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Effects of Different Methods of Ovulation Induction on Sex Hormones in Serum, and Meat of Rabbit Does

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ABSTRACT

High indicators of reproductive function in rabbits can be achieved using hormonal inducers of ovulation, in particular analogs of gonadotropin-releasing hormone, serum, and chorionic gonadotropins. Therefore, the aim of this study was to evaluate the dynamics of sex hormones in the blood serum and meat of rabbit does during ovulation stimulation over 5 consecutive pregnancies. For this purpose, 60 *Hyla* rabbit does were randomly divided into five groups of 12, ensuring four animals per group with three replicates. Animals of the first and second experimental groups, animals received intramuscular injections of serum gonadotropin, 40 IU and 25 IU respectively, three days prior to artificial insemination. Females of the third and fourth groups were administered combined doses of serum and chorionic gonadotropins (40 IU and 24 IU, respectively) during the same period. Rabbits of the control group were stimulated to ovulate by subcutaneous injection of 0.2 ml analog of gonadotropin-releasing hormone after artificial insemination. Long-term administration of gonadotropins revealed dose-dependent effects. Hyperprogesteronemia was detected in rabbit does (40 IU), while hyperandrogenia was noted in females (24 IU) during the combined administration of gonadotropins. The use of serum gonadotropin at a dose of 25 IU contributed to an increase in the level of follicle-stimulating, luteinizing hormone and progesterone while decreasing 17β-estradiol. A high dose (40 IU) in rabbit does did not cause significant fluctuations of hormones in blood serum, but decreased luteinizing hormone and progesterone. Long-term use of a gonadotropin-releasing hormone analog was accompanied by a pronounced decrease in the level of luteinizing hormone, as well as 17β-estradiol. However, the meat of all experimental animals did not increase the content of steroid hormones (testosterone and 17β-estradiol). It can be concluded that ovulation stimulation in rabbit does using a serum gonadotropin dose of 25 IU and the recommended dose of a gonadotropin-releasing hormone analog does not negatively impact the hormonal balance.

Keywords: Analogue of gonadotropin-releasing hormone, Artificial insemination, Equine chorionic gonadotropin, Human chorionic gonadotropin, Rabbit does

INTRODUCTION

Modern rabbit breeding is an industry that not only provides humanity with a useful product and valuable fur but is also widely used in scientific research, particularly for testing and evaluating assisted reproductive technologies (Viudes-de-Castro et al., 2017; Casares-Crespo et al., 2018; Bakeer et al., 2022). One such technology is artificial insemination (AI), which has become widely used in the practice of rabbit farms as a highly effective reproductive technology (Vicente et al., 2012; Gardela et al., 2020). The effectiveness of AI depends on both the physiological state of the female and the quality of males' ejaculate, especially its redox status (Casares-Crespo et al., 2016; Sanchez-Rodriguez et al., 2020; Koshevoy et al., 2021). The use of AI in rabbits is physiologically justified since the rabbit needs induced ovulation after insemination. In addition, the effectiveness of hormonal treatment in rabbit does has been demonstrated to increase induction of ovulation and stimulation of estrus by administration of gonadotropin-releasing hormone (GnRH), its analogues, serum or chorionic gonadotropins (Arias-Alvarez et al., 2013; Viudes-de-Castro et al., 2019). Ovulation should be induced with the hormone drugs exogenous administration, which can be performed intramuscularly, subcutaneously, or intravaginally. In addition, the effectiveness of hormone drugs is improved by the simultaneous use of organic phytoremedies (such as *Yucca schidigera*) and stimulants (Štochmal'ová et al., 2015; Elkomy et al., 2021; Viudes-de-Castro et al., 2023). The use of this group of drugs is limited by their negative effects (high levels of hormones in meat) on the health of rabbits and offspring, as well as the negative impact on humans when consuming products obtained from processed animals. It has become especially relevant given the "One Health" concept (Hughes

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and Watson, 2018; Miller and Leach, 2023). A high probability of developing side effects and morphological damage to the functional tissue of human ovaries are associated with incorrectly defined dosage and/or long-term use of gonadotropins (Herkert et al., 2022). The resulting negative consequences of gonadotropin use are related to the difference between recombinant forms of gonadotropins and endogenous hormones, which not only affects their pharmacodynamics but also modifies the cellular response (Banker & Garcia-Velasco, 2015; Casarini and Simoni, 2021). In rabbits does, there is evidence of the negative effect of different doses of intramuscular injections of lecirelin during insemination on the fertilization frequency, the total number of rabbits per litter, the number of stillbirths, and abortion rates (Zapletal and Pavlik, 2008). Although common, the effectiveness of AI with the introduction of GnRH analogs to the sperm dose, such as buserelin or [des-Gly10, D-Ala6]-LH-RH ethylamide, is limited due to the proteolytic activity of enzymes in sperm, which reduces the efficacy of added hormones and necessitates doses 15-25 times higher (Quintela et al., 2004; Gogol, 2016). High doses or long-term use of hormonal ovulation inducers cause negative side effects on the reproductive system of females and a decrease in offspring growth rates due to the lack of feeding when rabbits are weaned early (Karsch et al., 1997; Rebollar et al., 2008).

