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INFLUENCE OF CERTAIN TEMPERAMENTAL TRAITS ON THE LEVEL OF SEX HORMONES IN BLOOD PLASMA OF FEMALE BULL TERRIERS

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Summary. To evaluate the influence of certain temperamental characteristics on the level of sex hormones in the blood plasma of female Bull Terriers, an experiment was conducted with 15 female Bull Terriers. Pregnancy screening was performed on 21st–28th days after ovulation). The material for the study were blood plasma samples of bitches obtained at different stages of the sexual cycle, in which the levels of estradiol, follicle stimulating hormone, luteinizing hormone, and progesterone were measured. The assessment of temperamental traits was performed at the design stage of the experiment using the standardized C-BARQ methodology. One-way analysis of variance was used to determine the strength of the influence of individual temperament traits on the level of sex hormones in the blood plasma of bitches. According to the results of the experiment, it was found that the degree of fear and anxiety in bitches significantly affects the content of follicle-stimulating hormone, estradiol, and luteinizing hormone in the blood plasma of bitches three days before, during and 120–150 days after the LH surge ($\eta^2_\chi = 0.27–0.55$ ($P \le 0.05$). The excitability of bitches affects the level of follicle stimulating hormone, luteinizing hormone, and progesterone on the day of the LH surge — $\eta^2_\chi = 0.32$ ($P \le 0.05$). In addition, the level of excitability affects the level of progesterone on the 9th day after the surge — $\eta^2_\chi = 0.35$ ($P \le 0.05$) and the level of luteinizing hormone on the 2nd, 4th, 55th–60th days and after the LH surge — $\eta^2_\chi = 0.26–0.43$ ($P \le 0.05$). The degree of aggression affects the luteinizing hormone level two days after the surge and the progesterone level on 55th–60th days after the LH surge ($\eta^2_\chi = 0.34–0.36$; $P \le 0.05$). Training ability and obedience affect follicle stimulating hormone levels (the day before the surge, and on 4th, 9th, and 35th–40th days after the LH surge ($\eta^2_\chi = 0.30–0.52$; $P \le 0.05$), luteinizing hormone (on 9th day after LH surge, $\eta^2_\chi = 0.54$; $P \le 0.01$), and progesterone (on 35th–40th days after LH surge, $\eta^2_\chi = 0.34$; $P \le 0.05$)

Keywords: dogs, estradiol, follicle-stimulating hormone, luteinizing hormone, progesterone

Introduction. The brain plays a critical role in regulating the activity of all body systems. Several parts of the brain have been implicated in the reproductive process (Okafor, Okpara and Ibeabuchi, 2022). These include the cerebral cortex, the insula, the pons, the hypothalamic-pituitary-gonadal (HPG) axis, and the pineal gland. Mammalian reproduction is primarily controlled and regulated by the HPG axis. Anatomically, the HPG axis consists of: the hypothalamus (specifically the infundibular nucleus, a homologue of the human arcuate nucleus, where the neurons that produce KNDy and GnRH are located); the anterior part of the pituitary gland, where the gonadotropes secrete luteinizing hormone and follicle stimulating hormone; and the 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 direct and reverse feedback (Tena-Sempere, 2005). Hormones of various origins (pituitary, placenta, and ovary) are involved in the control of the canine sexual cycle (Conley et al., 2023; Gobello, 2007). The hypothalamus, in turn, controls reproduction by regulating the secretory activity of the pituitary (Everett, 1969). In response to exogenous and endogenous stimuli, it produces several peptide neurohormones that regulate the function of the anterior pituitary. These neurohormones are released from the median eminence into the capillaries of the pituitary veins where they are transported to the adenohypophyseal cells to stimulate or inhibit the release of specific trophic hormones (Rance et al., 2010). In addition, in response to environmental stimuli, the hypothalamus produces neurohypophyseal hormones that are transported along long axons of the hypothalamic-pituitary tract for storage in the neural lobe of the pituitary and subsequent release into the systemic circulation (Opel, 1979). The pituitary gland, as one of the major endocrine organs of an animal, plays a critical role in the regulation of various physiological processes in mammals through the secretion of various hormones (Cooper and Withers, 2008). In addition to hormones that regulate reproductive function (follicle-stimulating and luteinizing hormones), the pituitary gland secretes growth hormone, prolactin, adrenocorticotropic hormone, melanocyte-stimulating hormone, and thyroid-stimulating hormone, which can directly or indirectly affect the reproductive function of mammals (Hong, Payne and Jane, 2016).

