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ORIGINAL ARTICLE

Assessment of the level of sex hormones in the blood of domestic animals when using contraceptives

A.P. Paliy[1*], E.A. Dotsenko[1], L.M. Kovalenko[2], A.V. Telyatnikov[3], K.O. Rodionova[3], I.V. Nikolenko[3], O.V. Matsenko[4], K.A. Sinyagovskay[4], M.V. Kazakov[5], A.P. Palii[6]

[1]National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", 83 Pushkinska St, Kharkiv, 61023, Ukraine
[2]Sumy National Agrarian University, 160 Gerasim Kondratieva St, Sumy, 40021, Ukraine
[3]Odesa State Agrarian University, 13 Panteleimonovskaya St, Odesa, 65012, Ukraine
[4]Kharkiv State Zooveterinary Academy, 1 Akademicheskaya St, urban settlement Malaya Danilivka, Dergachivsky district, Kharkiv region, 62341, Ukraine
[5]Luhansk National Agrarian University, 23 Svobody St, Sloviansk, Donetsk region, 84122, Ukraine
[6]Kharkiv Petro Vasylenko National Technical University of Agriculture, 44 Alchevskih St, Kharkiv, 61002, Ukraine
**Corresponding author E-mail: paliy.dok@gmail.com*
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Many methods and tools are used to control sexual function in dogs and cats. However, there are many unresolved issues related to the diagnosis of changes in the reproductive system of animals, multiplicity, dosage and method of contraception, development of innovative methods of reproductive suppression. The study aimed to scientifically substantiate the effectiveness of hormonal drugs with the active substance megestrol acetate in cats and dogs of different breeds and genders. The experiments used two hormonal drugs containing megestrol acetate 5 mg each. Quantitative determination of the concentration of sex hormones in the blood of animals using hormonal drugs was carried out following current guidelines and legal acts. Experiments have shown that the studied contraceptives are well tolerated by cats and dogs and do not cause side effects and changes in the clinical condition of animals. We found that the studied hormonal drugs, when administered orally, cause a probable decrease ($p<0.001$) in progesterone, follicle-stimulating, and luteinizing hormones in female cats and dogs, as well as a probable decrease ($p<0.001$) in testosterone levels in cats and male dogs compared to indicators in control groups. We determined that the studied hormonal drugs exhibit antiestrogenic and anovulatory effects when used following the recommended regimens.
Keywords: megestrol acetate, dogs, cats, follicle-stimulating hormone, luteinizing hormone, progesterone, testosterone, blood serum.

Introduction

Sexual suppression and contraception in female pets are the least studied issue in veterinary medicine compared to other areas of animal reproduction (Luvoni, 2000; Coffey, 2008; Shakhova et al., 2020). Most applied research in regulation and suppression of the female reproductive system cannot be considered sufficiently substantiated (Carroll & Lynch, 2016). In recent years, considerable attention has been paid to the study of behavioral responses of the body and the regulation of reproductive function in cats and dogs (Mertens, 2006). The effects of exogenous sex hormones on the hormonal status of female cats and dogs during the sexual cycle and changes in the level of progesterone in the blood of animals remain insufficiently studied (Rutteman & Misdorp, 1993; Wiebe & Howard, 2009; Aspinal, 2011). It is known that progesterone plays a significant role in the process of animal reproduction (Kim et al., 2009; Reynaud et al., 2015). It is crucial for successful ovulation in the ovaries and the multifaceted role of the fallopian tube in mammalian reproduction. Its effects are mediated by the progesterone receptor (PGR), which is strongly expressed in the ovaries, in particular, granulosa cells of preovulatory follicles in response to the burst of luteinizing hormone (LH), which occurs immediately before ovulation, and in the fallopian tube, mainly luminal epithelial cells and muscle cells (Akison & Robker, 2012). Areas of the brain that are sensitive to hormonal stimulation of sexual receptivity also contribute to the animal's procedural behavior (Takahashi, 1990; Lightfoot, 2008).

Keeping and raising pets requires in-depth knowledge of their physiological needs, among which the control of sexual function is of paramount importance. To this end, it is proposed to use some drugs that suppress behavioral characteristics and create an alternative to surgery (Reichler, 2009; Novotny et al., 2012; Vasetska, 2020).

