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STUDY OF THE INFLUENCE OF BODY CONDITION ON THE DYNAMICS OF SEX HORMONES IN THE BLOOD OF BITCHES

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The physiological mechanisms of regulation of the reproductive function of bitches are well studied, however, the issue of individual characteristics of the body on the dynamics of sex hormones in the blood remained out of the attention of researchers. Therefore, we set ourselves the goal of studying the influence of body condition on the dynamics of sex hormones in the blood of bitches. The experiment was carried out on 15 bitches of the bull terrier breed with different body conditions according to the Body Condition Scoring scale. Three groups of animals were formed for the experiment: the control group was the ideal weight (the average BCS score was 5 points); I research group – low weight (average BCS score – 3.6 points); Group II – overweight (average BCS score – 6.6 points). For the first time, the dynamics of the content of sex hormones (progesterone, luteinizing hormone, follicle-stimulating hormone, estadiol) in the blood of bull terrier bitches with different body conditions was studied. New scientific data on the breed features of humoral regulation of the sexual cycle in the body of bitches have been obtained. The results of the studies indicate that factors such as overweight and low weight of bitches have an impact on the dynamics of sex hormones in the blood plasma of bitches during the sexual cycle. In particular, a significant effect of excess weight on the level of luteinizing hormone (P≤0.05) and progesterone (P≤0.05) at the stage of diestrus, on the level of progesterone (P≤0.05) at the stage of estrus was established. Low bitch weights were accompanied by higher levels of follicle-stimulating hormone at the proestrus stage (P≤0.05). Thus, our findings highlight that the characteristics of the corpus luteum of bitches and the synthesis of progesterone are influenced by many factors that have received little scientific attention to date.

*Keywords***.** *bitches, sexual cycle, progesterone, luteinizing hormone, follicle-stimulating hormone, estadiol.*

ДОСЛІДЖЕННЯ ВПЛИВУ СТАНУ ТІЛА НА ДИНАМІКУ СТАТЕВИХ ГОРМОНІВ В КРОВІ СУК

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Фізіологічні механізми регуляції репродуктивної функції сук вивчені досить добре, однак, питання індивідуальних особливостей організму на динаміку статевих гормонів у крові залишилось поза увагою дослідників. Тому, ми поставили перед собою за мету вивчити вплив стану тіла на динаміку статевих гормонів в крові сук. Експеримент проведено на 15 суках породи бультер'єр з різним станом тіла відповідно до шкали Body Condition Scoring. Для експерименту було сформовано три групи тварин: контрольна – ідеальна вага (середня оцінка BCS – 5 ба-

лів); І дослідна група – низька вага (середня оцінка BCS – 3,6 бала); ІІ дослідна група – надмірна вага (середня оцінка BCS – 6,6 бала). Уперше досліджено динаміку вмісту статевих гормонів (прогестерону, лютеїнізуючого гормону, фолікулостимулюючого гормону, естадіолу) у крові сук породи бультер'єр з різним станом тіла. Отримані нові наукові дані щодо породних особливостей гуморальної регуляції статевого циклу в організмі сук. Отримані результати досліджень свідчать, що такі фактори, як надмірна вага і низька вага сук мають вплив на динаміку статевих гормонів в плазмі крові сук потягом статевого циклу. Зокрема, встановлено достовірний вплив надмірної ваги на рівень лютеїнізуючого гомону (P≤0,05) та прогестерону (P≤0,05) на стадії діеструса, на рівень прогестерону (P≤0,05) на стадії еструса. Низька вага сук супроводжувалась більшим рівнем фолікулостимулюючого гормону на стадії проеструса (P≤0,05). Таким чином, наші висновки підкреслюють, що на характеристики жовтого тіла сук та синтез прогестерону впливають багато факторів, яким на сьогоднішній день приділяється мало наукової уваги.

Ключові слова. суки, статевий цикл, прогестерон, лютеїнізуючий гормон, фолікулостимулюючий гормон, естадіол.

Introduction. The domestic dog is the most common pet that accompanies people approx. 15,000 years. And today there is about 343 miscellaneous dog breeds (Alvares et al., 2019). It makes dogs the most frequent patients of veterinarians. The problem of canine reproductive medicine today is becoming more and more relevant due to the fact that the available therapeutic approaches are based mainly on clinical experience and empirical data, rather than on an understanding of basic physiological processes that are not yet fully understood (Kowalewski, 2023). The dog is one of the most important laboratory animals and is one of the best laboratory animal models. They are models for investigating comparative aspects of reproductive physiology in other mammals and humans (Kowalewski, 2018).

