



Ветеринарне благополуччя та якість продукції тваринництва

Veterinary welfare and quality of livestock products

DOI 10.32900/2312-8402-2025-133-162-174

UDC 636.71:612.6:591.18

ANALYSIS OF INDIVIDUAL DYNAMICS OF PROGESTERONE LEVELS IN FEMALE DOGS DEPENDING ON AGE, BODY CONDITION, AND TEMPERAMENT

Olga BOBRYTSKA, Doctor of Veterinary Sciences, Professor,

<https://orcid.org/0000-0002-5368-8094>

Valeria FORKUN, PhD student

<https://orcid.org/0009-0000-4810-4114>

Larisa VODOPIANOVA, Candidate of Biological Sciences, Associate
Professor

<https://orcid.org/0000-0002-9331-1689>

Iryna ZHUKOVA, Doctor of Veterinary Sciences, Professor,

<https://orcid.org/0000-0003-4488-3899>

State Biotechnological University, Kharkiv, Ukraine

*The aim of this study was to examine the influence of individual characteristics of female dogs (*Canis familiaris*) of the Bull Terrier breed on the dynamics of progesterone levels in their blood throughout the estrous cycle. The experiment involved 25 clinically healthy female dogs divided into three age groups: young (1.4 years), middle-aged (4.8 years), and older dogs (8.6 years). The health status of the animals was assessed through clinical examinations and laboratory analyses, including the determination of reproductive status using folliculometry, vaginal cytology, and blood progesterone level analysis. Blood samples were collected from the jugular vein during key periods of the estrous cycle and analyzed using an enzyme-linked immunosorbent assay ("Progesterone – ELISA," HEMA, Ukraine). Additionally, the impact of body condition (evaluated using the BCS scale) and temperament (assessed using the C-BARQ questionnaire) on hormonal status was investigated. To this end, 12 groups of female dogs were formed according to behavioral characteristics (aggression, fear and anxiety, excitability, learning ability, and obedience). All experimental procedures complied with the requirements of the Ukrainian Law "On the Protection of Animals from Cruelty" and the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. The results of two-factor ANOVA revealed that, regardless of age, body weight, or temperament, the stage of the estrous cycle was the determining factor influencing blood progesterone levels ($F=79.8-173.0$; $P<0.001$). Among individual characteristics, body weight had the greatest impact ($F=16.3$; $P<0.001$), while age differences showed a statistically significant but less pronounced effect ($F=3.65$; $P<0.05$). The temperament of female dogs also significantly affected progesterone levels, particularly in terms of aggression ($F=10.68$; $P<0.001$), fear and*



anxiety ($F=9.21$; $P<0.001$), and excitability ($F=7.6$; $P<0.001$). In contrast, learning ability and obedience did not show a significant effect on hormonal status ($F=0.91$; $P=0.41$). Analysis of interaction effects revealed a significant relationship between body weight, behavioral characteristics (aggression, fear), and the stage of the estrous cycle ($F=2.24-2.92$; $P<0.005-0.001$). This indicates that individual physiological and behavioral traits influence the endocrine regulation of reproductive function in female dogs. The findings expand the understanding of regulatory mechanisms in the estrous cycle of dogs and emphasize the need to consider individual characteristics when planning veterinary interventions and breeding programs.

Keywords: progesterone, estrous cycle, dogs, body condition, temperament, reproductive function, hormonal regulation.

АНАЛІЗ ІНДИВІДУАЛЬНОЇ ДИНАМІКИ РІВНЯ ПРОГЕСТЕРОНУ У СУК ЗАЛЕЖНО ВІД ВІКУ, СТАНУ ТІЛА ТА ТЕМПЕРАМЕНТУ

Ольга БОБРИЦЬКА, доктор ветеринарних наук, професор,
<https://orcid.org/0000-0002-5368-8094>

Валерія ФОРКУН, аспірантка
<https://orcid.org/0009-0000-4810-4114>

Лариса ВОДОП'ЯНОВА, кандидат біологічних наук, доцент
<https://orcid.org/0000-0002-9331-1689>

Ірина ЖУКОВА, доктор ветеринарних наук, професор,
<https://orcid.org/0000-0003-4488-3899>

Державний біотехнологічний університет, Харків, Україна

*Метою цього дослідження було вивчення впливу індивідуальних характеристик сук (*Canis familiaris*) породи бультер'єр на динаміку рівня прогестерону в їх крові протягом естрального циклу. В експерименті брали участь 25 клінічно здорових собак, розділених на три вікові групи: молоді (1,4 року), середнього віку (4,8 року) та старші (8,6 року). Стан здоров'я тварин оцінювали за допомогою клінічних та лабораторних досліджень, включаючи визначення репродуктивного статусу за допомогою фолікулометрії, вагінальної цитології та аналізу рівня прогестерону в крові. Зразки крові брали з яремної вени в ключові періоди естрального циклу та аналізували за допомогою імуноферментного аналізу («Прогестерон – ІФА», НЕМА, Україна). Додатково досліджували вплив стану тіла (оцінюваного за шкалою BCS) та темпераменту (оцінюваного за допомогою опитувальника C-BARQ) на гормональний статус сук. Для цього було сформовано 12 груп тварин за поведінковими характеристиками (агресія, страх і тривога, збудливість, здатність до навчання та слухняність). Усі експериментальні процедури відповідали вимогам Закону України «Про захист тварин від жорстокого поводження» та принципам Європейської конвенції про захист хребетних тварин, що використовуються в експериментальних та наукових цілях. Результати двофакторного дисперсійного аналізу (ANOVA) показали, що незалежно від віку, маси тіла чи темпераменту, стадія естрального циклу була визначальним фактором, що впливав на рівень прогестерону в крові ($F=79,8-173,0$; $P<0,001$). Серед індивідуальних характеристик найбільший вплив мала маса тіла ($F=16,3$; $P<0,001$), тоді як вікові відмінності показали*