The highest manifestation of nervous activity of animals is their behavior (Danchuk et al., 2020b), which is undoubtedly limited by the state of the nervous system, including temperament (Danchuk et al., 2020a). Temperament is considered as a relatively stable group of personality traits (Strelau, 2008). The genetic basis of temperamental traits such as anxiety and aggression has been established, but phenotype has a greater influence on the formation of temperament (Hecht et al., 2021;

Zapata et al., 2022; Morrill et al., 2022). To date, a number of dependencies of temperament on age, sex, weight, and breed of animals have been established, which largely shape the individual characteristics of the animal body (Casey et al., 2014; Fratkin et al., 2013; Hsu and Sun, 2010; Riemer et al., 2014; Sherman et al., 1996).

However, there are no data in the available literature on the influence of temperament on the dynamics of sex hormones in the blood of bitches. Therefore, the **purpose of our work** was to determine the degree of influence of individual temperament traits on the dynamics of sex hormones in blood plasma of female Bull Terriers during the estrous cycle.

Materials and methods. The experiment was performed on 15 bitches of the Bull Terrier breed. The bitches were inseminated by different methods (both natural and artificial). Pregnancy screening was performed on 21^{st}–28^{th} days after ovulation). Parturition in the pregnant group was observed on 65 ±1 days after a rise in blood levels of luteinizing hormone. All animals were free of infectious and invasive diseases at the time of the study. The health status of the animals was assessed by clinical examination and laboratory tests. Blood samples were collected from the jugular vein of bitches on -3^{rd}, -1^{st}, 0, 2^{nd}, 4^{th}, 9^{th}, 23^{rd}–30^{th}, 35^{th}–40^{th}, 55^{th}–60^{th}, and 120^{th}–150^{th} days after the luteinizing hormone (LH) surge. Plasma levels of estradiol (Dog E2 ELISA Kit, ICNE2KT, Innovative Research, USA), follicle stimulating hormone (Dog Follicle Stimulating Hormone (FSH) ELISA Kit, Abbexa Ltd, United Kingdom), luteinizing hormone (Dog Luteinizing Hormone (LH) ELISA Kit, Abbexa Ltd, United Kingdom) and progesterone (Progesterone ELISA, HEMA, Ukraine) were measured. Measurements were performed on an ELx800 universal microplate reader (Bio-Tek Instruments, USA).

Temperament traits were assessed during the design phase of the experiment using the uniform Canine Behavioral Assessment and Research Questionnaire (C-BARQ) methodology, which is designed to provide dog owners and professionals with standardized assessments of canine temperament and behavior. The C-BARQ is a standardized dog behavior assessment tool developed at the University of Pennsylvania and accepted as a basic tool for determining animal temperament traits (Serpell, 2023). Of the 14 major criteria of dog behavior, we selected the four most important, namely aggression, fear and anxiety, excitability, and training and obedience, to meet our objective (Table 1). Owners were interviewed using the standard C-BARQ questionnaire (Serpell, 2015).

In order to determine the strength of the influence of certain temperamental traits on the level of sex hormones in the blood plasma of female Bull Terriers, a one-way analysis of variance was performed with the help of MS Excel 2019 using the built-in function 'Data Analysis.'

Table 1 — Correlation of C-BARQ characteristics with different dog temperament traits

Characteristics according to C-BARQ	Temperament traits		
Aggression	Calm	Moderately aggressive	Aggressive
Fear and anxiety	Animals without a strong sense of fear or anxiety (courage)	Animals with a moderate level of anxiety or a sense of fear	Animals that express a sense of fear (timidity, fearfulness)
Excitability	Calm	Moderately excitable	Excessively excitable
Training and obedience	Obedient (well trained)	Sometimes not obedient	Not obedient

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017).

Results. Using a one-factor analysis of variance, a reliable strength of influence (η^2_χ) of individual temperament characteristics on the level of sex hormones in blood plasma was established (Figs 1–4). It was proved that a high degree of fear and anxiety in bitches significantly affects the content of FSH in the blood plasma of bitches only three days before the LH surge — $\eta^2_\chi = 0.27$ ($P \leq 0.05$). At the same time, the level of bitches' excitability had a significant effect on the hormone content on the day of the LH surge — $\eta^2_\chi = 0.32$ ($P \leq 0.05$). It should be noted that bitch aggression did not significantly affect the level of FSH in blood plasma throughout the experiment, while the ability to be trained and obedience proved to be a significant factor in influencing the dynamics of FSH in animal plasma. In particular, a significant effect of the ability to train and obedience of bitches on the FSH content in blood plasma the day before the LH surge ($\eta^2_\chi = 0.36$; $P \leq 0.05$), on the 4^{th} and 9^{th} day after the LH surge, respectively, $\eta^2_\chi = 0.52$ ($P \leq 0.01$) and $\eta^2_\chi = 0.26$ ($P \leq 0.05$), and on the 35^{th}–40^{th} day after the LH surge ($\eta^2_\chi = 0.30$; $P \leq 0.05$). At the stage of sexual rest (anestrus), the selected temperament traits did not have a significant effect on the content of follicle-stimulating hormone in the blood of bitches.