Compared to other types of pets, the sexual cycle is relatively long. The sexual cycle of bitches is aseasonal and monoestrous with a mandatory anestestrus between them.

A peculiarity of this type of animal is that the corpus luteum (Corpora lutea; CL) is the only major source of circulating steroids in females, regardless of their reproductive status. The dog is the only type of pet that does not produce steroids in the placenta, which emphasizes the central role CL in the control of the reproduction of dogs (Nogueira Aires et al., 2022). The phases of the sexual cycle of bitches are always separated by a resting phase (anestrus). And the biggest difference between dogs and other mammals is the absence of the antiluteolytic principle in the absence of pregnancy, which leads to physiological pseudopregnancy. Pseudopregnancy can last at least as long as or even longer than the time span of a normal pregnancy (Tsuchida et al., 2022).

Unlike other pets, in dogs, the resumption of meiosis and the completion of chromosome division is stimulated by the preovulatory surge of LH and occurs before ovulation. Ovulation of immature eggs occurs, which require another 2-3 days of oviductive maturation, only then acquiring the stage of development of fertile secondary eggs (Bigler et al., 2023). Thus, unlike other types of pets, in which eggs ovulate as secondary oocytes, a dog ovulates primary eggs (Balogh et al., 2018).

Also, the specific features of the reproductive system are the absence of an increase in estrogen content during pregnancy and before birth of puppies. And luteal regression occurs despite an increase in pituitary hormones (PRL, LH) (Pereira et al.,

2021). Finally, from an endocrinological point of view, fetal placental relaxin is the only reliable marker of pregnancy in a dog, since gestational levels of sex hormones are elevated compared to pseudo-pregnancy, they cannot be used to detect pregnancy, since elevated levels of P4 can also be observed in pseudo-pregnancies, in addition to this, significant individual variations are also observed (Bonfim Neto et al., 2022).

Nowadays, scientific interest in the study of the influence of body weight of bitches on the humoral component of the sexual cycle is mainly associated with the comparison of breeds of different body sizes, which obviously does not provide complete information about the physiological characteristics of the sexual cycle, since late breeds sometimes differ quite in their metabolism and physiological differences. It has been found that the weight of the bitch also affects the weight of the average corpus luteum (CL) and DNA content ($P < 0.001$), bitches heavier than 20 kg had more and heavier CL, resulting in more total luteal tissue (Marinelli et al., 2009). The relationship between body size and total luteal mass among other species is well known, and the size of mature CL is relatively constant within a species under physiological circumstances (Reynolds & Redmer, 1999).

The physiological mechanisms that regulate the function of the corpus luteum are of increased scientific interest. However, although the function of individual, predominantly luteotrophic, factors has been characterized, a general understanding of the physiology of CL in dogs remains poorly understood (Kowalewski, 2018). Therefore, we set ourselves the goal of studying the influence of the state of the body on the dynamics of sex hormones in the blood of bitches, which will allow us to obtain basic information about the peculiarities of the regulation of the sexual cycle in bitches and develop methods for their correction.

Materials and methods. The experiment was carried out on 15 females (Canis familiaris) of the Bull Terrier breed with different body conditions. At the time of the research, all animals were free of infectious and invasive diseases. The health status of the animals was assessed through clinical examination and laboratory tests.

The body condition of all bitches was assessed on the Body Condition Scoring scale. BCS is a quantitative tool widely used by veterinarians to assess an animal's body condition, fat accumulation, and nutritional status. The BCS scale ranges from 1 (depleted) to 9 (dangerously obese). The ideal body score is usually 4 or 5 with a physiological amount of muscle and fat (Body Condition Scoring (BCS) Charts, 2023). It should be mentioned that only healthy bitches without signs of hypo- or hypertrophy were selected for groups of animals. And the deviations of the body structure of animals in the experimental groups were within physiological limits. Three groups of animals were formed for the experiment: the control group was the ideal weight (the average BCS score was 5 points); I research group – low weight (average BCS score – 3.6 points); group II – overweight (average BCS score – 6.6 points).