статистично значущий, але менш виражений вплив ($F=3,65$; $P<0,05$). Темперамент сук також суттєво впливав на рівень прогестерону, зокрема, щодо агресії ($F=10,68$; $P<0,001$), страху та тривоги ($F=9,21$; $P<0,001$) та збудливості ($F=7,6$; $P<0,001$). Натомість, здатність до навчання та слухняність не виявили значного впливу на гормональний статус ($F=0,91$; $P=0,41$). Аналіз ефектів взаємодії виявив значний зв'язок між масою тіла, поведінковими характеристиками (агресія, страх) та стадією естрального циклу ($F=2,24-2,92$; $P<0,005-0,001$). Це вказує на те, що індивідуальні фізіологічні та поведінкові риси впливають на ендокринну регуляцію репродуктивної функції у сук. Отримані результати розширюють розуміння регуляторних механізмів естрального циклу собак та підкреслюють необхідність врахування індивідуальних характеристик під час планування ветеринарних втручань та програм розведення.

Ключові слова: прогестерон, естральний цикл, собаки, стан тіла, темперамент, репродуктивна функція, гормональна регуляція.

Introduction. The reproductive function of dogs is a complex process that depends on numerous factors such as physiological condition, age, breed, temperament, and body condition. The reproductive cycles of dogs, in terms of physiological neurohumoral regulatory mechanisms, are unique among domestic animal species. To date, many physiological and clinical questions concerning the regulation of the estrous cycle in dogs remain unresolved (Concannon, 2009; Martin et al., 2009). Existing information on the individual characteristics of estrous cycle regulation in female dogs is fragmented and ambiguous. In particular, data on the impact of age, body condition, and temperament on the dynamics of sex hormones in the blood of Bull Terrier females are lacking. A better understanding of the mechanisms involved in the establishment and maintenance of pregnancy may be useful for elucidating the pathogenesis of some common disorders and contribute to the development of better clinical protocols, ensuring an individualized approach for each patient (de Carvalho Papa & Kowalewski, 2020).

The Corpus Luteum is a temporary endocrine gland formed after ovulation in the ovary at the site of the Graafian follicle. The corpus luteum produces the hormone progesterone, and its name derives from the characteristic yellow color of its content. In dogs, the corpus luteum (CL) is the sole source of progesterone (P4) and estradiol (E2) during diestrus (Papa & Hoffmann, 2011). The functional duration of the CL is determined by endocrine, paracrine, and autocrine factors (Sousa et al., 2016). Local growth factors, cytokines, and prostaglandins modulate CL function, creating a balance that leads to luteal regression in non-pregnant dogs or luteolysis during pregnancy (Mariusz P. Kowalewski, 2014). In pregnant dogs, the trophoblast acts as the fetomaternal compartment responsible for synthesizing prostaglandin F2 α (PGF2a), which actively participates in prepartum luteolysis (Mariusz Pawel Kowalewski et al., 2010). Progesterone (P4) is a steroid hormone responsible for preparing the endometrium for implantation of the fertilized egg and for maintaining pregnancy. After implantation, the corpus luteum continues to produce progesterone during the early stages of pregnancy until the placenta develops and takes over progesterone production for the remainder of gestation (Holesh, Bass, & Lord, 2023). Progesterone plays a pivotal role in fertility. The expression and activation of the progesterone receptor (PGR) are essential for ovulation (Park et al., 2020). Additionally, progesterone is critical for preparing the uterine environment for implantation, embryonic development, and regulation of the estrous cycle (Pereira, Mainigi, & Strauss III, 2021).

Progesterone levels are widely used as a clinical biomarker in the reproductive management of dogs (Conley, Gonzales, Erb, & Christensen, 2023). The variability in



circulating P4 levels among dogs is associated with the number of ovulations and corpora lutea (Concannon, Butler, Hansel, Knight, & Hamilton, 1978). In many species, plasma progesterone concentrations increase with the rate of ovulation and the number of CL (Knox, Vatzias, Naber, & Zimmerman, 2003). Adult dogs exhibit higher efficiency in P4 synthesis compared to younger ones, indicating that luteal endocrine activity undergoes maturation as dogs transition from youth to adulthood (Marinelli, Rota, Carnier, Da Dalt, & Gabai, 2009). Beyond its primary function, progesterone also belongs to the group of neurosteroids. It is metabolized in all regions of the central nervous system (Hanukoglu, Karavolas, & Goy, 1977) and possesses neuromodulatory, neuroprotective, and neurogenic properties (Schumacher et al., 2004). These effects are mediated through interactions with non-nuclear progesterone receptors such as mPR and PGRMC1, as well as other receptors like $\sigma 1$ and nACh (Singh, Su, & Ng, 2013).