The balance of sex hormones, follicle-stimulating, luteinizing, estradiol, progesterone, and testosterone, in the body of rabbits, plays an important role in the implementation of sexual function and full fertilization, pregnancy, and childbirth. The follicle-stimulating hormone has a chemical nature, it is a complex glycoprotein, which stimulates the follicles' development until ovulation, enhances the synthesis of estrogens, and increases the sensitivity of the gonads to lutropin. This gonadotropic hormone is secreted by basophilic cells of adenohypophyses. The synthesis of follicle-stimulating hormone (FSH) is regulated by releasing hormones from the hypothalamic area of the brain (foliberin) as well as by the principle of feedback involving the content of androgens and estrogens in the blood (Laborde et al., 1981; Moore and Hasler, 2017). Rabbit does can not produce a sufficient amount of progesterone (P4) in the first reproductive cycle due to insufficient development of the corpus luteum. It is formed in the ovary from a ruptured tertiary (Graafian) follicle after ovulation and secretes P4 to support pregnancy. The corpus luteum will degenerate closer to the end of the estrous if pregnancy does not occur (Salem et al., 2020). Low concentrations of progesterone before artificial insemination change dynamically with the onset of pregnancy (Ubilla et al., 2001). It is known that human chorionic gonadotropin (hCG) can provoke additional synthesis of P4 after binding to LH receptors (Salem et al., 2020). According to Stevenson et al. (2007), hCG induces additional natural synthesis of P4 from accessory luteal cells after binding to LH receptors.

The use of hormonal stimulation and synchronization of oestrus increases fertility and reduces the amount of IA necessary for pregnancy. Correct hormonal stimulation provides a prolonged effect on ovulation during 3-4 reproductive cycles, after which repeated use of inducers is necessary (Rebollar et al., 2006). In addition, the fertility of rabbit does increases after the injection of equine chorionic gonadotropin (eCG) with hCG or gonadotropin-releasing hormone (GnRH), despite their different biological effects and specific actions (De Rensis and López-Gatius, 2014; Hassanein et al., 2021). The hormonal agents commonly used for ovulatory stimulation in rabbit does include buserelin at a dose of 0.2 ml per animal administered subcutaneously, and chorionic gonadotropin at doses ranging from 20-25 IU administered intravenously (Arias-Alvarez et al., 2010; El-Ratel et al., 2020).

Therefore, the current study aimed to investigate the dynamics of sex hormone levels in blood serum and meat of rabbit does following the administration of serum gonadotropins (at doses of 40 IU and 25 IU) and chorionic gonadotropins (at doses of 40 IU and 24 IU), effects of a gonadotropin-releasing hormone analog. This investigation sought to evaluate the effects of their long-term use.

MATERIALS AND METHODS

Ethical approval

Experiments on rabbit does with the use of hormonal drugs were reviewed and approved by the Bioethics Committee of the State Biotechnological University (ethical permit No. 4-05 dated May 5, 2019). Treatment of females and the necessary manipulations were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (2006) and the General Ethical Principles of Animal Experiments adopted by the 1st National Congress on Bioethics (Kyiv, Ukraine, 2001).

Animals and experimental design

The experimental animals included 60 multiparous *Hyla* rabbit does, aged between 7 and 8 months, with weights ranging from 3.2 to 3.9 kg. During the research period, the experimental animals did not receive any medicines, vaccine prophylaxis, and antiparasitic treatments. To form five groups of animals, their total number was randomly divided into

groups of 12 animals each. Thus, four animals and three repetitions were used in each group, providing the necessary amount of data for statistical processing. The experimental design is shown in Figure 1. To determine the levels of hormones in rabbits, blood samples (1 ml in sodium citrate tube) were taken on day 7 of pregnancy, in the morning before feeding. This sampling was repeated consecutively over the course of five pregnancies (during the experiment, it was important to study the dynamics of changes during each pregnancy that occurred after the use of various ovulation inducers). Notably, animals of all experimental groups (1-4) received intramuscular injections of gonadotropins 3 days before AI. In contrast, rabbit does of the control group were subcutaneously injected with a GnRH analogue immediately after AI.