The terms of fertility of bitches were determined using folliculometry, vaginal cytology and analysis of the level of progesterone in the blood. Insemination of bitches was carried out by various methods (both natural and artificial). Pregnancy screening was performed on (or on the 21st-28th day after ovulation). The material for the studies was blood samples of bitches taken from the jugular vein at -3, -1, 0, 2, 4, 9, 23-30, 35-40, 55-60 and 120-150 days after a surge in luteinizing hormone (LH). In the blood plasma of bitches, the content of estradiol (Dog E2 ELISA Kit, ICNE2KT, Innovative Research, USA), follicle-stimulating hormone (Dog Follicle Stimulating Hormone (FSH) ELISA Kit, Abbexa LTD, Great Britain), luteinizing hormone (Dog Luteinizing Hormone (LH) ELISA Kit, Abbexa LTD, UK) and progesterone (Progesterone –

ELISA, HEMA, Ukraine). Measurements were carried out on a universal reader for the ELx800 microplate (Bio-Tek Instruments, USA).

The experiment was carried out in compliance with the requirements of the Law of Ukraine No. 3447-IV of 21.02.06 "On the Protection of Animals from Cruel Treatment" and is fully consistent with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg, 1986).

Results. The hypothalamic-pituitary-gonadal (HPG) axis primarily controls and regulates mammalian reproduction. Anatomically, the HPG axis consists of:

- the hypothalamus (especially the infundibular nucleus, a homolog of the human arcuate nucleus, where neurons producing KNDy and GnRH are located);
- the anterior part of the pituitary gland, where gonadotropes secrete luteinizing and follicle-stimulating hormones;
- gonads, which are responsible for the production of both sex steroids and gametes, under the influence of sex hormones.

As in other endocrine systems, the HPG axis is regulated by forward and reverse feedback (Tena-Sempere, 2005). Hormones of various origins (pituitary, placenta, and ovary) are involved in the control of the sexual cycle of dogs (Conley et al., 2023; Gobello, 2007).

FSH acts on the appropriate mammalian target organs, namely the testes, and ovaries, to exert its biological functions through peripheral circulation (Wang et al., 2021). Since FSH is a crucial regulator of the hypothalamic-pituitary-gonadal system, it plays an indispensable role in reproductive activity. Some reproductive disorders are associated with impaired secretion of FSH, and its concentration was lower in polycystic ovary syndrome (BAIRD et al., 1977). A lack of FSH can cause impaired spermatogenesis and infertility (Rougier et al., 2019). In females, FSH stimulates follicular growth and development and increases oxygen uptake by parietal granulosa cells to promote the synthesis of the corresponding protein (Widayati & Pangestu, 2020).

Studies have shown an increase in the level of FSH in the blood plasma of bitches depending on the body condition from the third to the first day up to a surge of LH of 2.1–2.4 times ($P \leq 0.001$). It should be noted that three days before the LH surge, the FSH content in the blood plasma of bitches of the first experimental group (with low weight) was 1.57 (1.28–2.00) ng/ml, which is significantly higher by 31.3% ($P \leq 0.05$) than in the bitches of the control group (Fig. 1).

The level of FSH in bitches by the second day after the LH surge continues to increase, reaches its maximum, and in the bitches of the control group is 13.3 (11.12–15.27) ng/ml. In overweight bitches (II experimental group), the level of FSH in the blood on the day of the LH surge (day 0) and two days after it is 32.1% ($P \leq 0.05$) and 31.9% ($P \leq 0$, respectively, 05), while in the females of the II experimental group only the corresponding tendency was noted. It is known that the sensitivity of the hypothalamic-pituitary-gonadal axis is relatively reduced in overweight individuals (Kim et al., 2015; Pasquali, 2006), among overweight women, a lower steady-state FSH level was often accompanied by a low E2 level. De Pergola (2006) established a negative correlation of body mass index with FSH and LH levels ($p < 0.001$ and $p < 0.01$, respectively) (De Pergola et al., 2006). However, a significantly lower level of FSH in overweight bull terrier bitches was found only within two days of the LH surge.

