Part 1. Veterinary medicine

UDC 619:615.357.036/.038:591.147.8:636.7/.8 **DOI 10.36016/JVMBBS-2022-8-1-2-1**

CORRECTION OF THE SEXUAL FUNCTION IN DOMESTIC ANIMALS BY MEGESTROL ACETATE

Paliy A. P. [1], Dotsenko K. A. [1], Pavlichenko O. V. [2], Palii A. P. [2], Rodionova K. O. [3]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: paliy.dok@gmail.com
[2] State Biotechnological University, Kharkiv, Ukraine
[3] Odesa State Agrarian University, Odesa, Ukraine

Summary. Correction of sexual function in animals is a topical issue today, despite a large amount of scientific data on this problem. The study aimed to scientifically substantiate the effectiveness of hormonal veterinary drugs in cats and dogs of different breeds and genders. Veterinary drugs with the active substance megestrol acetate were used in the experiments. The studies were performed on clinically healthy cats and dogs of both sexes, different breeds, and ages vaccinated against infectious diseases and treated with antiparasitic drugs. The study was conducted according to the current regulatory documentation. It has been found that the use of hormonal drugs to interrupt/delay estrus in female cats at a dose of 5 mg of megestrol acetate for 8 days leads to a significant decrease in follicle-stimulating hormone, and the content of luteinizing hormone in the blood of cats in the experimental groups reliably decreased throughout the experiment. The use of contraceptives based on megestrol acetate in doses of 2.5 mg for 14 days for cats leads to a reliable decrease in the concentration of testosterone in the serum. It was found that the use of experimental hormonal drugs for female dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day, leads to a reliable decrease in concentrations of follicle-stimulating and luteinizing hormones in serum compared to the control. Oral administration of experimental hormonal drugs to male dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight for 8 days, led to a reliable decrease in the concentration of testosterone in the serum of males. It was found that the estrous cycle in female cats and dogs of the experimental groups after the cessation of hormonal drugs was completely restored, which indicates the safety of drugs with an active ingredient megestrol acetate

Keywords: dogs, cats, hormonal drugs

Introduction. The issue of preserving the reproductive potential of animals is a very important issue in veterinary science and practice (Shakhova et al., 2021). In this regard, veterinary hormonal contraceptives, which are used to regulate the sexual cycle and correct behavior in cats and dogs, are especially popular (Wildt, Brown and Swanson, 1998; Romagnoli, 2015).

Conservative method of contraception is the use of synthetic hormonal highly active drugs (Ginel et al., 2012; Attalah et al., 2016). The use of contraceptives often provides rapid suppression of undesirable behavior of the female during estrus and creates an alternative to surgery (Vasetska, 2020; Paliy et al., 2021).

The effects of exogenous sex hormones on the hormonal status of female cats and dogs during the sexual cycle and changes in progesterone levels in animal blood remain poorly understood (Akison and Robker, 2012).

Therefore, there is a need for research in the field of suppression of sexual function in females of small domestic animals (dos, cats) for more effective and safe use of hormonal contraceptives and the development of new, alternative, non-surgical, safe schemes and methods of drug suppression of sexual arousal with minimal risk of pathology of the reproductive system, breast and adverse effects on the body as a whole (Sarasola et al., 2002; Asa, 2018; Urfer and Kaeberlein, 2019). The estrous cycle in cats and dogs is manifested by morphological changes that are cyclically repeated in the reproductive system of females, which are associated with the maturation of gametes and their release into the abdominal cavity during ovulation. Hormones of the hypothalamic-adenohypophyseal system and ovary are involved in the regulation of the estrous or sexual cycle (Burke, 1976; Aspinall, 2011). The estrus corresponds to the period of sexual activity of females and coincides in time with the maturation of follicles in the ovaries (follicular phase of the sexual cycle). It is also called one of the stages of the vaginal cycle of animals (corresponds to the end of the sexual cycle) (Concannon, 2011).

Manifestations of normal physiological activity of the reproductive system in cats and dogs are accompanied by hypersexual, aggressive and antisocial behavior, which brings significant inconvenience to owners. For a long time, scientists have been developing non-surgical, safe for animals, easy to use, most effective, with regulated period of effect on the body drugs for the suppression of sexual function of animals (Diederichs et al., 1991; Junttila, Huohvanainen and Tiira, 2021).

Drugs that regulate the sexual cycle in female cats are also widely used because of the specific behavior that a cat exhibits during estrus (Greenberg et al., 2013; Driancourt and Briggs, 2020).

An important requirement for hormonal contraceptives used in veterinary practice is long-term prevention (suppression of estrus), short-term delay of estrus or interruption of estrus at different stages of the sexual cycle (Refsdal, 2000).

Scientists have found that progestin megestrol acetate is the most commonly used. Megestrol acetate is a synthetic progestogen, affects the hypothalamic-pituitary system of the animal, blocks the secretion of gonadotropic hormones by the adenohypophysis (follicle-stimulating and luteinizing). Decreased levels of these hormones in the blood of animals lead to impaired folliculogenesis in females, which provides antiestrogenic and antiovulatory effects on the gonads of animals, resulting in delayed, suppressed, and interrupted estrus and suppression of sexual desire (Luvoni, 2000; Han et al., 2014; Jang et al., 2014).

Sexual desire in cats and dogs after receiving the full course dose is renewed in 3–4 months. Megestrol acetate is well absorbed in the gastrointestinal tract and excreted with the urine within 1–2 days after administration to the animal's body (Mertens, 2006; Wiebe and Howard, 2009).

