traT). Notably, the presence of the papC gene was linked to higher necrosis scores in the endometrium. These findings suggest that papC-positive *E. coli* strains play a significant role in the severity of pyometra in dogs. The identification of specific virulence factors, such as papC, in *E. coli* isolates from pyometra cases underscores the importance of targeted therapeutic strategies. Recognizing the association between these virulence genes and disease severity can aid veterinarians in prognostic assessments and in tailoring more effective treatment plans for affected dogs.

Hormonal Factors

Proper endocrine function underlies normal cycling, coordinated rise and fall of progesterone, estrogen, LH, FSH, and prolactin drive cycle transitions (Concannon, 2009). Even subtle hormonal imbalances (e.g. hypothyroidism or luteal insufficiency) can lengthen interestrus period or cause pregnancy failure. For example, inadequate progesterone support in pregnancy (hypoluteoidism) leads to embrionic death and resorption or abortion if unrecognized (Hinderer et al., 2021).

Genetic Factors

Influence the onset and frequency of reproductive cycles in dogs. Breed and lineage differences play a significant role. Smaller breeds typically reach puberty earlier and cycle more frequently, exhibiting two or even three heats annually. In contrast, giant breeds or specific lineages may cycle only once per year. Research indicates that within the same litter, some bitches consistently experience longer anestrus phases than others, suggesting heritable variations in cycle length. Bitches with unusually long interestrus intervals naturally have fewer breeding opportunities and may display lower lifetime fertility. Conversely, very short interestrus intervals (less than approximately 4-5 months) can lead to uterine disease due to insufficient post-estrus involution, reducing fertility if breeding occurs too frequently (Kutzler, 2007).

Environmental Factors

Just like photoperiod, pheromones, management, and nutrition are crucial. A study found that improving the diet of breeding bitches with more protein sources, essential fatty acids, and various vitamins for two months before estrus led to better estrus presentation and early pregnancy outcomes (Calabrò et al., 2021). This highlights the importance of balanced nutrition and health in cycle regularity and fertility.

Table 1 Hormones involved in the estrus cycle of dogs

Hormone	Origin	Function	Level During Estrus Stages
Estrogen	Ovaries (Graafian follicles)	Stimulates the development of follicles in the ovaries, promotes sexual receptivity, and triggers physical signs of estrus (e.g., vulvar swelling, bloody discharge).	Proestrus: High, rising to peak; Estrus: Peaks then drops sharply; Diestrus: Low; Anestrus: Low

Hormone	Origin	Function	Level During Estrus Stages
Progesterone	Ovaries (Corpus luteum)	Maintains pregnancy, supports the development of the endometrium, and inhibits further estrous cycles.	Proestrus: Low; Estrus: Starts to rise; Diestrus: High, peaking mid-diestrus; Anestrus: Low
Luteinizing Hormone (LH)	Pituitary gland (anterior lobe)	Triggers ovulation and formation of the Corpus luteum, critical for the release of the mature egg.	Proestrus: Low; Estrus: Peaks sharply around the time of ovulation; Diestrus: Low; Anestrus: Low
Follicle-Stimulating Hormone (FSH)	Pituitary gland (anterior lobe)	Stimulates the growth and maturation of ovarian follicles and increases estrogen production.	Proestrus: Moderate, supporting follicle growth; Estrus: Drops just before ovulation; Diestrus: Low; Anestrus: Low
Prolactin	Pituitary gland (anterior lobe)	Supports milk production and maternal behaviors, also plays a role in maintaining the Corpus luteum in some species.	Proestrus: Low; Estrus: Low; Diestrus: Moderate to high (especially in pregnant dogs); Anestrus: Low
Oxytocin	Pituitary gland (posterior lobe)	Stimulates uterine contractions during labor and milk letdown during nursing.	Proestrus: Low; Estrus: Low; Diestrus: Low; Anestrus: Low; Parturition: High

Long-Term Effects of Hormonal Contraceptives and Gonadectomy on Reproductive Health

Recent research of Kutzler (2020) highlights concerns regarding gonadectomy (spay/neuter) and its impact on luteinizing hormone (LH) regulation. After removal of the gonads, the pituitary continues to secrete LH at high levels due to the loss of negative feedback. This chronic LH elevation may contribute to several long-term health effects, including:

- Urinary incontinence: Higher LH levels post-gonadectomy are associated with estrogen-sensitive urinary sphincter incompetence, particularly in female dogs.
- Orthopedic disorders: Persistently elevated LH may affect joint development, increasing the risk of cranial cruciate ligament rupture and hip dysplasia.
- Obesity and metabolic changes: LH elevation may contribute to altered metabolism and weight gain in neutered animals.
- Neoplasia: Hormone-sensitive cancers such as lymphoma, hemangiosarcoma, and mast cell tumors have been linked to early-age gonadectomy, possibly due to prolonged LH stimulation of non-gonadal tissues.