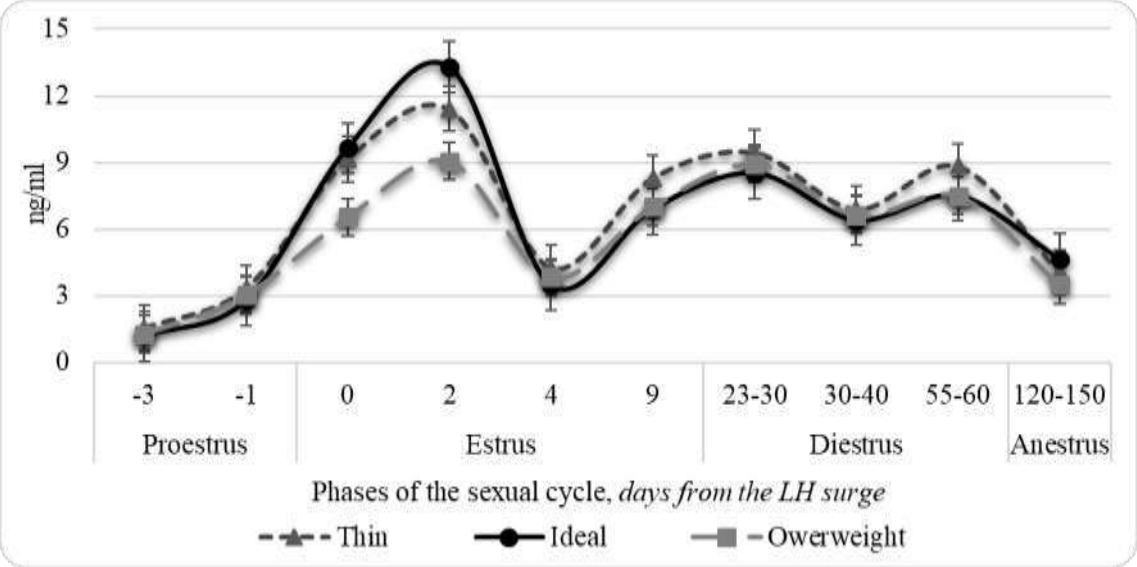

Fig. 1. **Dynamics of follicle-stimulating hormone content in the blood plasma of pregnant bitches of different body condition (n= 5; ng/ml).**

From the second to the fourth day after the LH surge, the level of FSH in the blood plasma of all experimental groups decreases by 2.4-2.7 times (P≤0.001) and ceases to differ significantly and reaches its minimum value at the level of (3.88, 2.5-6.6) ng/ml. Subsequently, by the 20-30th day after the LH surge, the level of FSH in the blood plasma of bitches increases by 2.2-2.5 times (P≤0.001), After that, by 55-60 days, it is restored to the previous values. Consequently, during pregnancy, the level of FSH in the blood plasma of bitches decreases slightly and increases before delivery. It should be noted that in pregnant bitches of different body conditions there are no significant differences in the content of the level of FSH in the blood, however, we note a tendency towards its higher level in bitches with low body weight (by 7.8-17.9%).

Estrogens are steroid hormones responsible for the growth and regulation of the female reproductive system and secondary sexual characteristics. Estradiol (E2) is the primary and most active hormone from the group of estrogens. Estrogens, mainly E2, are produced by the granulosa cells of the developing follicle and negatively affect LH production at the onset of the menstrual cycle (Holesh et al., 2023). However, once E2 levels reach a critical level, as eggs mature in the ovary in preparation for ovulation, estrogen begins to provide positive feedback on LH production, resulting in a surge. In addition to reproduction, E2 has many other effects that are important to the body (Tsutsumi & Webster, 2009). E2 has an inverse effect on the secretion of gonadotropic hormones during most stages of the estrous cycle (Kowalewski et al., 2011). The general pattern of E2 secretion roughly corresponds to the pattern of P4 secretion. It decreases towards the end of luteal life, indicating its source within the CL; no prenatal increase in E2 is observed (Kowalewski, 2017).

Kowalewski (2017) notes that pWith proestrus, E2 levels in bitches increase from initial low values of 5-10 pg/mL to preovulatory concentrations of 45-120 pg/mL (Kowalewski, 2017). Three days before the LH surge, the E2 content in the bitches of the control group was 45.1 (29.4–70.7) pg/ml, and before the LH surge, it increased by 1.4-1.6 times (P≤0.01), although the maximum peaks were shown one day before the LH surge (Fig. 2). Subsequently, during estrus, the content of the hormone in the blood plasma of bitches gradually decreases to values of 11.64 (8.9–19.7) pg/ml. At the beginning of diestrus, the dominance of P4 over E2 is noticeable (Feldman et al., 2014),

however, luteal formation and an increase in P4 levels have been associated with a new increase in E2 starting around day 10, in both pregnant and pseudo-pregnant dogs (Kowalewski, 2018). An increase in E2 levels in bitches during estrus to values of 34.6 (19.9-35.3), but preovulatory concentrations are not achieved. By the time of anestrus, there is a decrease in the level of E2 in the blood of bitches as a result of luteal regression. It should be outlined that no significant differences in the concentration of E2 in the blood of bitches with different body conditions were found during the experiment.