Megestrol acetate binds to cytoplasmic progesterone receptors. After transfer of progesterone receptor complexes from megestrol acetate to the cell nucleus, RNA synthesis is stopped, which in turn inhibits protein synthesis. This reduces the number of cytoplasmic estrogen receptors, that is estrogen cannot reach the target molecule and cause DNA damage in the cell nucleus.

Megestrol acetate posseses a high affinity for the progesterone receptors, and significant ability to bind to androgen and glucocorticoid receptors. Megestrol acetate reduces the release of follicle-stimulating hormone in the pituitary gland and, as a consequence, slows down the synthesis of estrogen in the ovaries relative to the synthesis of androgen in the testes. Megestrol acetate counteracts the stimulating effect of estrogen on the growth of hormone-receptor of positive cell lines, reduces the secretion of luteinizing hormone in the pituitary gland. According to animal studies, megestrol acetate reduces the secretion of prolactin in the pituitary gland. According to studies in animals, megestrol acetate reduces the secretion of adrenocorticotropic hormone in the pituitary gland (Colon et al., 1993; Kutzler and Wood, 2006).

Megestrol acetate is almost completely absorbed. After oral administration of one dose of megestrol acetate, the maximum concentration in blood plasma is reached within 2–3 h. The level of concentration in blood plasma depends on the dose, but is not directly proportional to it (Chainey, McCoubrey and Evans, 1970). The half-life is 15–20 h. At steady state, which is reached on the 3rd day of oral administration of the drug, the peak concentration in plasma is 90%. Megestrol acetate is metabolized in the liver. According to the results of the analysis of urine, which was collected within 7 days, megestrol acetate is excreted from the body in the urine by 56–78%, and in the feces for the same period — by 8–30%.

Along with the use of hormonal drugs, the issue of preserving the functions of germ cells for further reproduction of animals both naturally and through biotechnological methods remains relevant (Smorag et al., 2008; Shakhova et al., 2020).

The aim of the study was to scientifically substantiate the effectiveness of hormonal veterinary drugs in cats and dogs of different breeds and genders.

Materials and methods. Veterinary drugs were used in the experiments:

— composition of the drug No. 1 (1 tablet (0.25 g)): active substance: megestrol acetate (5 mg); excipients: lactose, calcium stearate;

— composition of the drug No. 2 (1 tablet (0.30 g)): active substance: megestrol acetate (5 mg); excipients: powdered sugar, calcium carbonate, carboxymethylcellulose sodium salt, polyvinylpyrrolidone, flavoring, magnesium stearate, sucram.

Studies of the effectiveness of veterinary drugs were conducted in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv) and in the animal shelter (Balaklia, Kharkiv Region).

The experiments were performed on clinically healthy cats and dogs of both sexes, different breeds and ages, vaccinated against infectious diseases and treated with antiparasitic drugs.

Cats and dogs were kept in cages on a standard diet with free access to water.

Prior to the experiment, the animals underwent clinical studies, including examination, palpation, thermometry, studies of respiratory rate, heart rate. Animal body weight, fatness, condition of skin, auricles, teeth, oral mucosa were recorded. The hormonal status of cats and dogs was studied, and the phases of the sexual cycle (follicular and luteal) were determined. During the quarantine period, cats and female cats of both experimental groups showed signs of sexual hunting. The obtained data were entered into individual registration cards of animals. Prior to the experiment, the animals of the experimental groups were treated against ectoparasites and helminths, after that they were quarantined for one month (Kotsiumbas, 2013).

To conduct research, control and research groups were formed on the principle of analogues, taking into account body weight, age and type of animal constitution.

At the first stage of the study, experiments were conducted to establish the effectiveness of veterinary drugs in female cats and cats. For this purpose, one control and two experimental groups of female cats with five animals in each were formed. The animals had a

normal estrous cycle. Female cats of the experimental group I on the 1st day of sexual hunting were given oral drug No. 1 for interruption of heat (1 tablet daily for 8 days from the beginning of heat).

Female cats of the second experimental group were given drug No. 2 following the similar scheme.

The cats in the control group were not given hormonal contraceptives. The animals received dry food and pure drinking water. Prior to administration, on the 1st, 3rd, 5th, and 8th days of the experiment, blood samples were taken from cats for endocrinological studies. Quantitative determination of progesterone, follicle-stimulating hormone, and luteinizing hormone was performed.

The next stage of research was to conduct an experiment on cats. At the appearance of signs of sexual arousal, hormonal drugs were given to cats 1–2 tablets per day for 14 days for sedation. Cats in the control group were not given hormonal contraceptives. On 1st, 3rd, 7th, and 14th days of the experiment, blood samples were taken from cats to quantify testosterone.

At the second stage of the study, a series of experiments were conducted to establish the effectiveness of hormonal veterinary drugs on female and male dogs. Females of the first experimental group were orally administered the drug No. 1 according to the scheme for interruption of the heat (1 tablet per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day). Females of the second experimental group were given drug No. 2 following a similar scheme.

Female dogs of the control group were not given hormonal contraceptives. The animals received dry food and pure drinking water. On 1st, 3rd, 4th, 7th, and 10th days of the experiment, blood samples were taken from female dogs to determine progesterone, follicle-stimulating hormone, and luteinizing hormone.

In the second experiment, hormonal drugs were given to female dogs following the scheme to delay heat (1 tablet per 10 kg of body weight for 7 days before the start of heat for 32 days). On 1st, 7th, 14th, and 32nd days of the experiment, blood samples were taken from bitches to quantify progesterone, follicle-stimulating hormone, and luteinizing hormone.