Dispersion analysis revealed a significant effect (η^2_χ) of only the level of fear and anxiety in bitches three days before the LH surge on plasma estradiol content in bitches — $\eta^2_\chi = 0.27$ ($P \leq 0.05$). At the same time, other temperamental traits had no significant effect on the hormonal balance during the sexual cycle (Fig. 2).

A reliable strength of influence (η^2_χ) of individual temperament traits on the level of luteinizing hormone in blood plasma was established (Fig. 3). The level of aggression of animals has the least influence on the dynamics of LH in the blood of bitches compared to other temperamental traits. In particular, a high level of aggression in bitches only significantly affects the content of LH in the blood plasma of bitches two days after the LH surge — $\eta^2_\chi = 0.36$ ($P \leq 0.05$). At the same time, the degree of fear and anxiety in bitches significantly affects the hormone content in blood plasma three days before the LH surge — $\eta^2_\chi = 0.48$ ($P \leq 0.05$) and 120th–150th days after the LH surge — $\eta^2_\chi = 0.55$ ($P \leq 0.01$).

The level of excitability of the bitches had a significant effect on hormonal contents during estrus and at the end of diestrus. Specifically, on the day of the LH surge and two and four days thereafter, the effect of excitability on LH content was $\eta^2_\chi = 0.31$ ($P \leq 0.05$), $\eta^2_\chi = 0.43$ ($P \leq 0.05$), and $\eta^2_\chi = 0.26$ ($P \leq 0.05$), respectively. In contrast, the effect of excitability was $\eta^2_\chi = 0.32$ ($P \leq 0.05$) on 55th–60th days after the LH surge. The ability to be trained and obedience affected the LH content in the blood plasma of the animals only on the 9th day after the LH surge ($\eta^2_\chi = 0.54$; $P \leq 0.01$).

Dispersion analysis revealed a significant effect (η^2_χ) of individual temperament traits on blood plasma progesterone levels in bitches (Fig. 4). In particular, the aggression of the bitches had a significant effect on the plasma level of P4 only on the 55th–60th days after the LH surge ($\eta^2_\chi = 0.34$; $P \leq 0.05$).

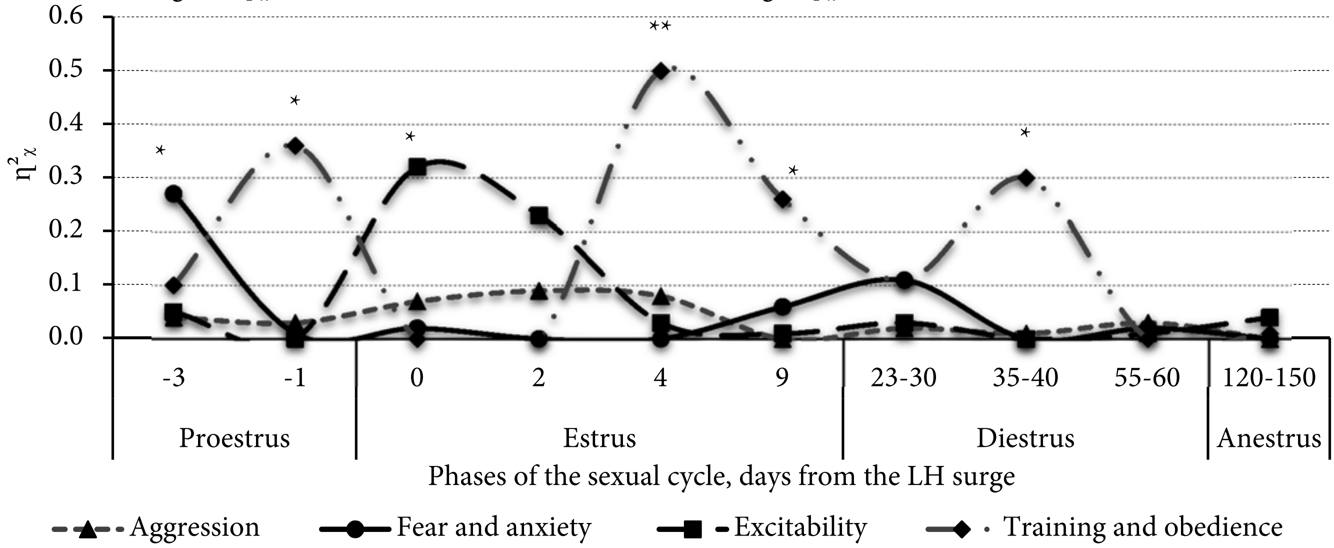

Figure 1. The strength of influence (η^2_χ) of individual temperament characteristics on the level of follicle-stimulating hormone in the blood plasma of bitches (n = 15; units). Reliable values: * — $P \leq 0.05$; ** — $P \leq 0.01$.