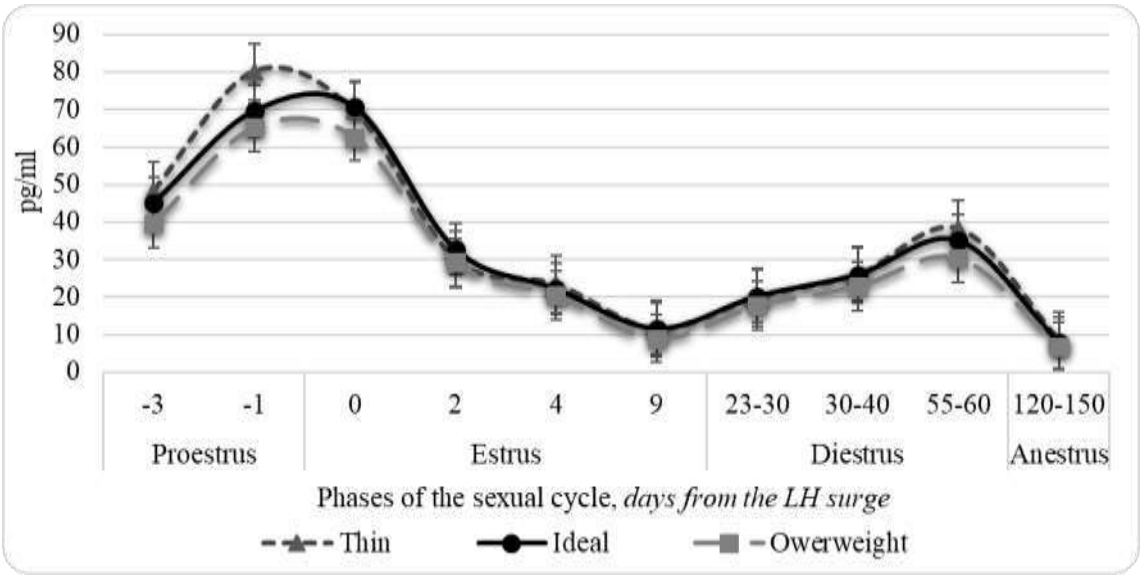

Fig. 2. **Dynamics of oestradiol content in blood plasma of pregnant bitches of different builds (n= 5; pg/ml).**

Luteinizing hormone (LH) is a glycoprotein hormone with a molecular weight of 28 kDa (Ezcurra & Humaidan, 2014) that is part of the HPG axis. Degradation and excretion remove LH and LHRH from the blood (Melnyk et al., 1976). LH release is stimulated by GnRH and inhibited by estrogens in women and testosterone in men. LH performs different functions that differ depending on the sex, but the common one is to promote the maturation of germ cells. In females, the hormone stimulates the synthesis of steroid hormones by the ovaries (Ilahi & Ilahi, 2022) and, to some extent, regulates the sexual cycle, playing a role in both ovulation and egg implantation (Kumar & Sait, 2011).

Thus, the surge of LH in bitches of the control group is characterized by an increase in the concentration of the hormone during the day (3.2-3.8 times; $P \leq 0.001$) from 3.56 (2.2–4.3) ng/ml to 13.6 (10.7–20.0) ng/ml (Fig. 3). In overweight bitches (II experimental group), the concentration of LH in the blood during the day before the surge increases only 3.1 times (against 3.7-3.8 times in the bitches of the control and I experimental groups), as a result of which the level of the hormone on the day of the surge was less by 38.5 % than in the bitches of the control group, however, due to the high variability of indicators, these differences have the character of a trend.