In an experiment on male dogs, when signs of sexual arousal, the drugs were given to calm down following the scheme: 1 tablet per 2.5 kg of body weight for 8 days, then 1–2 tablets per 2.5 kg for the next 8 days. On 1st, 5th, 7th, 10th, and 16th days of the experiment, blood samples were taken from males to quantify testosterone.

The blood of cats and dogs for testing was obtained from the forearm lateral subcutaneous vein (*v. cephalica*). Blood sampling was performed following the rules of asepsis and antiseptics. Serum was obtained by settling for 15 min in test tubes in a thermostat. The serum was separated from the clot with the help of a stainless steel rod. Centrifugation was performed at 3,000 rpm for 20 min. Serum was collected using a dosing pipette into sterile Eppendorf tubes.

The concentration of sex hormones, namely progesterone, follicle-stimulating hormone, and luteinizing hormone was determined in the serum of female cats and dogs. Testosterone levels were determined in the serum of male cats and dogs. Fresh serum was used for the studies.

Enzyme-linked immunosorbent assays of blood to determine the concentration of progesterone, follicle-stimulating hormone, and luteinizing hormone in the serum of female cats and dogs were performed using 'Granum' test systems (Ukraine).

The principle of competitive enzyme-linked immunosorbent assay was used to study progesterone. The test sample and conjugate (peroxidase-labeled progesterone) were added to the well with the immobilized antigen (specific anti-progesterone antibodies). The progesterone in the sample competes with the conjugate for binding to the antigenic surface of the well. After washing, the activity of the enzyme bound on the surface of the well was shown by the addition of substrate and measured at a wavelength of 450 nm. The intensity of the color reaction was inversely proportional to the amount of progesterone in the sample.

The principle of two-site enzyme-linked immunosorbent assay (sandwich method) was used to study luteinizing hormone. The test sample and conjugate (second peroxidase-labeled anti-luteinizing hormone antibodies) were added to the well with the immobilized antigen (specific luteinizing hormone antibodies). Luteinizing hormone from the sample binds to the antigen on the surface of the well and the conjugate.

Unbound material was removed by washing. After washing, the activity of the enzyme bound on the surface of the well was shown by adding the substrate and measured at a wavelength of 450 nm. The intensity of the color reaction was directly proportional to the amount of luteinizing hormone in the sample.

The principle of two-site enzyme-linked immunosorbent assay (sandwich method) was used to study follicle-stimulating hormone. The test sample and conjugate (second peroxidase-labeled anti-follicle-stimulating hormone antibodies) were added to the well with the immobilized antigen (specific anti-follicle-stimulating hormone antibodies). Follicle-stimulating hormone from the sample binds to the antigen on the surface of the well and the conjugate. Unbound material was removed by washing. After washing, the activity of the enzyme bound on the surface of the well was shown by the addition of substrate and is measured at a wavelength of 450 nm. The intensity of the color reaction is directly proportional to the amount of follicle-stimulating hormone in the sample.

The principle of competitive enzyme-linked immunosorbent assay was used to study testosterone. The test sample and conjugate (peroxidase-labeled testosterone) were added to the well with the immobilized antigen (specific anti-testosterone antibodies). The testosterone from the sample competes

with the conjugate for binding to the antigen on the surface of the well. After washing, the activity of the enzyme bound on the surface of the well was shown by the addition of substrate and measured at a wavelength of 450 nm. The intensity of the color reaction is inversely proportional to the amount of testosterone in the sample.

Statistical processing of the results was performed by Student's *t*-test with STATISTICA v. 10.0 for Windows (Rebrova, 2006).

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). The research program was reviewed and approved by the Bioethics Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in the current order.

Results and discussions. To achieve this goal, a series of experiments were conducted to determine the effectiveness of hormonal contraceptive veterinary drugs in female cats, cats, female and male dogs.

When studying the effectiveness of veterinary drugs in female cats and cats, we conducted clinical studies of animals in control and research groups. No deviations from the physiological norm were registered. Animals of the control and experimental groups were active, willingly took food and water, visible mucous membranes were pink, normal respiration and heart rate, average fatness. It was found that throughout the experiment, female cats periodically spontaneously aroused. In animals of the experimental groups, signs of sexual desire were registered before the use of veterinary drugs, as well as after discontinuation of drugs, when the sexual cycle resumed. It was found that the use of hormonal drugs leads to the suppression of signs of sexual desire in female cats of experimental groups.

Observations of the behavior of female cats showed that within 3–5 days of drug application, the sexual activity of animals was significantly reduced compared with the control group. In control group female cats, which did not received hormonal contraceptives during the experiment, showed clinical signs and behavioral changes characteristic of the stage of sexual arousal.

As a result of a comprehensive study of the effectiveness of experimental drugs, we determined the dynamics of the concentration of sex hormones in the serum of female cats and cats (Table 1).

Fig. 1 shows the dynamics of progesterone content in the serum of female cats receiving hormonal drugs to delay heat (M ± m, n = 5).

Fig. 1 shows that oral administration of hormonal drugs to delay heat at a dose of 5 mg of megestrol acetate every two weeks led to a reliable decrease in the concentration of progesterone in the serum, compared with control.

The data obtained (Table 1) indicate that the use of drugs for interruption of heat in female cats at a dose of 5 mg of megestrol acetate for 8 days led to a reliable decrease in follicle-stimulating hormone levels in animals of experimental groups I and II, namely: on the 3rd day — by 65.34% and 66.48% relative to control, on the 5th day — 48.18% and 49.27% respectively, on the 8th day — 73.10% and 69.66% respectively.