Unlike other domestic animals, the reproductive system of female dogs is characterized by the absence of increased estrogen levels during pregnancy and before parturition (Concannon, 2011), while luteal regression occurs despite elevated pituitary hormone levels (Concannon, 2009). Elevated progesterone levels are also observed during pseudopregnancy (Feldman, Nelson, Reusch, & Scott-Moncrieff, 2014). Furthermore, significant individual variations in sex hormone levels have been observed in female dogs (Concannon, Castracane, Temple, & Montanez, 2009). Previous studies have indicated considerable variation in progesterone levels depending on the breed and physiological condition of dogs. For instance, Luz (2006) demonstrated significant individual fluctuations in P4 levels in the plasma of mixed-breed dogs, whereas these variations were less pronounced in Beagle females. Moreover, the impact of age, body weight, and temperament on reproductive efficiency has also attracted researchers' attention; however, the consistency of data in these aspects remains insufficient.

This study aimed to determine the effects and differences in progesterone dynamics in the plasma of female dogs depending on their individual characteristics such as age, body weight, and temperament. The objective of the research was to identify key factors influencing progesterone levels in female dogs and their interactions, which could improve the understanding of hormonal regulation mechanisms and provide a scientific basis for optimizing canine breeding programs.

Materials and methods. The experiment was conducted on 25 female dogs (*Canis familiaris*) of the Bull Terrier breed of various ages, body conditions, and temperaments. At the time of the study, all animals were clinically healthy, without signs of infectious or parasitic diseases. Health status was assessed through clinical examinations and laboratory analyses. To evaluate the humoral component of the reproductive status of the females, the periods of fertility were determined using folliculometry, vaginal cytology, and analysis of progesterone levels in the blood. Blood samples were collected from the jugular vein during the following time points: -3, -1, 0, 2, 4, 9, 23-30, 35-40, 55-60, and 120-150 days after the luteinizing hormone (LH) surge. The progesterone content in plasma was measured using the "Progesterone – ELISA" kit (HEMA, Ukraine) with a universal microplate reader ELx800 (Bio-Tek Instruments, USA).

To assess the effect of age on blood progesterone levels, all female dogs were divided into three age groups:

- Middle-aged animals: average age 4.8 years (range 3.5 to 6.2 years);
- Young animals: average age 1.4 years (range 15 to 18 months);
- Older animals: average age 8.6 years (range 8 to 9 years).

Body condition was evaluated at the start of the experiment using the Body Condition Score (BCS) scale. This scale is a widely recognized tool for assessing body



condition, fat accumulation, and nutritional status, with a range from 1 (emaciated) to 9 (morbidly obese). An ideal body condition score typically ranges from 4 to 5, reflecting optimal physiological levels of muscle and fat (Pennsylvania, 2023).

Temperament traits were assessed at the planning stage of the experiment using the standardized C-BARQ (Canine Behavioral Assessment & Research Questionnaire) method. This instrument, developed at the University of Pennsylvania, is widely used to evaluate the behavioral characteristics of dogs. Four main criteria were selected for assessment: aggression, fear and anxiety, excitability, and learning and obedience. Owners were surveyed using the standardized C-BARQ questionnaire (Serpell, 2015). For the experiment, 12 groups of dogs (5 females in each) were formed based on temperament traits. The same dog could belong to multiple groups depending on its behavioral characteristics. Materials, sampling schemes, and analyses corresponded to previous experimental protocols.

The obtained results of progesterone levels in the blood of female dogs of different ages, body conditions, and temperaments were statistically analyzed using two-factor analysis of variance with replication via the "Data Analysis" tool in Microsoft Excel 2019.

The experiment was conducted in compliance with the requirements of the Law of Ukraine No. 3447-IV of February 21, 2006, "On the Protection of Animals from Cruelty," as well as the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986).

Research results. The dynamics of progesterone (P4) levels in the blood of female dogs throughout their estrous cycle exhibit significant fluctuations, which are characterized by distinct changes depending on the phase of the cycle. During the Proestrus phase, an initial increase in progesterone levels is observed. On day -3, the hormone level was 0.61 ng/ml, rising to 1.40 ng/ml on day -1, which indicates a 129.5% increase. This reflects the body's preparation for the next phase, accompanied by the gradual activation of luteal cells.

In the Estrus phase, progesterone levels rise sharply. On day 0, the hormone concentration reached 5.87 ng/ml, which is a 319% increase compared to the previous day. This period is characterized by the highest hormonal activity, preparing the body for ovulation and potential fertilization. On the second day, progesterone levels continued to increase, reaching 6.90 ng/ml, indicating a 17.6% rise. In the following days (4th and 9th), progesterone levels rose further to 8.65 ng/ml and 9.49 ng/ml, respectively, highlighting the gradual achievement of the hormonal peak in this phase.

The Diestrus phase is characterized by the highest progesterone levels, reaching a maximum of 33.36 ng/ml on days 30-40. Compared to day 9, this represents a 251.4% increase, indicating the active function of the corpus luteum, which supports the luteal phase and potential pregnancy. However, by days 55-60, progesterone levels began to decline to 16.11 ng/ml, demonstrating a 51.7% reduction. This indicates the conclusion of the luteal phase and the body's preparation for a period of rest.

During the Anestrus phase, which is the phase of reproductive quiescence, progesterone levels drop to minimal values. On days 120-150, the hormone level was 0.30 ng/ml, representing a 98.1% decrease compared to the peak observed in the Diestrus phase. Such dynamics indicate the completion of the estrous cycle and the inactivity of the reproductive system during this period.

Thus, the dynamics of progesterone levels in Bull Terrier females reflect the complex physiological processes accompanying the estrous cycle. The maximum progesterone values in the Diestrus phase underscore the importance of this hormone for maintaining the luteal phase and pregnancy. Conversely, the minimal values in the Anestrus phase signify the conclusion of hormonal activity and the body's return to a state



of rest. These findings have significant practical implications for veterinary reproductive medicine, particularly for breeding planning and diagnosing hormonal disorders.