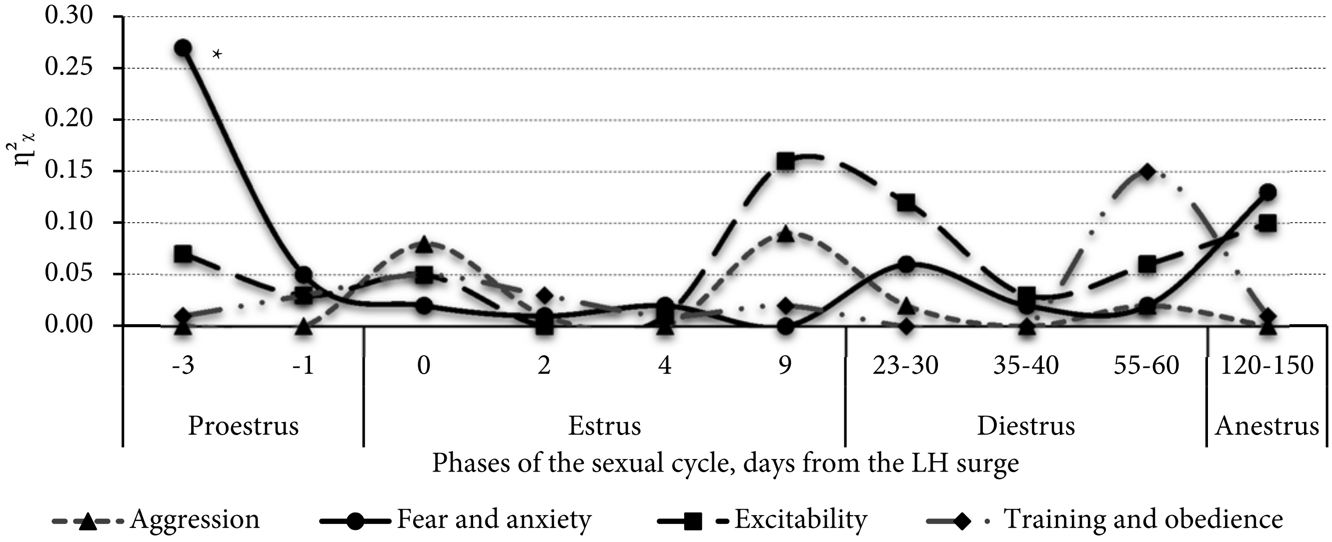

Figure 2. The strength of influence (η^2_χ) of individual temperament traits on the level of estradiol in the blood plasma of bitches (n = 15; units). Reliable values: * — $P \leq 0.05$.