It was found that the content of luteinizing hormone in the blood of female cats of experimental groups I and II reliably decreased during the entire experimental period: on the 1st day — by 15.51% and 20.32% relative to control, on the 3rd day — 10.17% and 6.78% respectively, on the 7th day — 42.98% and 45.71% respectively, on the 14th day — 46.38% and 44.60% respectively. It should be noted that when using the drugs in females of the experimental groups we did not register changes in the general clinical state, animals willingly consumed food and water.

To study the effectiveness of hormonal drugs to delay heat in female cats, the concentration of sex hormones was determined (Table 2).

From Table 2 it is seen that oral administration of experimental hormonal drugs to female cats at a dose of 5 mg of megestrol acetate every 2 weeks, leads to a reliable decrease in follicle-stimulating hormone levels in animals of experimental groups I and II, namely: on the 3rd day — 51.98% and 57.43% compared to control, on the 5th day — 45.66% and 49.13% respectively, on the 8th day — 65.31% and 63.78% respectively.

It has been found that the content of luteinizing hormone in the blood of female cats of experimental groups I and II reliably decreased throughout the experiment period: on the 1st day — 18.75% and 10.68% compared to control, on the 3rd day — 27.04% and 30.53% respectively, on the 7th day — 50.65% and 45.29% respectively, on the 14th day — 34.69% and 31.23% respectively.

Experiments have shown that the use of contraceptives based on megestrol acetate in doses of 2.5 mg for 14 days in cats leads to a reliable decrease in serum testosterone (Table 3).

Table 3 shows that application of hormonal drugs to cats of experimental groups I and II at a dose of 2.5 mg of megestrol acetate for two weeks causes a reliable decrease in the concentration of testosterone in the serum: on the 3rd day — 56.85% and 52.15% relative to control, on the 7th day — 67.16% and 66.42% respectively, on the 14th day — 55.20% and 52.65% respectively.

According to the results of observations, it was found that the use of hormonal drugs in cats reduces the clinical manifestations of sexual arousal.

According to the results of the studies, the application of experimental hormonal drugs to female dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day, leads to a reliable decrease in concentrations of follicle-stimulating and luteinizing hormones in blood serum, compared with control indicators (Table 4).

Table 1 — The concentration of hormones in the blood serum of female cats that received hormonal drugs for the interruption of heat (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	5th	8th
Follicle-stimulating hormone, ng/ml					
Control	0.49 ± 0.01	0.56 ± 0.04	1.76 ± 0.04	2.74 ± 0.03	1.45 ± 0.01
Experimental I	0.58 ± 0.03	0.55 ± 0.06	0.61 ± 0.05**	1.42 ± 0.02**	0.39 ± 0.04**
Experimental II	0.56 ± 0.01	0.57 ± 0.03	0.59 ± 0.02**	1.39 ± 0.04**	0.44 ± 0.02**
Luteinizing hormone, nmol/l					
Control	3.26 ± 0.04	3.74 ± 0.14	6.49 ± 0.04	14.33 ± 0.15	20.18 ± 0.13
Experimental I	3.20 ± 0.06	3.16 ± 0.07**	5.83 ± 0.01**	8.17 ± 0.01**	10.82 ± 0.07***
Experimental II	3.32 ± 0.01	2.98 ± 0.02**	6.05 ± 0.02**	7.78 ± 0.03**	11.18 ± 0.11***

Notes:** — $p < 0.01$,*** — $p < 0.001$ according to the indicator in the control.