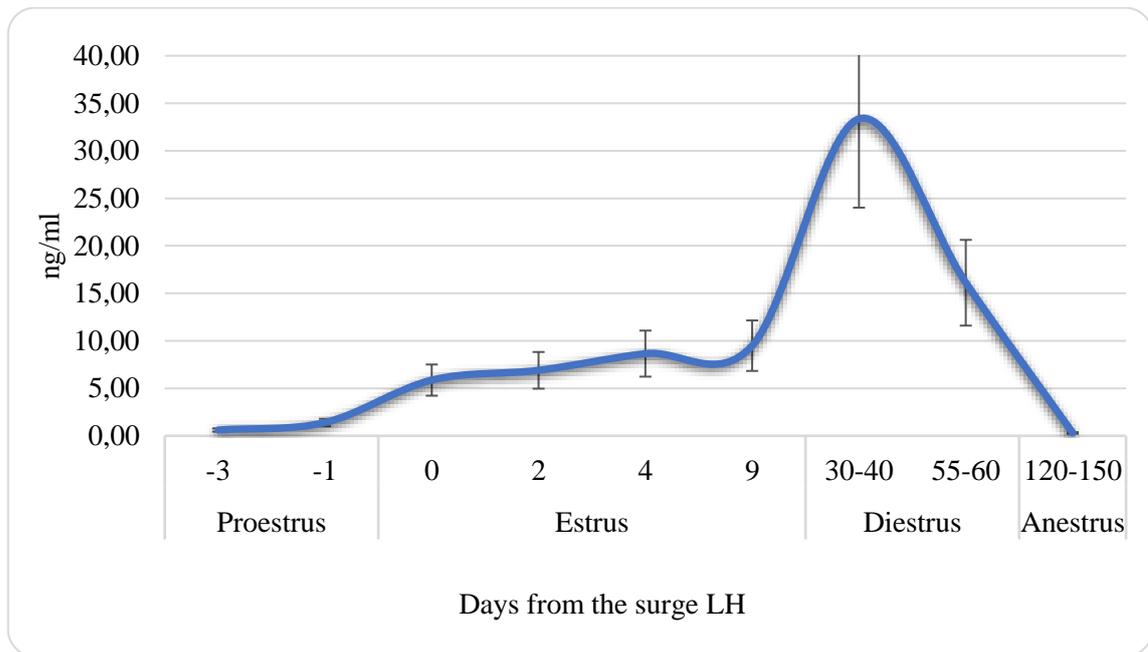


Fig. 1. Progesterone levels in the blood of Bull Terrier female dogs throughout the estrous cycle (ng/ml, n=25).

A two-factor analysis of variance (ANOVA), the results of which are presented in Table 1, revealed the influence of individual characteristics of female dogs on their blood progesterone levels. Regardless of the studied characteristic (temperament, age, or body weight), the stage of the estrous cycle was the determining factor influencing progesterone levels ($F = 79.8-173.0 > FU = 2.11$; $P < 0.001$). Among the individual characteristics of the dogs, body weight had the most significant effect on progesterone levels ($F = 16.3 > FU = 3.09$; $P < 0.001$).

A minor but statistically significant effect of age on blood progesterone levels was also observed ($F = 3.65 > FU = 3.09$; $P < 0.05$).

The temperament of the female dogs also influenced their blood progesterone levels. Specifically, the impact of aggression was significant ($F = 10.68 > FU = 3.09$; $P < 0.001$), as well as the levels of fear and anxiety ($F = 9.21 > FU = 3.09$; $P < 0.001$) and excitability ($F = 7.6 > FU = 3.09$; $P < 0.001$). In contrast, traits related to trainability and obedience did not significantly influence blood progesterone levels ($F = 0.91 < FU = 3.09$; $P = 0.41$).

It should be noted that the analysis of progesterone levels in the blood of female dogs with varying body weight, aggression levels, and levels of fear and anxiety revealed a significant interaction between factors ($F = 2.24-2.92 > FU = 1.8$; $P < 0.005-0.001$). This indicates a statistically significant interrelationship between the individual characteristics of female dogs and their estrous cycle.



Table 1

Two-factor analysis of variance of progesterone levels in the blood of Bull Terrier female dogs with varying age, body weight, and temperament

Factor	SS	df	MS	F	P-value	F critical
Age of animals						
Age	146,8	2	73,42	3,65	0,03	3,09
Stage of estrous cycle	11242,9	7	1606,13	79,82	< 0,001	2,11
Interaction	197,6	14	14,12	0,70	0,768	1,80
Body weight						
Body weight	356,7	2	178,34	16,3	< 0,001	3,09
Stage of estrous cycle	13255,6	7	1893,65	173,03	< 0,001	2,11
Interaction	447,8	14	31,98	2,92	< 0,001	1,80
Aggression level						
Aggression	269,3	2	134,67	10,68	< 0,001	3,09
Stage of estrous cycle	13448,2	7	1921,16	152,42	< 0,001	2,11
Interaction	427,2	14	30,51	2,42	0,006	1,80
Fear and anxiety level						
Fear and anxiety	268,1	2	134,03	9,21	< 0,001	3,09
Stage of estrous cycle	13016,6	7	1859,51	127,71	< 0,001	2,11
Interaction	455,9	14	32,56	2,24	0,011	1,80
Excitability level						
Excitability level	189,4	2	94,72	7,60	< 0,001	3,09
Stage of estrous cycle	11592,7	7	1656,11	132,83	< 0,001	2,11
Interaction	250,8	14	17,92	1,44	0,151	1,80
Trainability and obedience						
Trainability	35,6	2	17,81	0,91	0,407	3,09
Stage of estrous cycle	12389,7	7	1769,96	90,21	< 0,001	2,11
Interaction	96,2	14	6,87	0,35	0,985	1,80

Note. SS – sum of squares; df – degrees of freedom (number of factor levels -1); MS – mean square; F – factor evaluation criterion for its effect on the dependent variable; P – significance level; F critical – critical value of the factor.