Given these potential risks, Kutzler (2020) suggests that veterinarians and breeders consider alternative sterilization methods, such as ovary-sparing spay (hysterectomy without ovary removal) or vasectomy in males. These techniques prevent reproduction while preserving natural hormone regulation, mitigating some of the negative effects linked to LH hypersecretion. Integrating these findings into reproductive management strategies can help optimize canine health while maintaining effective population control.

CONCLUSION

The estrous cycle in female dogs consists of four hormonally regulated phases: proestrus, estrus, diestrus, and anestrus. Cycle variability is influenced by genetic, environmental, microbial, and endocrine factors. While traditional methods such as vaginal cytology and hormonal assays remain essential for estrus detection, artificial intelligence-based approaches offer promising advancements in cycle prediction.

Despite extensive research on hormonal regulation, gaps remain in understanding the interplay between endocrine function, microbial colonization, and genetic predisposition. The vaginal and uterine microbiome significantly impact reproductive health, with pathogens like *Escherichia coli* and *Brucella canis* linked to infertility and disorders, such as pyometra.

The prolonged anestrus phase complicates assisted reproductive technologies, limiting the success of artificial insemination and embryo transfer. Additionally, hormonal contraceptives and gonadectomy result in chronic luteinizing hormone elevation, increasing the risk of metabolic disorders, orthopedic issues, and hormone-sensitive neoplasia. Alternative sterilization methods, such as ovary-sparing spay, may help mitigate these risks.

Further research is needed to clarify the complex interactions between endocrine regulation, microbial dynamics, and genetic factors influencing cycle variability. Advancements in artificial intelligence-driven estrus detection and reproductive management strategies will be key to optimizing fertility and overall canine health.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept and Design – AM, BP, BČ; Supervision – AM, BČ; Literature review, analysis and interpretation of data - AM, SF, BP,BČ; Writing and Critical review - AM, SF, BP, BČ

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ESTRUSNI CIKLUS KOD KUJA: MEHANIZMI, FAKTORI UTJECAJA I KLINIČKI ZNAČAJ

SAŽETAK

Estrusni ciklus ženki pasa je složen, hormonalno reguliran proces koji obuhvata proestrus, estrus, diestrus i anestrus. Njegova varijabilnost je pod utjecajem genetskih faktora pasmine, sredine i mikrobne dinamike reproduktivnog trakta. Metode otkrivanja estrusa, uključujući vaginalnu citologiju, hormonske eseje, ultrazvuk i termografiju i dalje predstavljaju osnove upravljanja reproduktivnim zdravljem, dok noviji pristupi zasnovani na vještačkoj inteligenciji omogućavaju napredak u polju predviđanja ciklusa i praćenja. Uprkos opsežnom istraživanju hormonskih mehanizama, postoji značajan raskorak u razumijevanju odnosa između endokrine regulacije, mikrobne kolonizacije i genetske predispozicije u varijabilnosti ciklusa. Upotreba hormonalnih kontraceptiva i gonadektomija izazivaju dugotrajne fiziološke promjene, uključujući porast luteinizirajućeg hormona povezanog s metaboličkim poremećajima, ortopedskim komplikacijama i neoplazijama. Pored toga, utjecaji mikroba na reproduktivno zdravlje, posebno ulogu vaginalnog i uterinog mikrobioma na fertilnost i prijemčivost za bolest, zahtjevaju daljnja istraživanja. Prolongirana faza anestrusa kod pasa predstavlja značajan izazov za tehnologije potpomognute oplodnje ograničavajući efikasnost vještačke oplodnje i embriotransfera. Ovaj pregled daje kritički osvrt na postojeće tehnologije otkrivanja estrusnog ciklusa, razmatra faktore koji utječu na interindividualnu varijabilnost i evaluira dugotrajne implikacije reproduktivnih intervencija na fertilnost i cjelokupno zdravlje pasa.

Ključne riječi: Reproduktivne faze, reproduktivno zdravlje, ženke pasa