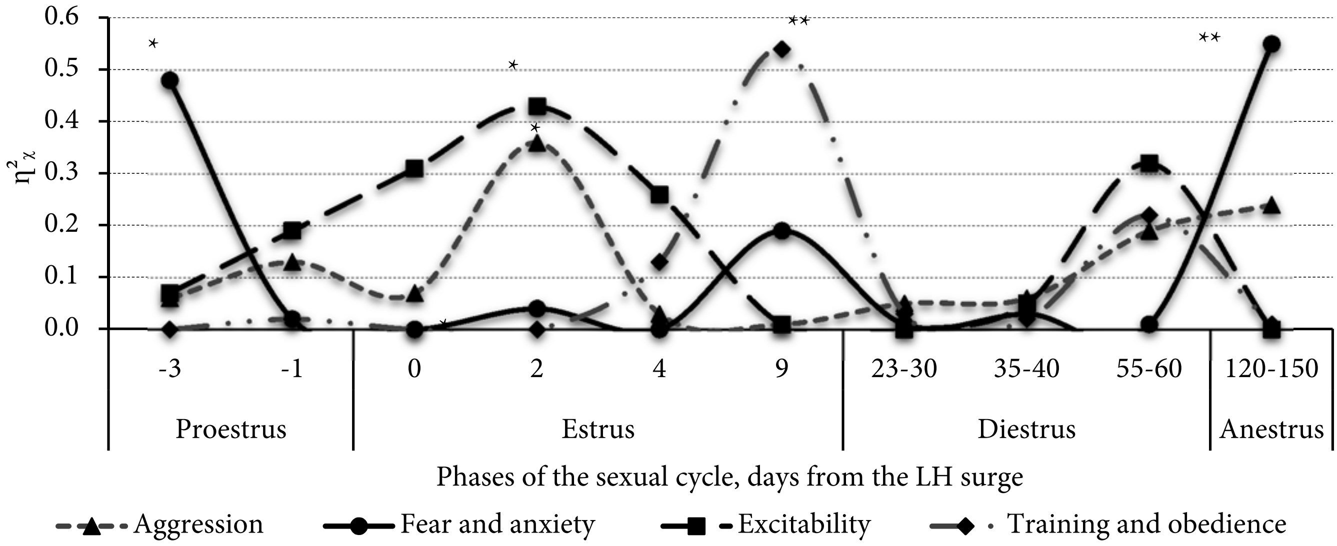

Figure 3. The strength of influence (η^2_χ) of individual temperament traits on the level of luteinizing hormone in the blood plasma of bitches (n = 15; units). Reliable values: * — P ≤ 0.05; ** — P ≤ 0.01.

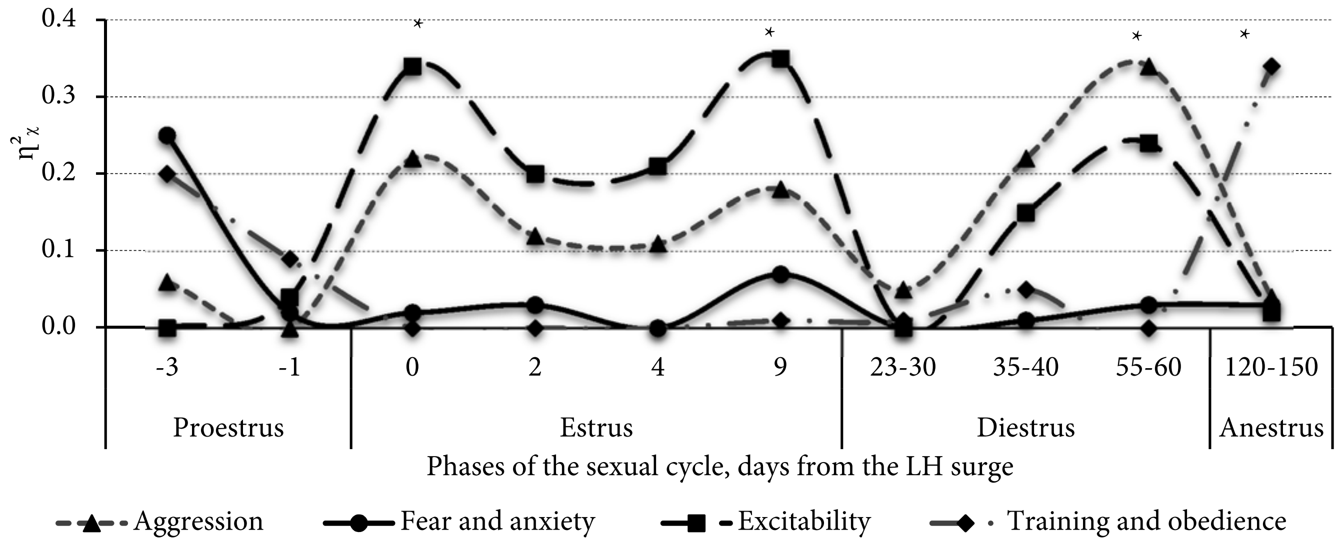

Figure 4. The strength of influence (η^2_χ) of individual temperament traits on the level of progesterone in the blood plasma of bitches (n = 15; units). Reliable values: * — P ≤ 0.05.

At the same time, the level of excitability of bitches had a significant effect on the hormone content on the day of the LH surge — $\eta^2_\chi = 0.34$ ($P \leq 0.05$) and on the 9th day after the LH surge — $\eta^2_\chi = 0.35$ ($P \leq 0.05$). In addition, a significant effect of trainability and obedience of bitches on the hormone content in blood plasma is manifested only on the 35th–40th days after the LH surge ($\eta^2_\chi = 0.34$; $P \leq 0.05$). No significant effect of the level of fear and anxiety in bitches on P4 content was found.