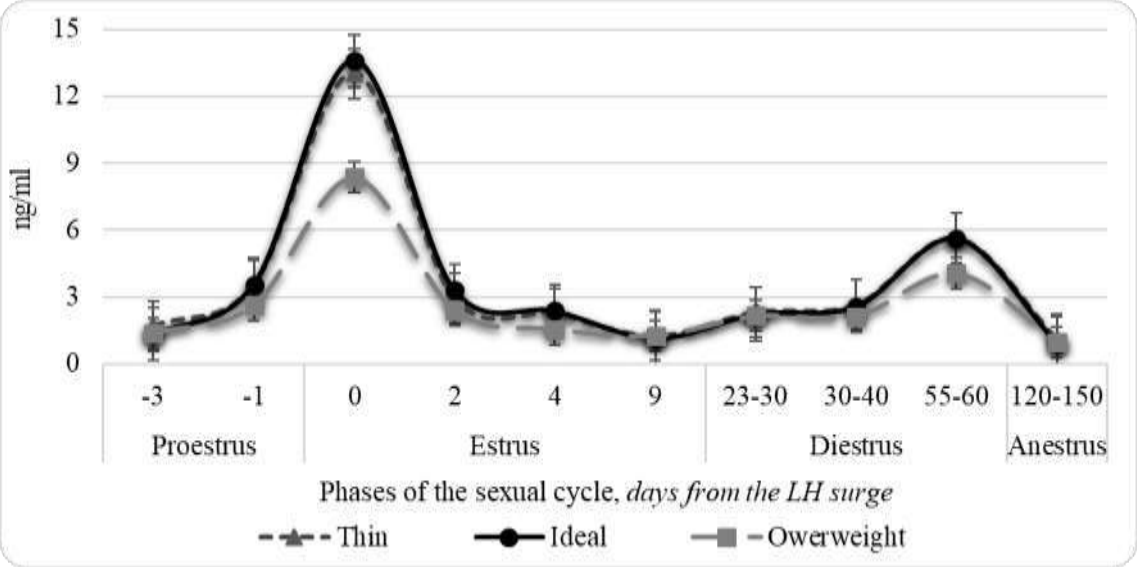

Fig. 3. **Dynamics of luteinizing hormone content in the blood plasma of pregnant bitches of different complexion (n= 5; ng/ml).**

Luz (2006) investigated mixed breeds and found significant fluctuations in individual variations in plasma P4 levels (Luz et al., 2006), however, in the study of P4 levels in the group of beagle bitches during the sexual cycle, these deviations were significantly reduced (Marinelli et al., 2009). In our studies, the established variability of this indicator was less than in these works, obviously due to the selection of groups of analogues by age and, most importantly, by breed. It is known that the size of the animal (the size of the breed) significantly affects the dynamics of sex hormones in their blood (Luz et al., 2006).

Endocrinologically, estrus begins 0-1 day from the LH surge (Kowalewski, 2018). Later, by the second day after the surge, the concentration of LH in bitches decreases by 3.4-4.3 times ($P \leq 0.001$), returning to previous values. During estrus flush, LH in bitches gradually decreases to 1.15 (0.8–1.5) ng/ml. And the subsequent increase in the diestrus stage is associated with pregnancy (or pseudopregnancy) of bitches. In particular, from the 9th to the 23-30th day after the surge, the level of LH in the blood of bitches increases by 1.71-1.94 times ($P \leq 0.001$), and to a lesser extent in overweight bitches. The LH content reaches its highest values at the stage of diestrus on the 55-60th day – 5.1 (3.1-7.0) ng/ml, and it was found that the content of the hormone in the blood of bitches was significantly lower (by 27.9%; $P \leq 0.001$) overweight.

At the stage of anestrus, the level of LH in the blood of bitches returns to baseline values of 0.98 (0.3-1.4) ng/ml. The researchers note that in the anestrus phase, the level of LH in the blood of bitches is quite low (<1 ng/ml) with small pulse intervals (4–24 cph) (Concannon et al., 2009).

Progesterone (P4) is a steroid hormone that prepares the endometrium to implant a fertilized egg in the uterus and maintain pregnancy. If the fertilized egg implants, the corpus luteum secretes progesterone early in pregnancy until the placenta develops and produces progesterone for the rest of the pregnancy (Holesh et al., 2023). Progesterone levels are widely used as a clinical biomarker in female reproductive management (Conley et al., 2023). The degree of variation in circulating levels of P4 among bitches is related to the multiple and variable number of ovulations and corpora lutea (Concannon P. W. et al., 1978). In many species, plasma concentrations of progesterone increase with the rate of ovulation and the number of CLs (Knox et al., 2003). Adult bitches have a higher efficiency of P4 synthesis than young bitches, suggesting that

luteal endocrine activity changes from young age to adulthood during the maturation process (Marinelli et al., 2009b).