Drugs used to interrupt the sexual cycle in pets are divided into two groups: herbal remedies and chemical hormonal agents: monohormonal – one hormone and bihormonal – two hormones in a fixed combination (Rhodes, 2015; Asa, 2018).

Herbal preparations (natural) are safer than hormonal drugs but have a significantly weaker effect on the animal's body. They contain a variety of herbs, the effect of which is limited to a calming effect (Riemer et al., 2021).

The active ingredients of chemical-hormonal drugs are various compounds, but they all belong to progestogens, which are steroid hormones. Depending on the species and physiological characteristics of the animals, these drugs are administered orally or administered subcutaneously to create a more prolonged effect (Kutzler & Wood, 2006; Urfer & Kaeberlein, 2019). Unreasonable use increases the likelihood of pathologies of the genital system, such as endometritis, plyometrics, tumors of the mammary glands, and genitals (Sleeckx et al., 2011). Therefore, their use should be justified, and the safety of the hormonal agents used should be established at the stages of their development and testing (Jewgenow et al., 2006; Munks, 2012; Driancourt & Briggs, 2020).

Synthetic progestogens include megestrol acetate – a drug that affects the hypothalamic-pituitary system of the animal and blocks the release of gonadotropic hormones (follicle-stimulating and luteinizing). Lack of these hormones in the blood delays the maturation of follicles in the ovaries of females, and in males testosterone synthesis is blocked, resulting in cessation of estrus and sexual desire in animals. In male dogs and cats, megestrol reduces sexual activity and related behavior (Nelson et al., 1973; Li et al., 2015).

The mechanism of action of megestrol acetate has two main directions of action on the body: anti gonadotropic activity is manifested in the suppression of the secretion of gonadotropic hormones and delayed ovulation, which is clinically a consequence of delayed estrus; the progestogenic effect is a direct effect on the endometrium of the uterus, which activates the secretory phase, which inhibits the implantation of the embryo, enhancing the contraceptive properties of the drug (Wanke et al., 2006).

Megestrol acetate is one of the most common synthetic analogs of progesterone. It inhibits the release of follicle-stimulating hormone, which leads to inhibition of estrus (Jang et al., 2014). Megestrol acetate is rapidly and completely absorbed from the gastrointestinal tract. The time to reach the maximum concentration in the serum is 2-3 hours and it circulates in the body for a long time (2-3 days). A small amount is deposited in adipose tissue. Excretion from the body by 50-70% is carried out by the kidneys, 30% – by the intestine (Greenberg et al., 2013). The endocrinological effect of megestrol acetate is manifested by a decrease in the secretion of gonadotropic hormones (FSH, LH) (Concannon & Meyers-Wallen, 1991).

Today, the scale of use of hormonal drugs for pets is increasing, which in turn necessitates the development of innovative, safe, and effective ways to suppress sexual function in animals.

Materials and methods

Our work aimed to substantiate scientifically in a comparative aspect the effectiveness of hormonal drugs with the active substance megestrol acetate in cats and dogs of different breeds and genders.

The experiments used hormonal drugs of the following compositions:

No 1 – 1 ml of the drug contains the active substance: megestrol acetate – 5 mg, excipients: propylene glycol, benzyl alcohol — transparent oily liquid, colorless or yellowish with a specific odor of the components. The drug was administered orally (fed to the animal with food or forced to enter directly into the oral cavity) in the following doses: Female cats: to interrupt estrus – 1 ml daily for 8 days from the beginning of estrus. Did not apply if 2 days have passed since the beginning of estrus; to delay estrus – 1 ml every 2 weeks. Cats: if there are signs of sexual arousal to calm down – 0.5 ml per day for 2 weeks. Bitches: to interrupt estrus – 1 ml per 2.5 kg of body weight in the first 3 days, from the 4^{th} to the10^{th} days half the daily dose was given; to delay estrus – 1 ml per 10 kg of body weight 7-15 days before an estrus. Males: for sexual soothing – 1 ml per 2.5 kg of body weight for 8 days, then – 0.5 ml per 2.5 kg for the next 8 days.