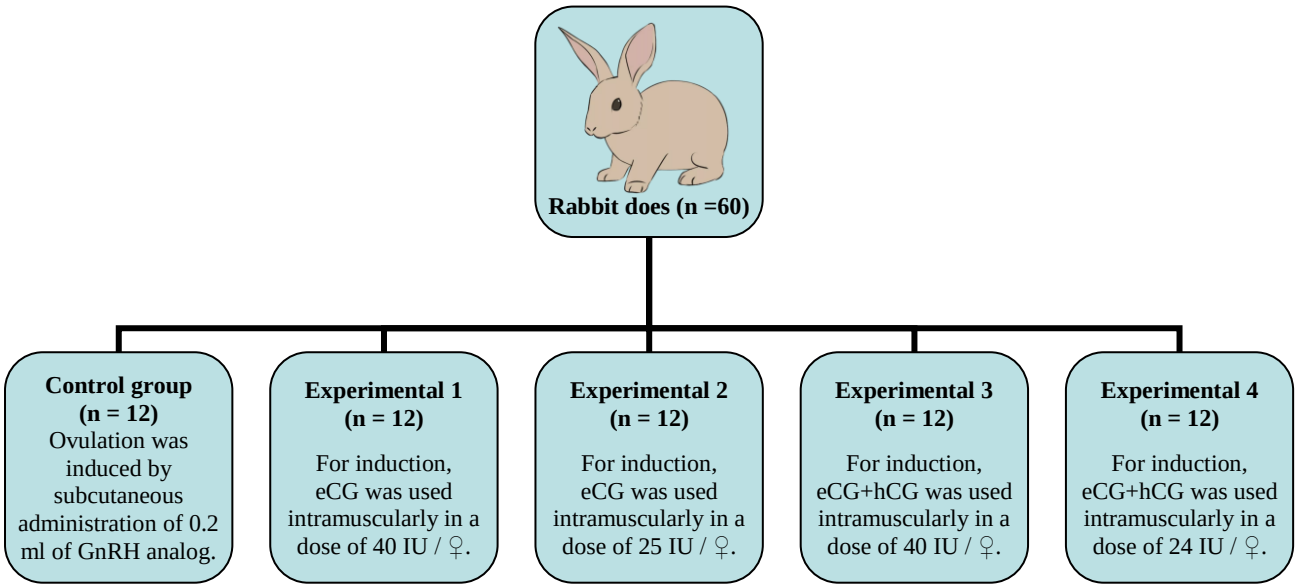


Figure 1. Groups of animals in the experiment and used dosages of ovulation inducers. GnRH: Gonadotropin-releasing hormone; eCG: Equine chorionic gonadotropin; hCG: Human chorionic gonadotropin.

Feeding and housing of rabbit does

All rabbit does were fed a ration containing ingredients and chemical composition as in Table 1. Each doe was fed *ad libitum* on this commercial diet throughout the experiment. Water was available through automatic drinkers attached to the galvanized wire cages. A complete feed diet (2750 kcal ME/kg, 18.5% CP, and 12.5% CF) was used as a commercial diet for feeding rabbits ad-libitum according to their physiological stage (El-Desoky et al., 2021).

The ration indicated in Table 1 included nitrogen-free extract 59.45 g/kg dry matter (DM), crude protein 17.54 g/kg DM, crude fiber 12.53 g/kg DM, ash 9.43 g/kg DM, and ether extract 2.05 g/kg DM. The humidity in the rooms where the rabbits were kept was 55.0 ± 5.0%, and the air temperature was 25.0 ± 1.0ºC. All animals were individually housed in galvanized wire batteries (70 × 50 × 40 cm) located in a naturally ventilated and lighted (12 hours of light: 12 hours of dark) room. About 5 days pre-kindling wooden nest boxes (containing straw or hay) with dimensions of 50 cm (length) ×30 cm (width) × 30 cm (height) attached to the dam's cages). The bedding was replaced daily by a new one to avoid any contamination from urine or fecal material.