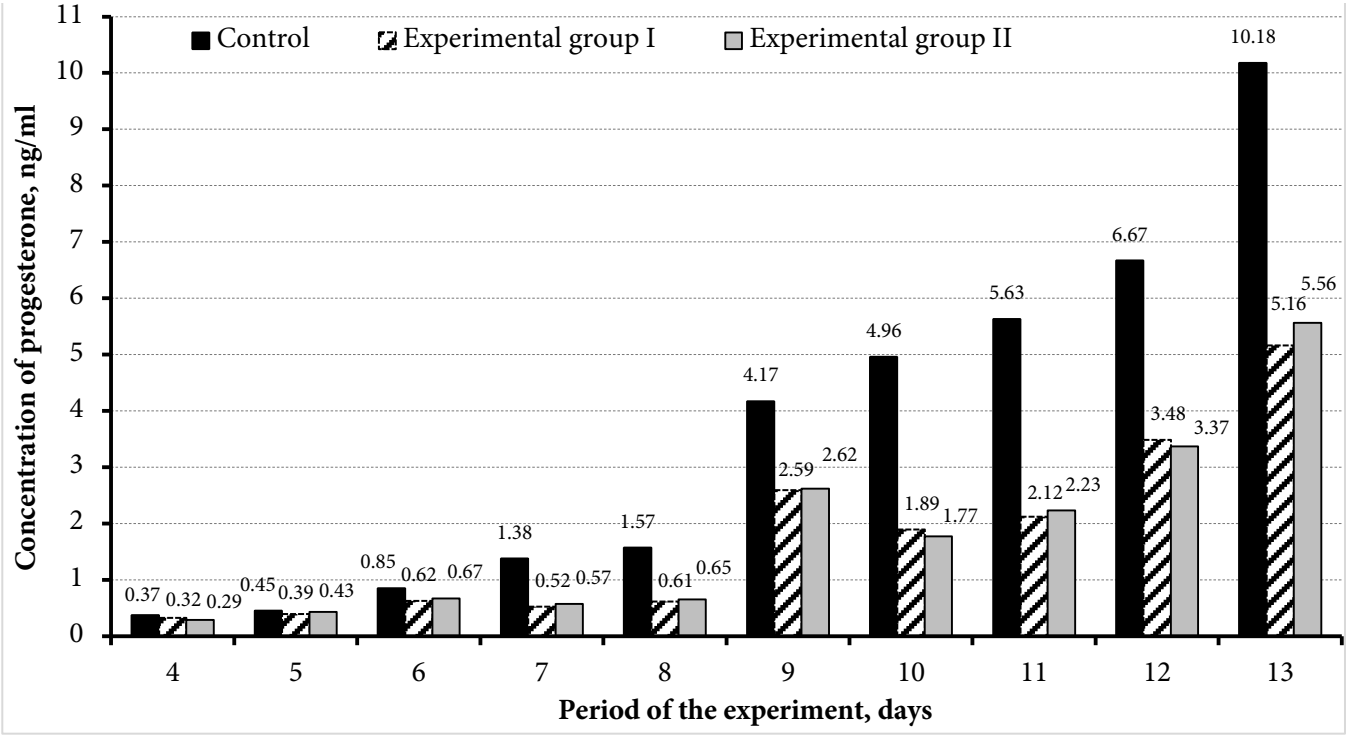

Figure 1. Dynamics of progesterone levels in the serum of female cats receiving hormonal drugs to delay heat (M ± m, n = 5)

Table 2 — The concentration of hormones in the serum of female cats receiving hormonal drugs to delay heat (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	7th	14th
Follicle-stimulating hormone, ng/ml					
Control	0.59 ± 0.02	0.64 ± 0.05	2.02 ± 0.06	1.73 ± 0.02	1.96 ± 0.04
Experimental I	0.61 ± 0.05	0.60 ± 0.02	0.97 ± 0.03*	0.94 ± 0.15*	0.68 ± 0.07**
Experimental II	0.57 ± 0.02	0.58 ± 0.07	0.86 ± 0.05*	0.88 ± 0.10**	0.71 ± 0.03**
Luteinizing hormone, nmol/l					
Control	4.16 ± 0.09	3.84 ± 0.11	8.32 ± 0.10	14.55 ± 0.21	19.31 ± 0.52
Experimental I	3.58 ± 0.07	3.12 ± 0.09*	6.07 ± 0.08**	7.18 ± 0.12**	12.61 ± 0.10**
Experimental II	4.27 ± 0.04	3.43 ± 0.05*	5.78 ± 0.11**	7.96 ± 0.15**	13.28 ± 0.19**

Notes:* — $p < 0.05$,** — $p < 0.01$ according to the indicator in the control.

Table 3 — The concentration of testosterone (nmol/l) in the serum of cats, which received hormonal drugs to calm down when signs of sexual arousal (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	7th	14th
Control	4.26 ± 0.01	4.29 ± 0.07	4.89 ± 0.07	5.45 ± 0.12	4.71 ± 0.04
Experimental I	4.15 ± 0.03	3.78 ± 0.04	2.11 ± 0.02*	1.79 ± 0.04*	2.11 ± 0.01*
Experimental II	3.96 ± 0.02	4.26 ± 0.03	2.34 ± 0.05*	1.83 ± 0.02*	2.23 ± 0.05*

Note.* — $p < 0.05$ according to the indicator in the control.

Table 4 — The concentration of hormones in the serum of female dogs, which received hormonal drugs (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	1st	3rd	5th	8th
Follicle-stimulating hormone, ng/ml					
Control	0.65 ± 0.01	0.73 ± 0.04	2.31 ± 0.01	2.97 ± 0.16	1.67 ± 0.19
Experimental I	0.56 ± 0.03	0.52 ± 0.06*	1.16 ± 0.03*	0.82 ± 0.01**	0.51 ± 0.05**
Experimental II	0.69 ± 0.05	0.68 ± 0.03*	1.57 ± 0.02*	0.94 ± 0.02**	0.46 ± 0.01**
Luteinizing hormone, nmol/l					
Control	3.21 ± 0.10	4.52 ± 0.15	8.12 ± 0.24	14.27 ± 0.15	19.57 ± 0.32
Experimental I	2.79 ± 0.12	4.18 ± 0.12	3.15 ± 0.17*	2.98 ± 0.12**	4.19 ± 0.25**
Experimental II	3.30 ± 0.14	4.26 ± 0.31	3.47 ± 0.29*	3.12 ± 0.11**	4.83 ± 0.42**

Notes:* — $p < 0.05$,** — $p < 0.01$ according to the indicator in the control.

Table 4 shows that oral administration of experimental drugs to female dogs at a dose of 5 mg of megestrol acetate per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4th to the 10th day, leads to delayed heat and a reliable decrease in follicle-stimulating hormone in animals of experimental groups I and II, namely: on the 1st day — 28.77% and 6.85% compared to control, on the 3rd day — 49.78% and 32.03% respectively, on the 5th day — 72.39% and 68.35% respectively, on the 8th day — 69.46% and 72.46% respectively.

It was found that the content of luteinizing hormone in the blood of female dogs of experimental groups I and II reliably decreased during the entire experiments: on the 3rd day — 61.21% and 57.16% respectively, on the 5th day — 79.11% and 78.14% respectively, on the 8th day — 78.59% and 75.32% respectively. It should be noted that during the application of drugs in female dogs of the experimental groups we did not register changes in the general clinical condition, animals willingly consumed food and water, the behavior did not differ from the animals of the control group.

Experimental drugs were used to delay heat (Table 5).

Table 5 shows that oral administration of drugs to female dogs in doses of 5 mg of megestrol acetate per 10 kg of body weight a week before the heat leads to a reliable decrease in the concentration of follicle-stimulating hormone in the blood of female dogs of the groups I and II on the 1st day — 31.33% and 24.09% compared to control, on the 3rd day — 69.70% and 67.15% respectively, on the 5th day — 83.41% and 81.52% respectively, on the 8th day — 82.39% and 77.84% respectively.