No 2 – 1 ml of the drug contains the active substance: megestrol acetate 5 mg, excipients: polyethylene glycol-400. Oily liquid from light gray to light yellow with a specific odor. The drug was used individually orally in the following doses: female cats: to prevent estrus – 1 ml of the drug for 10-14 days; to stop estrus – 1 ml of the drug daily for 3-5 days (from the moment of manifestation of signs of sexual arousal); as a contraceptive – 3 ml of the drug during the day after mating; cats: to soothe sexual arousal – 1 ml of the drug daily for 3-5 days; bitches: to prevent estrus – 1 ml of the drug per 10 kg of body weight once every 14 days; to stop estrus – 1 ml of the drug per 10 kg of body weight for 7 days (from the moment of manifestation of sexual arousal); as a contraceptive – 2 ml of the drug per 5 kg of body weight daily for 2 days after mating; males: to soothe sexual arousal – 1 ml of the drug per 10 kg of body weight daily for 5 days.

According to GOST 12.1.007-76 "Harmful substances. Classification and general safety requirements "studied hormonal drugs according to the degree of toxicity belong to low-hazard substances (4^{th} hazard class).

According to the purpose of the study, there have been carried out clinical trials of these drugs in the recommended doses to delay or interrupt estrus in bitches and cats, also we conducted a quantitative determination of the concentration of hormones in the blood of animals using the drugs.

The experiments used laboratory glassware, spectrophotometer, thermostat, dispensers, reagent kits for quantitative enzyme-linked immunosorbent assay of progesterone (PG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (TS) in the serum of cats and dogs, automatic single- and multi-channel dispensers of fixed or variable volume 5-1000 µl., general laboratory equipment, enzyme-linked immunosorbent assay with a wavelength of 450 nm.

Experimental studies were performed based on the laboratory of veterinary sanitation and parasitology of NSC "IECVM" (Kharkiv) and in the animal shelter (Balaklia, Kharkiv region). Before the experiment, disinfection of livestock facilities was performed with the determination of its quality (Paliy et al., 2020a; 2020b). The study was performed on clinically healthy cats and dogs of both sexes, different breeds, and ages vaccinated against infectious diseases. Cats and dogs were kept in cages on a standard diet with free access to water.

Before the experiment, the animals underwent clinical studies, which included examination, palpation, thermometry, the study of respiratory rate, heart rate. We recorded body weight of animals, fatness, condition of the skin, ears, teeth, mucous membranes of the oral cavity (Melihov & Shavrikova, 1999; Malcev et al., 2002). 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 of both experimental groups showed signs of sexual hunting. The obtained data were entered into individual registration cards of animals. Before the experiment, dogs and cats were treated against ectoparasites and helminths, after which they were quarantined for one month (Paliy et al., 2021).

Control and experimental groups were formed for experiments on the principle of analogs, taking into account body weight, age, and type of animal constitution (Stefanov et al., 2001; Kotsiumbas et al., 2006). The drugs were administered to the animals individually according to the instructions for use.

At the first stage of the research, a series of experiments were conducted to establish the effectiveness of veterinary drugs in female and male cats. The control group included female cats that did not receive synthetic progestogens and had a normal estrous cycle. According to the plan of the experiment, the animals of the experimental group on the first day of sexual hunting started to receive orally the drug No 1 according to the scheme for interruption of estrus (5 mg daily for 8 days). Female cats of the second experimental group were given the drug No 2 according to a similar scheme. Cats in the control group were given a solvent of 1 cm^3. Before administration, on the 1^{st}, 3^{rd}, 5^{th}, and 8^{th} days of the experiment, blood samples were taken from cats for endocrinological studies, namely, quantification of progesterone (PG), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (Polancev, 1998; Banks & Stabenfeldt, 1982). In the second experiment, the studied drugs were given to cats according to the scheme for delaying estrus (5 mg once every 14 days). Cats in the control group were given a solvent at a dose of 1 cm^3. On days 1, 3, 7, and 14 of the experiment, blood samples were taken from cats to quantify progesterone (PG), follicle-stimulating hormone (FSH), and luteinizing hormone (LH).

In the third experiment in male cats with signs of sexual arousal to calm down, the experimental drugs were given at a dose of 2.5 mg per day for 14 days. Cats in the control group were orally administered the solvent for the drug. On days 1, 3, 7, and 14 of the experiment, blood samples were taken from cats to quantify testosterone (TS).