Discussion. The C-BARQ was developed as a quantitative tool for measuring behavioral problems in domestic and working dogs (Serpell and Hsu, 2005). Today, this tool is used not only to characterize dog behavior, but also to create various regression models and factor analyses of the etiology of problematic behavioral traits (Canejo-Teixeira et al., 2018; González-Ramírez, Quezada-Berumen and Landero-Hernández, 2017). The population size and distribution of individual and group temperament traits in dogs have been studied, providing an explanation for the physiological origin of behavior (Zapata et al., 2022). The genetic basis of individual temperament traits has been identified (Hecht et al., 2021; Zapata et al., 2022; Morrill et al., 2022), which to some extent limits the condition of body systems, including the condition of the reproductive system. Specifically, we found that the level of fear and anxiety in bitches affected plasma levels of FSH, E2, and LH ($P \leq 0.05$). Temperamental traits such as aggression, anxiety, and learning ability differ significantly between dog breeds and are heritable, but the neural basis for these differences is unknown (Hecht et al., 2021). The level of aggression in bitches has a significant effect on the LH level two days after the surge ($P \leq 0.05$) and on the P4 level on 55th–60th days after the LH surge ($P \leq 0.05$). It is known that aggressive behavior is the most common type of undesirable behavior in dogs (Boyd et al., 2018). Some researchers note that the leading factor in aggressive behavior in dogs is the environment, not their temperament (Davis et al., 2012).

Significant correlations have been found between temperament and gray matter morphology. The neural connections involved in the 'flee or fight' response are associated with temperamental traits such as fear and aggression, which appear to be the main behaviors under selection pressure during domestication from wolf to dog (Hecht et al., 2021). At the same time, researchers have noted that dog excitability determines the diversity of temperament profiles (Rosati and Hare, 2013). We found that excitability limits the levels of sex hormones in the blood of bitches largely than other temperament traits. In particular, the level of excitability of bitches had a significant effect on the levels of FSH, LH, P4 on the day of the LH surge ($P \leq 0.05$) and on the level of P4 on the 9th day after the LH surge ($P \leq 0.05$) and on the levels of LH on the 2nd, 4th, 55th–60th days and after the surge ($P \leq 0.05$).

The ability to learn is largely associated with expansion in broad areas of the cerebral cortex, whereas fear, aggression, and other 'problem' behaviors are associated with expansion in distributed subcortical areas (Hecht et al., 2021). Recently, experiments in dogs have confirmed earlier experiments in rats on the effects of prenatal stress on the ability to learn (Leroy et al., 2009). It has been found that the relationship between dog and owner is reflected in the temperament of the animal (Somppi et al., 2022). The ability to be trained and obedience limits the content of FSH in the blood plasma on the day before the surge and on the 4th, 9th, and 35th–40th days after the LH surge ($P \leq 0.05$). Also, this characteristic of temperament affects the content of LH in the blood plasma of animals only on the 9th day after the LH surge ($P \leq 0.01$). In addition, the ability to train and obedience of bitches has an effect on the content of P4 only on the 35th–40th days after the LH surge ($P \leq 0.05$).

Researchers have noted quite significant individual differences in the levels of sex hormones in the blood of bitches (Luz et al., 2006). When the levels of P4 were studied in a group of Beagle bitches during the sexual cycle, these variations were significantly reduced (Marinelli et al., 2009). Thus, these differences are breed specific, which was confirmed in our studies. However, we are the first to identify individual differences in the humoral status of bitches that are associated with animal temperament traits.

Conclusions. It was found that the degree of fear and anxiety in bitches affects the content of follicle-stimulating hormone, estradiol and luteinizing hormone in the blood plasma of bitches three days before, during and 120–150 days after the LH surge ($\eta^2_\chi = 0.27–0.55$ ($P \leq 0.05$). The excitability of bitches affects the level of follicle stimulating hormone, luteinizing hormone, and progesterone on the day of the LH surge — $\eta^2_\chi = 0.32$ ($P \leq 0.05$). In addition, the level of excitability affects the level of progesterone on the 9th day after the surge — $\eta^2_\chi = 0.35$ ($P \leq 0.05$) and the level of luteinizing hormone on the 2nd, 4th, 55th–60th days and after the LH surge — $\eta^2_\chi = 0.26–0.43$ ($P \leq 0.05$). The degree of aggression affects the luteinizing hormone level two days after the surge and the progesterone level on 55th–60th days after the LH surge ($\eta^2_\chi = 0.34–0.36$; $P \leq 0.05$). The ability to be trained and to obedience affects the follicle stimulating hormone level (the day before the surge and on the 4th, 9th, and 35th–40th days after the LH surge — $\eta^2_\chi = 0.\ 30$-0.52; $P \leq 0.05$), luteinizing hormone (on 9th day after LH surge, $\eta^2_\chi = 0.54$; $P \leq 0.01$), and progesterone (on 35th–40th days after LH surge, $\eta^2_\chi = 0.34$; $P \leq 0.05$).

References

Boyd, C., Jarvis, S., McGreevy, P., Heath, S., Church, D., Brodbelt, D. and O'Neill, D. (2018) 'Mortality resulting from undesirable behaviours in dogs aged under three years attending primary-care veterinary practices in England', *Animal Welfare*, 27(3), pp. 251–262. doi: 10.7120/09627286.27.3.251.