Based on the results of one-way analysis of variance, a significant effect (η^2_χ) of specific temperament characteristics, age, and body condition on the level of progesterone (P4) in the plasma of bitches was established (Table 2). The analysis allowed identifying specific time periods during which certain factors significantly influenced the hormone concentration in plasma. The age of the bitches demonstrated a variable impact on progesterone levels at different time points. Specifically, in younger animals, the effect was statistically significant only on days 23–30 after the luteinizing hormone (LH) surge, $\eta^2_\chi = 0.43$ ($P \leq 0.05$). In older bitches, pregnancy had a significant effect on progesterone levels on day 9 after the LH surge ($\eta^2_\chi = 0.52$; $P \leq 0.05$) and on days 35–40 ($\eta^2_\chi = 0.50$; $P \leq 0.05$).

The temperament of the animals proved to be an important factor in regulating P4 levels. For instance, aggression significantly influenced progesterone levels only on days 55–60 after the LH surge ($\eta^2_\chi = 0.34$; $P \leq 0.05$). Conversely, the excitability of the animals



had a significant effect on the hormone level both on the day of the LH surge ($\eta^2_\chi = 0.34$; $P \leq 0.05$) and on day 9 after the surge ($\eta^2_\chi = 0.35$; $P \leq 0.05$). The trainability and obedience of the bitches significantly influenced progesterone levels only on days 35–40 after the LH surge ($\eta^2_\chi = 0.34$; $P \leq 0.05$). At the same time, no significant effect of fear or anxiety on P4 concentration in plasma was detected.

Table 2

The Impact Strength of Individual Characteristics of Bitches on Progesterone Levels in Their Blood Throughout the Reproductive Cycle

Individual Characteristics of Animals		Stage of the Reproductive Cycle									
		Proestrus		Estrus				Diestrus			Anestrus
		-3	-1	0	2	4	9	23–30	35–40	55–60	120–150
Age	Young	0,02	0,1	0,05	0,03	0,04	0,06	0,43*	0,15	0,01	0,10
	Old	0,31	0,34	0,12	0,27	0,4	0,52*	0,14	0,50*	0,35	0,29
Weight	Under-weight	0,15	0,19	0,01	0,3	0,01	0,02	0,03	0,11	0,03	0,08
	Over-weight	0,10	0,00	0,73	0,55	0,49	0,45*	0,03	0,48*	0,52*	0,00
Agression		0,06	0,00	0,22	0,12	0,11	0,18	0,05	0,22	0,34*	0,04
Fear		0,25	0,02	0,02	0,03	0,00	0,07	0,00	0,01	0,03	0,03
Excitability		0,00	0,04	0,34*	0,2	0,21	0,35*	0,00	0,15	0,24	0,02
Training and obedience		0,20	0,09	0,00	0,00	0,00	0,01	0,01	0,05	0,00	0,34*

Note: Values are significant at $P < 0.05$.

The results obtained indicate a complex interaction between physiological and behavioral characteristics of the animals, which manifests during different time periods. These findings emphasize the need to consider age and temperament traits when assessing the reproductive status and hormonal profile of bitches.

Discussion. Our study confirms and expands upon existing data, highlighting the significant interplay between individual physiological traits, body condition, and temperament with hormone dynamics in female dogs throughout their reproductive cycle. The findings align with the conclusions of Luz (2006), who reported substantial individual variations in plasma progesterone (P4) levels in mixed-breed dogs (Luz, Bertan, Binelli, & Lopes, 2006). However, our results demonstrate reduced variability due to group control based on age and, importantly, breed. This is consistent with additional findings by Luz, indicating that progesterone variability significantly decreases within specific breeds, such as Beagles (Marinelli, Rota, Carnier, Da Dalt, & Gabai, 2009). The significant role of body size in influencing reproductive hormone dynamics, as noted by Luz (Luz et al., 2006), is further corroborated in our study. The observed lower variability in our results can be attributed to the selection of standardized breed-based groups. Furthermore, our findings are in agreement with previous reports that large breeds exhibit distinct hormonal profiles compared to smaller breeds due to metabolic and physiological differences (Reynolds & Redmer, 1999).

Impact of age on reproductive parameters. While the literature presents conflicting evidence regarding the effect of age on reproductive efficiency, our results



align with retrospective observations from canine breeding clubs (Gresky, Hamann, & Distl, 2005; Mutembei, Mutiga, & Tsuma, 2002). These studies noted reduced litter sizes in older females and after multiple pregnancies, particularly in Dachshunds, where younger females (<2.5 years) had smaller litters. Moreover, Marinelli's (2009) report of higher ovulation rates in older females (Marinelli et al., 2009) corresponds to our observation that older females exhibited more corpora lutea (CL) but reduced progesterone production efficiency. Specifically, our data indicate that females aged 3–6 years demonstrate optimal progesterone levels compared to younger and older groups, reflecting age-related characteristics of luteal tissue.