In the second stage of the research, a series of experiments were conducted to establish the effectiveness of veterinary drugs on female and male dogs. Bitches of the first experimental group were orally administered the drug No 1 according to the scheme for interruption of estrus (5 mg per 2.5 kg of body weight in the first 3 days, from the 4^{th} to the10^{th} days – a half of the daily dose was given). Females of the second experimental group were given the drug No 2 according to a similar scheme. Bitches of the control group were given a solvent of the drug. On days 1, 3, 4, 7, 10 of the experiment, blood samples were taken from bitches to determine progesterone (PG), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). In the second experiment, the drugs were given to bitches according to the scheme for delaying estrus (5 mg per 10 kg of body weight 7 days before the beginning of estrus for 32 days). On days 1, 7, 14, and 32, blood samples were taken from bitches to quantify progesterone (PG), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). In the third experiment on males for sedation, when signs of sexual arousal, the experimental drugs were given in doses of 5 mg per 2.5 kg of body weight for 8 days, then 2.5 mg per 2.5 kg for the next 8 days. On days 1, 5, 7, 10, and 16 of the experiment, blood samples were taken from males to quantify testosterone (TS).

Blood for testing in cats and dogs was obtained from the lateral subcutaneous vein of the forearm (v. Cephalica). Blood sampling was performed following the rules of asepsis and antiseptics.

To obtain serum, the blood sample was settled for 15 minutes in test tubes in a thermostat. The separation of the serum from the clot on the inner wall of the tube was performed with a stainless steel rod and centrifuged at 3000 rpm for 20 minutes. Blood serum was collected using a dispenser pipette into sterile Eppendorf-type tubes.

Serum levels of progesterone (PG) and follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined in the serum of female cats and dogs (Goodrowe et al., 1989; Burke, 2006; Vasetska & Mass, 2016; 2017). Testosterone (TS) levels were determined in the serum of male cats and dogs. Fresh, impurity-free blood serum was used for the studies.

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

The principle of competitive enzyme-linked immunosorbent assay was used to study progesterone (PG). The test sample and conjugate (peroxidase-labeled progesterone) were added to the well of the plate with the immobilized antigen (specific anti-progesterone antibodies). The progesterone from the sample competes with the conjugate for binding to the antigenic surface of the well. After washing, the activity of the bound on the surface of the well plate enzyme was shown by adding the 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 (LH) (Directive 81/852/EEC and EU guideline; Shcherbakova, 2015; Zeynalov et al., 2017). The test sample and conjugate (second peroxidase-labeled anti-LH antibodies) were added to the well of the plate with the immobilized antigen (specific LH antibodies). LH 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 bound on the surface of the well plate enzyme 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 LH in the sample.

The principle of two-site enzyme-linked immunosorbent assay (sandwich method) (Directive 81/852/EEC and EU guideline; Commission Decision 2002/657/EC) was used to study follicle-stimulating hormone (FSH-ELISA). The test sample and conjugate (second peroxidase-labeled anti-FSH antibodies) were added to the well of the plate with the immobilized antigen (specific anti-FSH antibodies). FSH 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 bound on the surface of the well plate enzyme is manifested 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 FSH in the sample.

The principle of competitive enzyme-linked immunosorbent assay was used to study testosterone (Testosterone-ELISA). A test sample and a conjugate (peroxidase-labeled testosterone) were added to the well of a plate with 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 bound on the surface of the well plate enzyme was shown by adding the 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 research results was performed using statistical methods (Statistica 10.0 for Windows) to determine the arithmetic mean (M), the statistical error of the arithmetic mean (m), the probability of difference (P) between the arithmetic means of two variation series by the confidence factor for the difference of mean (t). The difference between the two values was considered reliable for *p≤0.05; **p≤0.01; ***p≤0.001 (Varaksin, 2006).

Experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On protection of animals from cruel treatment") and "General ethical principles of animal experiments", adopted by the First National Congress of Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1985)) (Council Directive 86/609/EEC).