During the experiment, the concentration of luteinizing hormone was reliably lower than the control level, in particular: on the 3rd day — 53.25% and 53.92% respectively, on the 5th day — 79.03% and 79.62% respectively, on the 8th day — 87.15% and 86.21% respectively. In female dogs of the control group, the concentration of hormones was within the physiological norm, according to the specific phase of the sexual cycle.

During the clinical examination of female dogs of experimental groups I and II, no signs of heat in female dogs were registered.

According to the results of experimental studies, it was found that hormonal drugs affect the level of testosterone in the serum of male dogs (Table 6). The results of the experiment showed that oral administration of experimental hormonal drugs to male dogs in doses of 5 mg of megestrol acetate per 2.5 kg of body weight for 8 days, led to a reliable decrease in testosterone concentration in serum of male dogs of both experimental groups on the 3rd day by 68.99% and 68.84% respectively, on the 7th day — 26.85% and 21.75% respectively, on the 14th day — 53.98% and 51.99% respectively. The concentration of testosterone in the blood of male dogs of the control group was within the physiological norm. It was found that the estrous cycle in female cats and dogs of the experimental groups after the cessation of hormonal drugs was completely restored, which indicates the safety of drugs with the active ingredient megestrol acetate.

Table 5 — The concentration of hormones in the serum of female dogs, which received hormonal drugs to delay heat (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	**1st**	**3rd**	**5th**	**8th**
Follicle-stimulating hormone, ng/ml					
Control	0.78 ± 0.04	0.83 ± 0.03	2.74 ± 0.09	2.11 ± 0.07	1.76 ± 0.04
Experimental I	0.71 ± 0.02	0.57 ± 0.03*	0.83 ± 0.06**	0.35 ± 0.01**	0.31 ± 0.02**
Experimental II	0.68 ± 0.08	0.63 ± 0.04**	0.90 ± 0.02**	0.39 ± 0.06**	0.39 ± 0.05***
Luteinizing hormone, nmol/l					
Control	2.84 ± 0.03	4.05 ± 0.05	7.53 ± 1.02	15.21 ± 1.13	19.22 ± 2.11
Experimental I	2.79 ± 0.07	3.99 ± 0.11	3.52 ± 0.04*	3.19 ± 0.12*	2.47 ± 0.18**
Experimental II	2.80 ± 0.12	4.16 ± 0.12	3.47 ± 0.08**	3.10 ± 0.15**	2.65 ± 0.23***

Notes:* — $p < 0.05$,** — $p < 0.01$,*** — $p < 0.001$ according to the indicator in the control.

Table 6 — The concentration of testosterone (nmol/l) in the serum of male dogs, which received hormonal drugs at the appearance of signs of sexual arousal for sedation (M ± m, n = 5)

Groups	Periods of the experiment, days				
	Before administration	**1st**	**3rd**	**7th**	**14th**
Control	5.87 ± 0.32	6.67 ± 0.54	6.58 ± 0.09	2.16 ± 0.13	4.52 ± 0.23
Experimental I	5.26 ± 0.16	3.11 ± 0.24	2.04 ± 0.13*	1.58 ± 0.10**	2.08 ± 0.15***
Experimental II	4.97 ± 0.12	3.34 ± 0.19	2.05 ± 0.18**	1.69 ± 0.18**	2.17 ± 0.19***

Notes:* — $p < 0.05$,** — $p < 0.01$,*** — $p < 0.001$ according to the indicator in the control.

It should be noted that the pituitary gland secretes gonadotropins — luteinizing hormone and follicle-stimulating hormone (Scanes et al., 2005). Follicle-stimulating hormone stimulates ovarian estrogen production and testicular testosterone, egg follicle maturation, and spermatogenesis (Smitz et al., 2016). Luteinizing hormone is responsible for ovulation and corpus luteum formation in the ovaries and testosterone synthesis by Leydig cells in the testes (Rama Raju et al., 2013).

The active substance of the studied drugs is megestrol acetate, a synthetic progestogen that affects the hypothalamic-pituitary system of the animal and blocks the release of gonadotropic hormones (follicle-stimulating and luteinizing) (Pirzada, 2002). The systemic effect of megestrol acetate is higher than administration in nanocrystalline form (Jang et al., 2014).

Therefore, our results are consistent with the results of other researchers on the high effectiveness of hormonal drugs with the active substance megestrol acetate for sexual function suppression in animals.

Along with the use of hormonal veterinary drugs, a scientifically sound approach to the use of drugs for the destruction of pathogens of infectious and parasitic animal diseases remains relevant (Mateus et al., 2011; Paliy et al., 2019, 2020).

Conclusions. According to the results of experimental studies of hormonal drugs with the active substance megestrol acetate, it was found that they are well tolerated by domestic animals and do not cause side effects and changes in their clinical condition.

It has been scientifically proven that hormonal drugs have anti-estrogenic and anti-ovulatory effects. It has been found that the tested hormonal drugs are effective for interruption and suppression of heat in female cats and dogs. They also inhibit sexual activity and regulate the behavior of male cats and dogs.

The prospect of further research is to develop a scientifically sound scheme for the use of veterinary drugs for domestic animals, depending on their physiological condition, general epizootic situation and housing conditions.

References

Akison, L. and Robker, R. (2012) 'The critical roles of progesterone receptor (PGR) in ovulation, oocyte developmental competence and oviductal transport in mammalian reproduction: Progesterone receptor regulation of mammalian ovulation and oviductal transport', *Reproduction in Domestic Animals*, 47, pp. 288–296. doi: 10.1111/j.1439-0531.2012.02088.x.