Body condition and hormonal regulation. Our findings on the impact of body condition align with existing data identifying obesity as a prevalent issue in dogs (Cave, Allan, Schokkenbroek, Metekohy, & Pfeiffer, 2012; O'Neill, Church, McGreevy, Thomson, & Brodbelt, 2014). Studies have shown that adipose tissue functions as an active endocrine organ, producing adipokines such as leptin, which negatively impact reproductive efficiency (Burke, 2022). Elevated leptin levels, often observed in overweight dogs, correlate with reduced fertility and altered hormonal profiles (Brannian, Schmidt, Kreger, & Hansen, 2001). In our study, dogs with both excess and insufficient body weight exhibited altered hormonal dynamics, particularly progesterone levels, during the reproductive cycle. This supports the hypothesis that deviations from optimal body condition can disrupt endocrine function.

Temperament and reproductive hormones. Temperament emerged as a key factor influencing hormonal regulation. Our findings on the effects of aggression, excitability, and fear align with reports linking these traits to physiological responses and reproductive efficiency (Hecht et al., 2021; Zapata, Eyre, Alvarez, & Serpell, 2022). Genetic predisposition to behavioral traits such as aggression and fear affects systemic functions, including the reproductive system (Morrill et al., 2022). Notably, excitability, identified as a central temperament trait in the evolutionary history of dogs (Rosati & Hare, 2013), demonstrated the strongest association with progesterone levels in our study.

In contrast, traits such as trainability and obedience had no significant effect on hormone levels, indicating that temperament traits directly linked to stress responses may play a more critical role in endocrine regulation. This observation aligns with studies highlighting the bidirectional relationship between stress, temperament, and reproductive health (Cobb, Branson, McGreevy, Lill, & Bennett, 2015; Czerwinski, Smith, Hynd, & Hazel, 2016).

Comparative and broader implications. The absence of menopause in female dogs and their theoretical ability to reproduce throughout life represents a significant physiological distinction from humans (Solano-Gallego & Masserdotti, 2016). However, extended interestrus intervals and reduced hormone levels in older dogs increase the risk of conditions such as pyometra (Blendinger & Bostedt, 1991). Our findings support the hypothesis that hormonal profiles and reproductive efficiency depend on both intrinsic and extrinsic factors, such as age, body condition, and temperament. Moreover, the interplay between body size, temperament, and reproductive characteristics underscores the importance of multifactorial approaches in breeding program planning and reproductive management for dogs.

While the influence of temperament on hormonal profiles is often overlooked in the literature, our study highlights its significance, particularly in light of genetic and environmental influences on behavioral traits (Boyd et al., 2018).

The results of the one-way analysis of variance showed that individual characteristics, such as temperament, age, and body condition, have a statistically significant effect on the level of progesterone in the plasma of bitches at different periods



of their reproductive cycle. This suggests that the physiological and behavioral characteristics of animals influence the functioning of the endocrine system, which, in turn, affects the reproductive process. The analysis demonstrated that the age of animals has a variable impact on progesterone levels depending on the time after the luteinizing hormone (LH) surge. In young bitches, a significant effect was observed on days 23–30 after the LH surge ($P \leq 0.05$). This may indicate optimal corpus luteum functionality in young animals at this stage of the cycle. In older bitches, a significant impact was observed on day 9 ($P \leq 0.05$) and on days 35–40 ($P \leq 0.05$) after the LH surge. This may be due to physiological changes in aging animals that affect progesterone synthesis and secretion (Concannon et al., 1978; Holesh et al., 2023). The temperament of bitches also affects progesterone levels at specific periods of the reproductive cycle. For example, aggression had a significant effect only on days 55–60 after the LH surge ($P \leq 0.05$), which may be associated with elevated stress levels affecting the endocrine system. The excitability level significantly influenced progesterone concentration on the day of the LH surge ($P \leq 0.05$) and on day 9 after the surge ($P \leq 0.05$). These results underline the connection between an animal's reactivity and hormonal regulation, which is crucial for the successful progression of the reproductive cycle (Sousa et al., 2016; Park et al., 2020). A significant effect of trainability and obedience on progesterone levels was recorded only on days 35–40 after the LH surge ($\eta^2_{\chi} = 0.34$; $P \leq 0.05$). It is possible that more controlled behavior in animals creates conditions conducive to stable endocrine system functioning. Using one-way analysis of variance, no significant effect of fear and anxiety levels on progesterone concentration in plasma was found. This may indicate that these behavioral characteristics have a lesser influence on hormonal status or that their effects are compensated for by other factors (Schumacher et al., 2004; Singh et al., 2013).

The obtained data indicate the complex nature of progesterone regulation, which depends on both physiological and behavioral characteristics of bitches. The study highlights the importance of considering age and individual traits of animals in their management, particularly in the context of reproductive management. Understanding these relationships can be useful for developing tailored approaches to enhance reproductive efficiency and animal welfare.

Conclusion. Our study provides new insights into progesterone dynamics in female dogs, emphasizing the critical roles of body condition, temperament, and age in modulating reproductive efficiency. The findings suggest that future research should focus on exploring the mechanistic basis of these relationships, particularly the role of temperament and its interaction with endocrine function. Furthermore, these results underline the need for comprehensive management strategies in canine breeding programs to optimize reproductive outcomes and overall health.