Results and discussion

At the first stage of the research, a series of experiments were conducted to establish the effectiveness of veterinary drugs in female and male cats. In the second stage of the research, a series of experiments were conducted to establish the effectiveness of veterinary drugs on female and male dogs. To study the effectiveness of the drugs, the level of sex hormones in the serum of cats and dogs was determined.

During the experimental period, a clinical study of animals of experimental and control groups has been conducted. No deviations from the physiological norm were registered. The animals of the experimental and control groups were active, willing to take food and water, mucous membranes were pink, standard respiration rate and heart rate, average fatness.

During a clinical examination of cats and bitches of the control group, it was found that throughout the experiment, the animals periodically spontaneously came to sexual hunting. In animals of the experimental groups, signs of sexual hunting were recorded before the use of drugs, as well as after discontinuation of drugs, when the sexual cycle resumed.

Observations on the behavioral characteristics of sexual hunting in female and male cats showed that within 3-5 days of drug use, sexual activity decreased significantly compared with the control group.

We determined the dynamics of the concentration of sex hormones in the serum of cats and dogs under conditions of oral administration of the drug No 1 and 2 (Fig. 1).

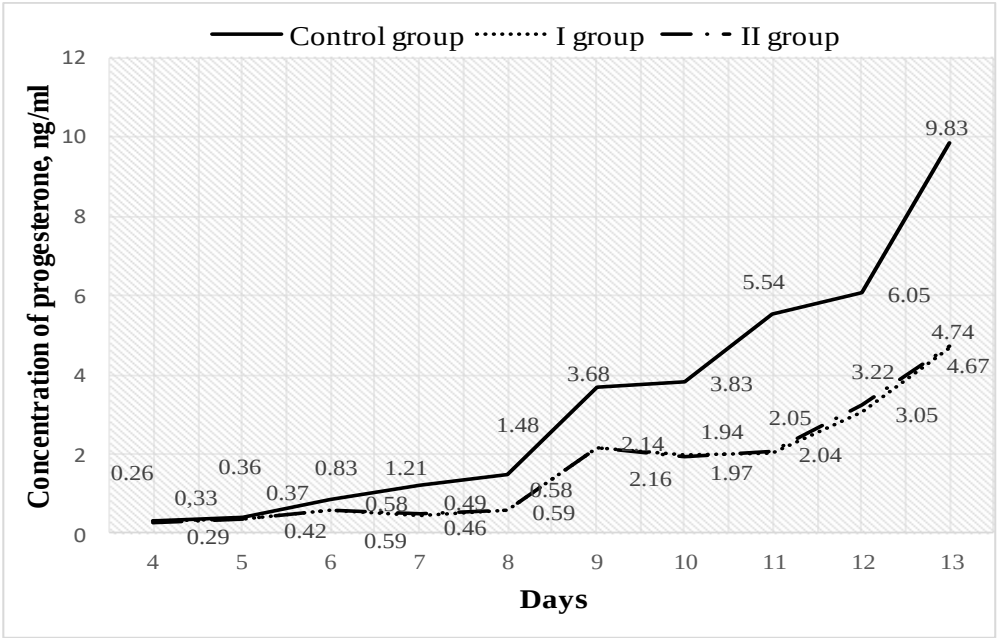

Fig 1. Serum levels of progesterone in female cats after the most commonly used progestogen-based compounds for the control of estrous (M±m, n=5)

Analysis of the results of quantitative progesterone studies showed that oral administration of the drugs No 1 and 2 to female cats every two weeks at doses of 5 mg (according to the active substance) led to a probable decrease in serum progesterone levels (Table 1) and delayed estrus in cats of experimental groups. Throughout the experiment, the concentration of progesterone under the influence of drugs remained at the basal level, which is characteristic of the anestrus phase of the sexual cycle.