Asa, C. S. (2018) 'Contraception in dogs and cats', *Veterinary Clinics of North America: Small Animal Practice*, 48(4), pp. 733–742. doi: 10.1016/j.cvsm.2018.02.014.

Aspinall, V. (2011) 'Reproductive system of the dog and cat: Part 1 — the female system', *Veterinary Nursing Journal*, 26(2), pp. 43–45. doi: 10.1111/j.2045-0648.2010.00013.x.

Attalah, E., Nasr, Y. S., El-Gammal, H. A. and Nour El-Dien, F. A. (2016) 'Optimisation and validation of a new analytical method for the determination of four natural and synthetic hormones using LC-ESI-MS/MS', *Food Additives and Contaminants: Part A*, 33(10), pp. 1545–1556. doi: 10.1080/19440049.2016.1227878.

Burke, T. J. (1976) 'Feline reproduction', *Veterinary Clinics of North America*, 6(3), pp. 317–331. doi: 10.1016/S0091-0279(76)50051-7.

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.

Chainey, D., McCoubrey, A. and Evans, J. (1970) 'The excretion of megestrol acetate by beagle bitches', *Veterinary Record*, 86(10), pp. 287–288. doi: 10.1136/vr.86.10.287.

Colon, J., Kimball, M., Hansen, B. and Concannon, P. W. (1993) 'Effects of contraceptive doses of the progestagen megestrol acetate on luteinizing hormone and follicle-stimulating hormone secretion in female dogs', *Journal of Reproduction and Fertility. Supplement*, 47, pp. 519–521. PMID: 8229972.

Concannon, P. W. (2011) 'Reproductive cycles of the domestic bitch', *Animal Reproduction Science*, 124(3–4), pp. 200–210. doi: 10.1016/j.anireprosci.2010.08.028.

Diederichs, W., Stief, C. G., Lue, T. F. and Tanagho, E. A. (1991) 'Sympathetic inhibition of papaverine induced erection', *Journal of Urology*, 146(1), pp. 195–198. doi: 10.1016/S0022-5347(17)37751-0.

Driancourt, M. A. and Briggs, J. R. (2020) 'Gonadotropin-releasing hormone (GnRH) agonist implants for male dog fertility suppression: A review of mode of action, efficacy, safety, and uses', *Frontiers in Veterinary Science*, 7, p. 483. doi: 10.3389/fvets.2020.00483.

Ginel, P. J., Sileo, M. T., Blanco, B., Garfia, B. and Quintavalla, F. (2012) 'Evaluation of serum concentrations of cortisol and sex hormones of adrenal gland origin after stimulation with two synthetic ACTH preparations in clinically normal dogs', *American Journal of Veterinary Research*, 73(2), pp. 237–241. doi: 10.2460/ajvr.73.2.237.

Greenberg, M., Lawler, D., Zawistowski, S. and Jöchle, W. (2013) 'Low-dose megestrol acetate revisited: A viable adjunct to surgical sterilization in free roaming cats?', *The Veterinary Journal*, 196(3), pp. 304–308. doi: 10.1016/j.tvjl.2013.01.038.

Han, J., Wang, Q., Wang, X., Li, Y., Wen, S., Liu, S., Ying, G., Guo, Y. and Zhou, B. (2014) 'The synthetic progestin megestrol acetate adversely affects zebrafish reproduction', *Aquatic Toxicology*, 150, pp. 66–72. doi: 10.1016/j.aquatox.2014.02.020.

Jang, K., Yoon, S., Kim, S.-E., Cho, J.-Y., Yoon, S. H., Lim, K. S., Yu, K.-S., Jang, I.-J. and Lee, H. (2014) 'Novel nanocrystal formulation of megestrol acetate has improved bioavailability compared with the conventional micronized formulation in the fasting state', *Drug Design, Development and Therapy*, 8, p. 851–858. doi: 10.2147/DDDT.S62176.

Junttila, S., Huohvanainen, S. and Tiira, K. (2021) 'Effect of sex and reproductive status on inhibitory control and social cognition in the domestic dog (*Canis familiaris*)', *Animals*, 11(8), p. 2448. doi: 10.3390/ani11082448.

Kotsiumbas, I. Ya. (ed.) (2013) *Clinical Studies of Veterinary Drugs and Feed Additives [Klinichni doslidzhennia veterynarnykh preparativ ta kormovykh dobavok]*. Lviv: SAM. ISBN 9668714091. [in Ukrainian].

Kutzler, M. and Wood, A. (2006) 'Non-surgical methods of contraception and sterilization', *Theriogenology*, 66(3), pp. 514–525. doi: 10.1016/j.theriogenology.2006.04.014.

Luvoni, G. C. (2000) 'Current progress on assisted reproductionin dogs and cats: *In vitro* embryo production', *Reproduction Nutrition Development*, 40(5), pp. 505–512. doi: 10.1051/rnd:2000114.

Mateus, A., Brodbelt, D. C., Barber, N. and Stärk, K. D. C. (2011) 'Antimicrobial usage in dogs and cats in first opinion veterinary practices in the UK', *Journal of Small Animal Practice*, 52(10), pp. 515–521. doi: 10.1111/j.1748-5827.2011.01098.x.

Mertens, P. A. (2006) 'Reproductive and sexual behavioral problems in dogs', *Theriogenology*, 66(3), pp. 606–609. doi: 10.1016/j.theriogenology.2006.04.007.

Paliy, A., Sumakova, N., Petrov, R., Shkromada, O., Ulko, L. and Palii, A. (2019) 'Contamination of urbanized territories with eggs of helmiths of animals', *Biosystems Diversity*, 27(2), pp. 118–124. doi: 10.15421/011916.