References

- Blendinger, K., & Bostedt, H. (1991). The age and stage of estrus in bitches with pyometra. Statistical inquiry and interpretive study of the understanding of variability. *Tierärztliche Praxis*, 19(3), 307–310.
- Boyd, C., Jarvis, S., McGreevy, P. D., Heath, S., Church, D. B., Brodbelt, D. C., & O'Neill, D. G. (2018). Mortality resulting from undesirable behaviours in dogs aged under three years attending primary-care veterinary practices in England. *Animal Welfare*, 27(3), 251–262.
- Brannian, J. D., Schmidt, S. M., Kreger, D. O., & Hansen, K. A. (2001). Baseline non-fasting serum leptin concentration to body mass index ratio is predictive of IVF outcomes. *Human Reproduction*, 16(9), 1819–1826.
- Burke, C. (2022). The role of energy and weight: from conception to adulthood. *The*



- Veterinary Nurse*, 13(4), 183–187.
- Cave, N. J., Allan, F. J., Schokkenbroek, S. L., Metekohy, C. A. M., & Pfeiffer, D. U. (2012). A cross-sectional study to compare changes in the prevalence and risk factors for feline obesity between 1993 and 2007 in New Zealand. *Preventive Veterinary Medicine*, 107(1–2), 121–133.
- Cobb, M., Branson, N., McGreevy, P., Lill, A., & Bennett, P. (2015). The advent of canine performance science: Offering a sustainable future for working dogs. *Behavioural Processes*, 110, 96–104. <https://doi.org/10.1016/j.beproc.2014.10.012>
- Concannon, P. W. (2009). Endocrinologic control of normal canine ovarian function. *Reproduction in Domestic Animals = Zuchthygiene*, 44 Suppl 2, 3–15. Retrieved from <https://api.semanticscholar.org/CorpusID:37515855>
- Concannon, P. W. (2011). Reproductive cycles of the domestic bitch. *Animal Reproduction Science*, 124 3-4, 200–210. Retrieved from <https://api.semanticscholar.org/CorpusID:33165495>
- Concannon, P. W., Butler, W. R., Hansel, W., Knight, P. J., & Hamilton, J. M. (1978). Parturition and Lactation in the Bitch: Serum Progesterone, *Cortisol and Prolactin. Biology of Reproduction*, 19(5), 1113–1118. <https://doi.org/10.1095/BIOLREPROD19.5.1113>
- Concannon, P. W., Castracane, V. D., Temple, M., & Montanez, A. (2009). Endocrine control of ovarian function in dogs and other carnivores. *Animal Reproduction*, 6, 172–193. Retrieved from <https://api.semanticscholar.org/CorpusID:43599259>
- Conley, A. J., Gonzales, K. L., Erb, H. N., & Christensen, B. W. (2023). Progesterone Analysis in Canine Breeding Management. *Veterinary Clinics: Small Animal Practice*, 53(5), 931–949.
- Czerwinski, V. H., Smith, B. P., Hynd, P. I., & Hazel, S. J. (2016). The influence of maternal care on stress-related behaviors in domestic dogs: What can we learn from the rodent literature? *Journal of Veterinary Behavior: Clinical Applications and Research*, 14, 52–59. <https://doi.org/10.1016/J.JVEB.2016.05.003>
- Feldman, E. C., Nelson, R. W., Reusch, C., & Scott-Moncrieff, J. C. (2014). Canine and feline endocrinology-e-book. Elsevier health sciences.
- Gresky, C., Hamann, H., & Distl, O. (2005). Influence of inbreeding on litter size and the proportion of stillborn puppies in dachshunds. *Berliner Und Munchener Tierarztliche Wochenschrift*, 118(3–4), 134–139.
- Hanukoglu, I., Karavolas, H. J., & Goy, R. W. (1977). Progesterone metabolism in the pineal, brain stem, thalamus and corpus callosum of the female rat. *Brain Research*, 125(2), 313–324. [https://doi.org/10.1016/0006-8993\(77\)90624-2](https://doi.org/10.1016/0006-8993(77)90624-2)
- Hecht, E. E., Zapata, I., Alvarez, C. E., Gutman, D. A., Preuss, T. M., Kent, M., & Serpell, J. A. (2021). Zapata. *Brain Structure & Function*, 226(8), 2725–2739. <https://doi.org/10.1007/s00429-021-02368-8>
- Holsh, J. E., Bass, A. N., & Lord, M. (2023). Physiology, Ovulation. *StatPearls*. Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK441996/>
- Kakhanouskaya K. Y., S. I. V. (2017). Kinetic studies of cow milk lactoperoxidase. *Belarus. State Univ. Biol.*, 2, 66–71.
- Knox, R. V, Vatzias, G., Naber, C. H., & Zimmerman, D. R. (2003). Plasma gonadotropins and ovarian hormones during the estrous cycle in high compared to low ovulation rate gilts. *Journal of Animal Science*, 81(1), 249–260.
- Kowalewski, Mariusz P. (2014). Luteal regression vs. prepartum luteolysis: Regulatory mechanisms governing canine corpus luteum function. *Reproductive Biology*, 14(2), 89–102. <https://doi.org/10.1016/J.REPBIO.2013.11.004>