Table 1. The concentration of hormones in the serum of female cats receiving hormonal drugs for interruption of estrus (M±m, n=5)

Experimental groups	Terms of research, days				
	before administration	1	3	5	8
		Follicle-stimulating hormone (FSH), ng/ml			
control	0.55±0.02	0.54±0.01	1.81±0.01	2.62±0.01	1.34±0.01
1	0.54±0.01	0.54±0.01	0.81±0.01***	1.21±0.01***	0.48±0.04***
2	0.54±0.02	0.53±0.01	0.82±0.01***	1.23±0.01***	0.49±0.06***
		Luteinizing hormone (LH), nmol/l			
control	3.19±0.02	3.61±0.23	7.21±0.14	12.45±0.40	18.91±0.53
1	3.21±0.16	3.22±0.15**	5.71±0.05***	7.83±0.46***	9.05±0.51***
2	3.20±0.03	3.28±0.11**	5.73±0.16***	7.99±0.70***	8.54±0.49***

Notes: ** – $p<0.01$ according to the indicator in control; *** – $p<0.001$ according to the indicator in control.

Table 1 shows that the concentration of follicle-stimulating hormone in the serum of female cats treated with the drugs No 1 and 2 orally according to the scheme for interruption of estrus at doses of 5 mg (1.0 cm^3) daily for eight days, was reliably lower ($p<0.001$) on the 3rd, 5th and 8th day of the experiment, compared with the indicators in control.

Serum luteinizing hormone levels in female cats treated with drugs No 1 and 2 orally for interruption of estrus at doses of 5 mg daily for 8 days were significantly lower on days 1, 3, 5, and 8 of the experiment compared to control. During clinical observation of experimental and control animals, no changes in clinical condition were observed during the use of drugs.

Experiments have also been performed on estrus retention in animals through hormonal drugs (Table 2).

Table 2. The concentration of hormones in the serum of cats that received hormonal drugs to delay estrus (M±m, n=5)

Experimental groups	Terms of research, days				
	before administration	1	3	7	14
		Follicle-stimulating hormone (FSH), ng/ml			
control	0.53±0.01	0.60±0.03	1.92±0.02	1.64±0.01	1.84±0.02
1	0.54±0.04	0.58±0.01	0.81±0.01***	0.82±0.01***	0.73±0.02***
2	0.54±0.04	0.56±0.01	0.82±0.02***	0.82±0.02***	0.74±0.01***
		Luteinizing hormone (LH), nmol/l			
control	3.28±0.10	3.71±0.17	7.92±0.19	13.35±0.43	18.00±0.66
1	3.39±0.33	2.76±0.39***	5.22±0.14***	6.38±0.52***	9.18±0.14***
2	3.37±0.27	2.65±0.30***	5.29±0.24***	6.63±0.27***	8.68±0.45***

Notes: *** – $p<0.001$ according to the indicator in control.

Table 2 shows that oral administration of drugs No 1 and 2 to female cats at doses of 5 mg (1.0 cm^3) every two weeks leads to a reliable ($p<0.001$) decrease in the level of follicle-stimulating and luteinizing hormones in the serum, indicating the anovulatory effect of these drugs.

It should be noted that the pituitary gland secretes gonadotropins luteinizing hormone (LH), and follicle-stimulating hormone (FSH). FSH stimulates estrogen production by the ovaries and testosterone by the testes, maturation of the follicle with the ovum, and spermatogenesis. Luteinizing hormone (LH) is responsible for ovulation and corpus luteum formation in the ovaries and testosterone synthesis by Leydig cells in the testes. 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). Therefore, it is necessary to conduct a study of these hormones in the serum of cats and dogs.

According to the results of experimental studies, it was found that oral administration to cats of the studied hormonal drugs in doses of 2.5 mg (0.5 cm^3) for 14 days leads to a reliable decrease in the concentration of testosterone in the serum of cats (Table 3).

Table 3. The concentration of testosterone in the serum of cats which received hormonal drugs at the appearance of signs of sexual arousal for sedation (M±m, n=5), nmol/l

Experimental groups	Terms of research, days				
	before administration	1	3	7	14
control	3.92±0.02	4.18±0.11	4.32±0.08	4.75±0.13	4.23±0.09
1	3.88±0.05	4.05±0.14	1.24±0.01***	0.61±0.08***	2.08±0.05***
2	3.91±0.04	4.01±0.09	1.25±0.01***	0.60±0.05***	2.06±0.03***

Notes: *** – p<0.001 according to the indicator in control.

Observations of animal behavior during the experimental period showed that drug No 1 has a calming effect on the appearance of signs of sexual arousal in cats.