Canejo-Teixeira, R., Almiro, P. A., Serpell, J. A., Baptista, L. V. and Niza, M. M. R. E. (2018) 'Evaluation of the factor structure of the Canine Behavioural Assessment and Research Questionnaire (C-BARQ) in European Portuguese', *PLoS One*, 13(12), p. e0209852. doi: 10.1371/journal.pone.0209852.

Casey, R. A., Loftus, B., Bolster, C., Richards, G. J. and Blackwell, E. J. (2014) 'Human directed aggression in domestic dogs (*Canis familiaris*): Occurrence in different contexts and risk factors', *Applied Animal Behaviour Science*, 152, pp. 52–63. doi: 10.1016/j.applanim.2013.12.003.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Conley, A. J., Gonzales, K. L., Erb, H. N. and Christensen, B. W. (2023) 'Progesterone analysis in canine breeding management', *Veterinary Clinics of North America: Small Animal Practice*, 53(5), pp. 931–949. doi: 10.1016/j.cvsm.2023.05.007.

Cooper, C. E. and Withers, P. C. (2008) 'Animal Physiology', in *Encyclopedia of Ecology. Vol. 3.* 2nd ed. Elsevier, pp. 228–237. doi: 10.1016/B978-0-444-63768-0.00456-X.

Danchuk, O. V., Broshkov, M. M., Karpovsky, V. I., Bobrytska, O. M., Tsvivlikhovsky, M. I., Tomchuk, V. A., Trokoz, V. O. and Kovalchuk, I. I. (2020a) 'Types of higher nervous activity in pigs: Characteristics of behavior and effects of technological stress', *Neurophysiology*, 52(5), pp. 358–366. doi: 10.1007/s11062-021-09892-7.

Danchuk, O. V., Karposvkii, V. I., Tomchuk, V. A., Zhurenko, O. V., Bobryts'ka, O. M. and Trokoz, V. O. (2020b) 'Temperament in cattle: A method of evaluation and main characteristics', *Neurophysiology*, 52(1), pp. 73–79. doi: 10.1007/s11062-020-09853-6.

Davis, A. L., Schwebel, D. C., Morrongiello, B. A., Stewart, J. and Bell, M. (2012) 'Dog bite risk: An assessment of child temperament and child-dog interactions', *International Journal of Environmental Research and Public Health*, 9(8), pp. 3002–3013. doi: 10.3390/ijerph9083002.

Everett, J. W. (1969) 'Neuroendocrine aspects of mammalian reproduction', *Annual Review of Physiology*, 31(1), pp. 383–416. doi: 10.1146/annurev.ph.31.030169.002123.

Fratkin, J. L., Sinn, D. L., Patall, E. A. and Gosling, S. D. (2013) 'Personality consistency in dogs: A meta-analysis', *PLoS One*, 8(1), p. e54907. doi: 10.1371/journal.pone.0054907.

Gobello, C. (2007) 'New GnRH analogs in canine reproduction', *Animal Reproduction Science*, 100(1–2), pp. 1–13. doi: 10.1016/j.anireprosci.2006.08.024.

González-Ramírez, M. T., Quezada-Berumen, L. and Landero-Hernández, R. (2017) 'Assessment of canine behaviors using C-BARQ in a sample from Northern Mexico', *Journal of Veterinary Behavior*, 20, pp. 52–58. doi: 10.1016/j.jveb.2017.03.007.

Hecht, E. E., Zapata, I., Alvarez, C. E., Gutman, D. A., Preuss, T. M., Kent, M. and Serpell, J. A. (2021) 'Neurodevelopmental scaling is a major driver of brain–behavior differences in temperament across dog breeds', *Brain Structure and Function*, 226(8), pp. 2725–2739. doi: 10.1007/s00429-021-02368-8.

Hong, G. K., Payne, S. C. and Jane, J. A. (2016) 'Anatomy, physiology, and laboratory evaluation of the pituitary gland', *Otolaryngologic Clinics of North America*, 49(1), pp. 21–32. doi: 10.1016/j.otc.2015.09.002.

Hsu, Y. and Sun, L. (2010) 'Factors associated with aggressive responses in pet dogs', *Applied Animal Behaviour Science*, 123(3–4), pp. 108–123. doi: 10.1016/j.applanim.2010.01.013.

Leroy, H., Depiereux, E., Giffroy, J.-M. and Diederich, C. (2009) 'Effect of prenatal environment on learning abilities in puppies and in adult dogs', *Journal of Veterinary Behavior*, 4(6), p. 253. doi: 10.1016/j.jveb.2009.06.008.