- Kowalewski, Mariusz Pawel, Beceriklisoy, H. B., Pfarrer, C., Aslan, S., Kindahl, H., Küçükaslan, I., & Hoffmann, B. (2010). Canine placenta: A source of prepartal prostaglandins during normal and antiprogesterin-induced parturition. *Reproduction*, 139(3), 655–664. <https://doi.org/10.1530/REP-09-0140>
- Luz, M. R., Bertan, C. M., Binelli, M., & Lopes, M. D. (2006). Plasma concentrations of 13,14-dihydro-15-keto prostaglandin F2-alpha (PGFM), progesterone and estradiol in pregnant and nonpregnant diestrus cross-bred bitches. *Theriogenology*, 66(6–7), 1436–1441. <https://doi.org/10.1016/j.theriogenology.2006.01.036>
- Marinelli, L., Rota, A., Carnier, P., Da Dalt, L., & Gabai, G. (2009). Factors affecting progesterone production in corpora lutea from pregnant and diestrus bitches. *Animal Reproduction Science*, 114(1–3), 289–300. <https://doi.org/10.1016/J.ANIREPROSCI.2008.10.001>
- Marinelli, L., Rota, A., Carnier, P., Da Dalt, L., & Gabai, G. (2009). Factors affecting progesterone production in corpora lutea from pregnant and diestrus bitches. *Animal Reproduction Science*, 114(1–3), 289–300. <https://doi.org/10.1016/J.ANIREPROSCI.2008.10.001>
- Martin, N., Höftmann, T., Politt, E., Hoppen, H. O., Sohr, M., Günzel-Apel, A. R., & Einspanier, A. (2009). Morphological examination of the corpora lutea from pregnant bitches treated with different abortifacient regimes. *Reproduction in Domestic Animals*, 44(SUPPL. 2), 185–189. <https://doi.org/10.1111/J.1439-0531.2009.01430.X>
- Morrill, K., Hekman, J., Li, X., McClure, J., Logan, B., Goodman, L., Carmichael, E. (2022). Ancestry-inclusive dog genomics challenges popular breed stereotypes. *Science*, 376(6592), eabk0639.
- Mutembei, H. M., Mutiga, E. R., & Tsuma, V. T. (2002). An epidemiological survey demonstrating decline in reproductive efficiency with age and non-seasonality of reproductive parameters in German shepherd bitches in Kenya. *Journal of the South African Veterinary Association*, 73(1), 36–37.
- O' Neill, D. G., Church, D. B., McGreevy, P. D., Thomson, P. C., & Brodbelt, D. C. (2014). Prevalence of disorders recorded in dogs attending primary-care veterinary practices in England. *PloS One*, 9(3), e90501.
- Papa, P. C., & Hoffmann, B. (2011). The Corpus Luteum of the Dog: Source and Target of Steroid Hormones? *Reproduction in Domestic Animals*, 46(4), 750–756. <https://doi.org/10.1111/J.1439-0531.2010.01749.X>
- Papa, P., & Kowalewski, M. P. (2020). Factors affecting the fate of the canine corpus luteum: Potential contributors to pregnancy and non-pregnancy. *Theriogenology*. Retrieved from <https://api.semanticscholar.org/CorpusID:211261844>
- Park, C. J., Lin, P.-C., Zhou, S., Barakat, R., Bashir, S. T., Choi, J. M., Lydon, J. P. (2020). Progesterone receptor serves the ovary as a trigger of ovulation and a terminator of inflammation. *Cell Reports*, 31(2).
- Pennsylvania, U. of. (2023). The C-BARQ is designed to provide dog owners and professionals with standardized evaluations of canine temperament and behavior. Retrieved from <https://vetapps.vet.upenn.edu/cbarq/>
- Pereira, M. M., Mainigi, M., & Strauss III, J. F. (2021). Secretory products of the corpus luteum and preeclampsia. *Human Reproduction Update*, 27(4), 651–672.
- Reynolds, L. P., & Redmer, D. A. (1999). Growth and development of the corpus luteum. *Journal of reproduction and fertility-supplement-*, 181–191.
- Rosati, A. G., & Hare, B. (2013). Chimpanzees and bonobos exhibit emotional responses to decision outcomes. *PloS One*, 8(5), e63058.



- Schumacher, M., Guennoun, R., Robert, F., Carelli, C., Gago, N., Ghoumari, A., ... De Nicola, A. F. (2004). Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. *Growth Hormone & IGF Research*, 14(SUPPL. A), 18–33. <https://doi.org/10.1016/J.GHIR.2004.03.007>
- Serpell, J. A. (2015). The C-BARQ questionnaire. University of Pennsylvania Vet Med.
- Singh, M., Su, C., & Ng, S. (2013). Non-genomic mechanisms of progesterone action in the brain. *Frontiers in Neuroscience*, 7(7 SEP), 60052. <https://doi.org/10.3389/FNINS.2013.00159/BIBTEX>
- Solano-Gallego, L., & Masserdotti, C. (2016). Reproductive system. *Canine and Feline Cytology*, 313.
- Sousa, L. M. M. D. C., Silva, R. dos S., da Fonseca, V. U., Leandro, R. M., Di Vincenzo, T. S., Alves-Wagner, A. B., ... De Papa, P. C. (2016). Is the canine corpus luteum an insulin-sensitive tissue? *Journal of Endocrinology*, 231(3), 223–233. <https://doi.org/10.1530/JOE-16-0173>
- Zapata, I., Eyre, A. W., Alvarez, C. E., & Serpell, J. A. (2022). Latent class analysis of behavior across dog breeds reveal underlying temperament profiles. *Scientific Reports*, 12(1), 15627. <https://doi.org/10.1038/s41598-022-20053-6>