Oral administration to bitches of drugs No 1 and No 2 in doses of 5 mg (1.0 cm^3) per 2.5 kg of body weight in the first 3 days, and half the daily dose from the 4^{th} to the 10^{th} day, leads to a probable decrease in serum concentrations of follicle-stimulating and luteinizing hormones, compared with the control group (Table 4 & 5).

Table 4. The concentration of hormones in the serum of bitches who received hormonal drugs to interrupt estrus (M±m, n=5)

Experimental groups	Terms of research, days				
	before administration	1	3	5	8
			Follicle-stimulating hormone (FSH), ng/ml		
control	0.59±0.02	0.58±0.05	1.92±0.04	2.85±0.08	1.49±0.02
1	0.60±0.02	0.45±0.02***	0.55±0.08***	0.91±0.04***	0.36±0.06***
2	0.58±0.04	0.43±0.01***	0.58±0.06***	0.88±0.06***	0.39±0.04***
			Luteinizing hormone (LH), nmol/l		
control	2.28±0.05	4.21±0.13	7.39±0.36	13.78±0.28	20.85±0.77
1	2.31±0.05	4.01±0.18	2.28±0.16***	2.94±0.09***	3.29±0.15***
2	2.30±0.06	4.11±0.61	2.42±0.25***	2.92±0.19***	3.40±0.32***

Notes: *** – p<0.001 according to the indicator in control.

Table 5. The concentration of hormones in the serum of bitches who received hormonal drugs to delay estrus (M±m, n=5)

Experimental groups	Terms of research, days				
	before administration	1	3	5	8
			Follicle-stimulating hormone (FSH), ng/ml		
control	0.67±0.05	0.72±0.02	2.09±0.04	1.81±0.06	1.97±0.03
1	0.65±0.04	0.49±0.01***	0.71±0.04***	0.29±0.03***	0.40±0.01***
2	0.64±0.04	0.51±0.02***	0.70±0.03***	0.32±0.04***	0.41±0.03***
			Luteinizing hormone (LH), nmol/l		
control	2.50±0.06	3.91±0.09	6.79±2.62	12.22±5.73	17.18±8.83
1	2.18±0.05	3.89±0.10	2.20±0.09***	1.79±0.16***	2.15±0.29***
2	2.21±0.11	3.82±0.17	2.18±0.11***	1.89±0.14***	2.13±0.37***

Notes: *** – p<0.001 according to the indicator in the control.

Table 5 shows that oral administration to bitches of drug No 1 and drug No 2 in doses of 5 mg (1.0 cm^3) per 10 kg of body weight for 7-15 days before estrus leads to a reliable decrease in the concentration of follicle-stimulating and luteinizing hormones in the serum (p<0.001). In the bitches of the control group, the concentration of hormones was within the physiological norm and corresponded to a specific phase of the sexual cycle of animals.

During the examination of animals of the experimental groups, no clinical signs of estrus in bitches were registered.

According to the results of experimental studies, it was found that the studied hormonal drugs affect the level of testosterone in the serum of male dogs (Table 6).

The results of the experiments showed that oral administration to males of the drug No 1 and No 2 in doses of 5 mg (1.0 cm^3) 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 of both experimental groups. The concentration of testosterone in the blood of males of the control group was within the physiological norm.

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

Experimental groups	Terms of research, days				
	before administration	1	3	7	14
control	5.15±0.54	5.59±0.23	6.58±0.09	0.12±0.21	3.63±0.30
1	4.88±0.20	2.41±0.16	2.04±0.13***	0.99±0.14***	1.77±0.12***
2	5.27±0.09	2.34±0.07	2.05±0.18***	1.13±0.11***	1.86±0.13***

Notes: *** – p<0.001 according to the indicator in control.

During the clinical examination of males of the experimental groups, signs of sedation of sexual arousal were found, while males of the control group during the experimental period showed signs of sexual arousal.
According to the results of studies, oral administration to bitches of drug No 1 and drug No 2 in doses of 5 mg (1.0 cm^3) per 10 kg of body weight 7-15 days before estrus leads to a reliable decrease in the concentration of progesterone in the serum, compared with indicators of the control group (Fig 2).