Luz, M. R., Bertan, C. M., Binelli, M. and 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), pp. 1436–1441. doi: 10.1016/j.theriogenology.2006.01.036.

Marinelli, L., Rota, A., Carnier, P., Da Dalt, L. and Gabai, G. (2009) 'Factors affecting progesterone production in corpora lutea from pregnant and diestrous bitches', *Animal Reproduction Science*, 114(1–3), pp. 289–300. doi: 10.1016/j.anireprosci.2008.10.001.

Morrill, K., Hekman, J., Li, X., McClure, J., Logan, B., Goodman, L., Gao, M., Dong, Y., Alonso, M., Carmichael, E., Snyder-Mackler, N., Alonso, J., Noh, H. J., Johnson, J., Koltookian, M., Lieu, C., Megquier, K., Swofford, R., Turner-Maier, J., White, M. E., Weng, Z., Colubri, A., Genereux, D. P., Lord, K. A. and Karlsson, E. K. (2022) 'Ancestry-inclusive dog genomics challenges popular breed stereotypes', *Science*, 376(6592), p. eabk0639. doi: 10.1126/science.abk0639.

Okafor, I., Okpara, U. and Ibeabuchi, K. (2022) 'The reproductive functions of the human brain regions: A systematic review', *Journal of Human Reproductive Sciences*, 15(2), p. 102. doi: 10.4103/jhrs.jhrs_18_22.

Opel, H. (1979) 'The hypothalamus and reproduction in the female', *Poultry Science*, 58(6), pp. 1607–1618. doi: 10.3382/ps.0581607.

Rance, N. E., Krajewski, S. J., Smith, M. A., Cholanian, M. and Dacks, P. A. (2010) 'Neurokinin B and the hypothalamic regulation of reproduction', *Brain Research*, 1364, pp. 116–128. doi: 10.1016/j.brainres.2010.08.059.

Riemer, S., Müller, C., Virányi, Z., Huber, L. and Range, F. (2014) 'The predictive value of early behavioural assessments in pet dogs — A longitudinal study from neonates to adults', *PLoS One*, 9(7), p. e101237. doi: 10.1371/journal.pone.0101237.

Rosati, A. G. and Hare, B. (2013) 'Chimpanzees and bonobos exhibit emotional responses to decision outcomes', *PLoS One*, 8(5), p. e63058. doi: 10.1371/journal.pone.0063058.

Serpell, J. A. (2015). The C-BARQ Questionnaire. Available at: https://kenneltocouch.org/wp-content/uploads/2021/05/dog-aggression-questionnaire.pdf.

Serpell, J. A. (2023). C-BARQ. Available at: https://vetapps.vet.upenn.edu/cbarq.

Serpell, J. A. and Hsu, Y. A. (2005) 'Effects of breed, sex, and neuter status on trainability in dogs', *Anthrozoös*, 18(3), pp. 196–207. doi: 10.2752/089279305785594135.

Sherman, C. K., Reisner, I. R., Taliaferro, L. A. and Houpt, K. A. (1996) 'Characteristics, treatment, and outcome of 99 cases of aggression between dogs', *Applied Animal Behaviour Science*, 47(1–2), pp. 91–108. doi: 10.1016/0168-1591(95)01013-0.

Simmonds, R. C. (2017) 'Chapter 4. Bioethics and animal use in programs of research, teaching, and testing', in Weichbrod, R. H., Thompson, G. A. and Norton, J. N. (eds.) *Management of Animal Care and Use Programs in Research, Education, and Testing*. 2nd ed. Boca Raton: CRC Press, pp. 35–62. doi: 10.1201/9781315152189-4.

Somppi, S., Törnqvist, H., Koskela, A., Vehkaoja, A., Tiira, K., Väätäjä, H., Surakka, V., Vainio, O. and Kujala, M. V. (2022) 'Dog–owner relationship, owner interpretations and dog personality are connected with the emotional reactivity of dogs', *Animals*, 12(11), p. 1338. doi: 10.3390/ani12111338.

Strelau, J. (2008). *Temperament as a Regulator of Behavior: After Fifty Years of Research*. Clinton Corners, NY: Eliot Werner Publications.

Tena-Sempere, M. (2005) 'Hypothalamic KiSS-1: The missing link in gonadotropin feedback control?', *Endocrinology*, 146(9), pp. 3683–3685. doi: 10.1210/en.2005-0652.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Zapata, I., Eyre, A. W., Alvarez, C. E. and Serpell, J. A. (2022) 'Latent class analysis of behavior across dog breeds reveal underlying temperament profiles', *Scientific Reports*, 12(1), p. 15627. doi: 10.1038/s41598-022-20053-6.