The production of progesterone is influenced by many factors that have received little scientific attention to date. Due to the high variability among and within breeds, much of the literature on canine reproductive endocrinology has been obtained with homogeneous experimental groups of beagles (Marinelli et al., 2009). Although this approach has led to important advances in the understanding of basic physiological mechanisms, more specific characteristics of the species, such as variability in body size, have not yet been given the attention they deserve.

Proestrus is characterized by low levels of P4 in the blood of bitches, although these values begin to increase (to mean values of 1.4-1.7 ng/ml) compared to those at the stage of anestrus (mean values of 0.31-0.35 ng/ml). From a biological point of view, the onset of discourse is defined as the point of ovulation that induces the formation of CL. In bitches, the leading role at the stage of the sexual cycle – diestrus – is played by the secretion of P4. At the beginning of the diestrus, the dominance of P4 over E2 is established. (Kowalewski, 2017). At this time, the level of the hormone in the blood of the control group of bitches was 7 (5.11–9.1) ng/ml and does not differ significantly from the indicators of bitches of the experimental groups. During estrus, the level of P4 in the blood of bitches increases 1.5-1.7 times (P≤0.001), depending on the condition of the body.

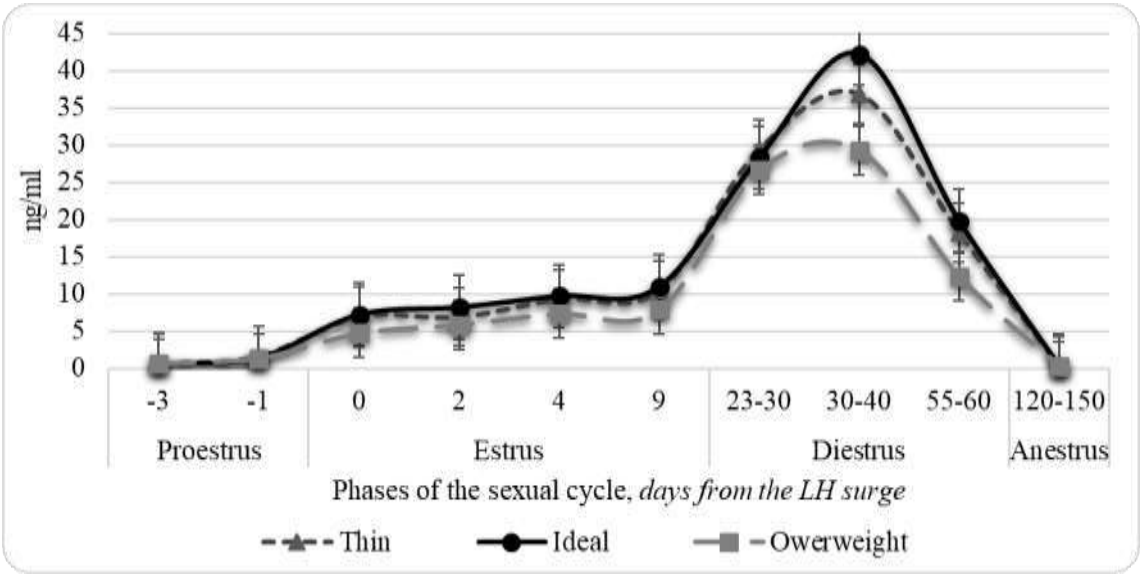

Fig. 4. **Dynamics of progesterone content in blood plasma of pregnant bitches of different complexion (n= 5; ng/ml)**

Unlike other domestic animals, in fully developed canine CLs, both morphologically and functionally, only one population of steroidogenic cells can be identified, which are activated at the onset of diestrus. As a consequence, P4 concentrations rise rapidly over 15 to 30 days, when CL is fully formed and maximum P4 concentrations are usually 30 to 35 ng/mL (up to 80 ng/mL or higher) (Kowalewski, 2018). Such effects on the reproductive system of high circulating concentrations of P4 are not observed in other domestic animal species. While early luteal phase develops CL, at least to some extent, gonadotropin-independent, and prostaglandins, in particular PGE2, are among the most important regulatory factors (Kowalewski, 2018).