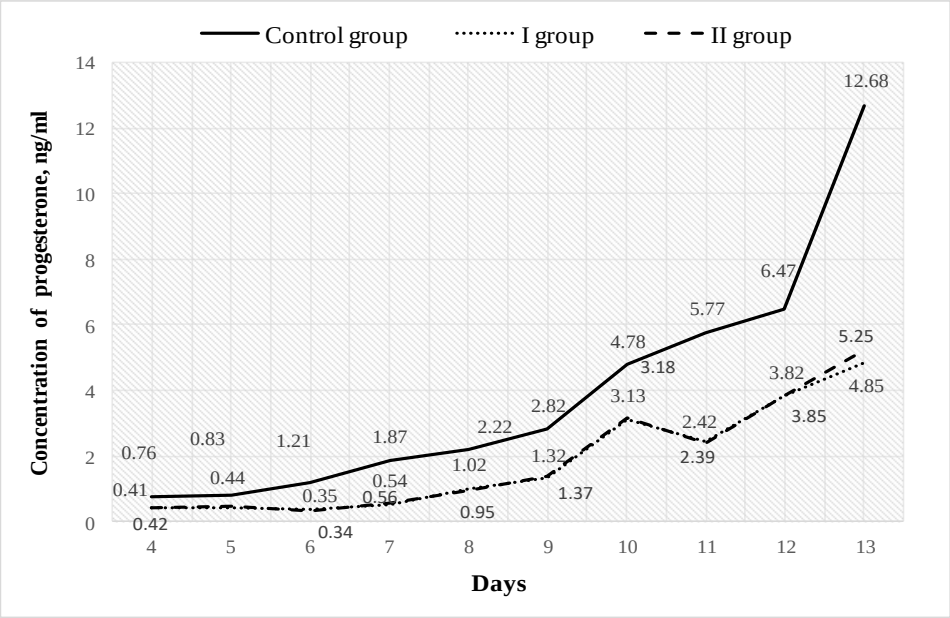

Fig 2. Serum levels of progesterone in female dogs after the most commonly used progestogen-based compounds for the control of estrous (M±m, n=5)

It should be noted that the estrous cycle in cats and bitches of the experimental groups after the cessation of the studied drugs was completely restored, which indicates the safety of drugs with an active ingredient, megestrol acetate.
It has been studied that iron, when used concomitantly with the formulation of megestrol acetate, prevents a sharp decrease in the number of erythrocytes and an increase in eosinophils in the blood of cats (Shcherbakova & Smolianinov, 2017). Changes in memory and cortisol suppression have been reported in humans using this drug (Mason et al., 2018). Megestrol acetate has been shown to inhibit the growth of HepG2 cells grown in vitro and in vivo. These data provide useful information for the clinical study of megestrol acetate to treat hepatocellular carcinoma (Zhang & Chow, 2004). It has been demonstrated that the antitumor activity of megestrol acetate can be enhanced by combination with pterostilbene, suggesting the possible use of a combination of pterostilbene and megestrol acetate for the treatment of endometrial cancer (Wen et al., 2017). Thus, the multifaceted use of megestrol acetate makes this drug promising and in demand in modern human and veterinary medicine. However, there are risks of uncontrolled use of this drug. Thus, synthetic progestins pollute the aquatic ecosystem and can cause harmful effects on aquatic life. Megestrol acetate has been shown to be a potent endocrine disruptor in fish, and its short-term effects adversely affect their populations (Han et al., 2014; Zhao et al., 2015).

Conclusions

Studies of hormonal drugs with the active substance megestrol acetate (5 mg) have shown that they are well tolerated by cats and dogs and do not cause side effects and changes in the clinical state of animals. The effectiveness of hormonal drugs in cats and dogs of different breeds and genders has been scientifically substantiated. In the tested doses, the investigated hormonal drugs show antiestrogenic and anovulatory effects. The studied hormonal drugs, when administered orally to female cats and dogs, cause a reliable decrease (p<0.001) in the levels of progesterone, follicle-stimulating, and luteinizing hormones, as well as a reliable decrease (p<0.001) in serum testosterone levels in male cats and dogs. Thus we verified the possibility of using

hormonal drugs to interrupt and suppress estrus in female cats and dogs, and inhibiting sexual activity, and regulating the behavior of male cats and dogs.

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