Paliy, A. P., Sumakova, N. V., Telyatnikov, A. V., Zhukova, I. O., Kasianenko, O. I., Shkromada, O. I., Suprun, Yu. O., Plyuta, L. V., Yevtushenko, I. D., Kovalenko, L. V., Dotsenko, E. A. and Palii, A. P. (2020) 'Study of the toxicity and effectiveness of an antiparasitic agent based on tinidazole and fenbendazole', *Ukrainian Journal of Ecology*, 10(6), pp. 272–279. doi: 10.15421/2020_293.

Paliy, A. P., Dotsenko, E. A., Kovalenko, L. M., Telyatnikov, A. V., Rodionova, K. O., Nikolenko, I. V., Matsenko, O. V., Sinyagovskay, K. A., Kazakov, M. V., and Palii, A. P. (2021) 'Assessment of the level of sex hormones in the blood of domestic animals when using contraceptives', *Ukrainian Journal of Ecology*, 11(3), pp. 205–212. Available at: https://www.ujecology.com/articles/assessment-of-the-level-of-sex-hormones-in-the-blood-of-domestic-animals-when-using-contraceptives.pdf.

Pirzada, O. L. (2002) 'Effect of megestrol caproate on the reproductive function of laboratory animals', *Bulletin of Experimental Biology and Medicine*, 133(6), pp. 574–576. doi: 10.1023/a:1020233925626.

Rama Raju, G., Chavan, R., Deenadayal, M., Govindarajan, M., Gunasheela, D., Gutgutia, R., Haripriya, G., Patel, N. and Patki, A. (2013) 'Luteinizing hormone and follicle stimulating hormone synergy: A review of role in controlled ovarian hyper-stimulation', *Journal of Human Reproductive Sciences*, 6(4), p. 227–234. doi: 10.4103/0974-1208.126285.

Rebrova, O. Yu. (2006) *Statistical Analysis of Medical Data: Using of STATISTICA Applied Package [Statisticheskiy analiz meditsinskikh dannykh: primenenie paketa prikladnykh programm STATISTICA]*. 3rd ed. Moscow: MediaSfera. ISBN 5890840134. [in Russian].

Refsdal, A. O. (2000) 'To treat or not to treat: A proper use of hormones and antibiotics', *Animal Reproduction Science*, 60–61, pp. 109–119. doi: 10.1016/S0378-4320(00)00094-4.

Romagnoli, S. (2015) 'Progestins to control feline reproduction: Historical abuse of high doses and potentially safe use of low doses', *Journal of Feline Medicine and Surgery*, 17(9), pp. 743–752. doi: 10.1177/1098612X15594987.

Sarasola, P., Jernigan, A. D., Walker, D. K., Castledine, J., Smith, D. G. and Rowan, T. G. (2002) 'Pharmacokinetics of

selamectin following intravenous, oral and topical administration in cats and dogs', *Journal of Veterinary Pharmacology and Therapeutics*, 25(4), pp. 265–272. doi: 10.1046/j.1365-2885.2002.00415.x.

Scanes, C. G., Jeftinija, S., Glavaski-Joksimovic, A., Proudman, J., Arámburo, C. and Anderson, L. L. (2005) 'The anterior pituitary gland: Lessons from livestock', *Domestic Animal Endocrinology*, 29(1), pp. 23–33. doi: 10.1016/j.domaniend.2005.04.002.

Shakhova, Y., Paliy, Anat., Paliy, And., Shigimaga, V., Kis, V. and Ivanov, V. (2020) 'Use of multicomponent cryoprotective media during cryopreservation of murine embryos by vitrification', *Problems of Cryobiology and Cryomedicine*, 30(2), pp. 203–206. doi: 10.15407/cryo30.02.203.

Shakhova, Y., Paliy, Anat., Paliy, And., Shkromada, O., Musiienko, Y. and Bondarenko, I. (2021) 'Influence of ways to thaw bull sperm on its quality', *Problems of Cryobiology and Cryomedicine*, 31(3), pp. 273–276. doi: 10.15407/cryo31.03.277.

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.

Smitz, J., Wolfenson, C., Chappel, S. and Ruman, J. (2016) 'Follicle-stimulating hormone: A review of form and function in the treatment of infertility', *Reproductive Sciences*, 23(6), pp. 706–716. doi: 10.1177/1933719115607992.

Smorag, Z., Katska-Ksiazkiewicz, L., Skrzyszowska, M., Jura, J., Gajda, B. and Bochenek, M. (2008) 'Animal reproduction biotechnology in Poland', *The International Journal of Developmental Biology*, 52(2–3), pp. 151–155. doi: 10.1387/ijdb.072325zs.

Urfer, S. R. and Kaeberlein, M. (2019) 'Desexing dogs: A review of the current literature', *Animals*, 9(12), p. 1086. doi: 10.3390/ani9121086.

Vasetska, A. (2020) 'Emergency contraception using progestin drugs in domestic cats', *Ukrainian Journal of Veterinary and Agricultural Sciences*, 3(2), pp. 3–6. doi: 10.32718/ujvas3-2.01.

Wiebe, V. J. and Howard, J. P. (2009) 'Pharmacologic advances in canine and feline reproduction', *Topics in Companion Animal Medicine*, 24(2), pp. 71–99. doi: 10.1053/j.tcam.2008.12.004.

Wildt, D. E., Brown, J. L. and Swanson, W. F. (1998) 'Reproduction in cats', in: Knobil, E. and Neill, J. D. (eds.) *Encyclopedia of Reproduction: Vol. 1*. New York: Academic Press, pp. 497–510. ISBN 9780122270208.

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].