The presence of a local mechanism that supports progesterone secretion after implantation still needs more research (Marinelli et al., 2009). Gudermuth (1998), by

measuring the concentration of fecal progesterone, found that the greatest increase in progesterone production during pregnancy occurs between 26 and 45 days after ovulation (Gudermuth et al., 1998). An increase of 2.6-3.4 times (P≤0.001) was found in the blood of bitches up to 20-30 days after a surge of LH level in the blood of bitches depending on the body condition with a peak on the 30-40th day after a surge of LH with an indicator of 36.9 (30.1–50.6) ng/ml. Subsequently, by the end of diestrus, the level of the hormone decreases by 2-2.4 times (P≤0.001). Excess weight negatively affects the content of P4 in the blood plasma of pregnant bitches in the second half of pregnancy. In particular, on the 30-40th and 55-60th day after the LH surge, the hormone content in the blood plasma was significantly less by 30.7% (P≤0.05) and 7.2% (P≤0.05), respectively, from the indicators of the bitches of the control group.

Diestrus ends when baseline P4 levels drop below <1 ng/mL and the cycle enters the anestrus stage. This can last up to 3 months after ovulation (Kowalewski, 2018). In bull terrier bitches in the anestrus stage, the level of P4 in the blood plasma did not depend on the condition of the body and ranged from 0.27-0.5 ng/ml.

Discussion. To date, no molecular-genetic bases have been established that provoke proestrus (Kowalewski, 2018). However, it is known that at the end of the anesterus, gonadotropin-releasing hormone (GnRH) impulses are amplified, followed by an increase in FSH release (Concannon, 2009).

For the first time, the phenomenon of preovulatory luteinization in bitches was described by von Bischoff in 1845 (Bischoff, 1845), the author notes that the difference between this process and that of other domestic animal species. Preovulatory luteinization is characterized by an increase in P4 levels 6 days before the LH surge (up to 0.6 to 1.0 ng/ml). During the LH surge, the P4 level rises, reaching a level of 5-10 ng/mL at the time of ovulation (Kowalewski, 2018). At the same time as the E2 level decreases, the P4 level increases, creating the conditions for an LH surge (Concannon et al., 2009). A surge of LH is considered to be when its level exceeds its basal levels by more than two times (Concannon, 2011). The surge is characterized by an initial increase in LH levels within 12 to 36 hours (Kowalewski, 2017).

The researchers point out that none of the progesterone parameters were influenced by the age or weight of the animal (Marinelli et al., 2009). However, we found that 4 and 9 days after the LH surge, the content of P4 in the blood of overweight bitches (II experimental group was at a significantly lower level, respectively, in bitches of the control group by 24.4% (P≤0.05) and 28.7% (P≤0.05).

Thus, excessive female body weight affects progesterone levels on days 4, 9, 35–40, and 55–60 after the LH surge, follicle-stimulating hormone levels on the day of the surge, and day two after the surge LH and the level of luteinizing hormone on the 55th–60th day after the LH surge (p≤0.05). The effect is characterized by a lower level (p≤0.05) of sex hormones in the blood of overweight bitches in the indicated periods of the sexual cycle. The low weight of bitches affects the level of follicle-stimulating hormone three days before the LH surge (p≤0.05).

Conclusions. For the first time, the dynamics of the content of sex hormones in the blood of bitches of the bull terrier breed with different body conditions was studied. The breed features of the humoral regulation of the sexual cycle in the body of bitches have been established and described. The results of the studies indicate that factors such as overweight and low weight of bitches have an impact on the dynamics of sex hormones in the blood plasma of bitches during the sexual cycle. A significant effect of overweight on the level of luteinizing hormone (P≤0, 05) and progesterone (P≤0.05) at the diestrus stage, at the level of progesterone (P≤0.05) at the estrus stage are determined. Low bitch weights were accompanied by higher levels of follicle-

stimulating hormone at the proestrus stage (P≤0.05). Thus, our findings highlight that the characteristics of the corpus luteum of bitches and the synthesis of progesterone are influenced by many factors that have received little scientific attention to date.

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None.

Conflict of interest

The authors declare no conflict of interest.

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