Table 1. Composition of diet ingredients for rabbit does

Ingredient	Content
Alfalfa hay (g/kg)	280
Wheat bran (g/kg)	250
Barley (g/kg)	180
Soybean meal (g/kg)	180
Yellow corn (g/kg)	60
Molasses (g/kg)	30
Barley grain (g/kg)	10
Di-calcium phosphate (g/kg)	10
NaCl and premix* (g/kg)	10

* 1 kg of premix (minerals and vitamins mixture) contains Vitamin A (20,000 IU), Vitamin D3 (15,000 IU), Vitamin B1 (0.33), Vitamin B2 (1.0 g), Vitamin B6 (0.33 g), Vitamin B5 (8.33 g), Vitamin B12 (1.7 mg), pantothenic acid (3.33 g), biotin (33 mg), folic acid (0.83 g), choline chloride (200 g), Vitamin E (8.33 g) and Vitamin K (0.33 g).

Blood sample collection and sex hormone assay

No sedation or anesthesia medications were administered to the animals during blood sampling procedures. Blood for the study was collected on day 7 of the experiment from the lateral saphenous veins, following the generally accepted method. Blood samples were taken simultaneously in the morning into tubes with anticoagulant (sodium citrate; BD Vacutainer®, Russia). The levels of follicle-stimulating and luteinizing hormones, 17β-estradiol, progesterone, and testosterone were determined in the obtained blood serum samples of rabbits using standard sets of ELISA Kit reagents (LifeSpan BioSciences Inc., USA) with the Stat Fax 303 plus enzyme immunoassay (Awaraness Technology, USA).

Sex hormones in meat assay

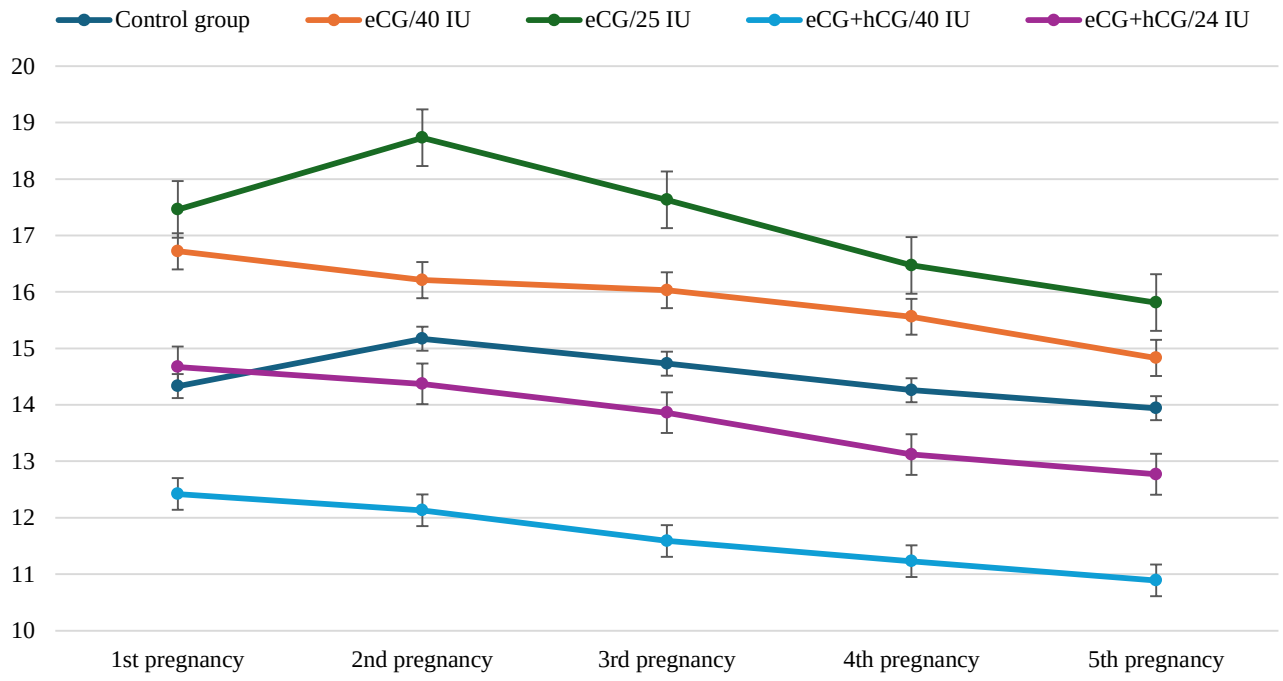
After euthanizing rabbits, 0.5 kg of muscle tissue from four animals in each group was collected and divided into three samples. These samples were then placed in polyethylene bags and stored at -18ºC. The surfaces of the muscle were cleaned from all fat and connective tissues. Testosterone and 17β-estradiol were determined using radioimmunoassay on the 411 analyzer (Germany). The analytic sensitivity of the testosterone assay was 0.005 mg/kg, and 17β-estradiol was 0.003 mg/kg (Rebaz et al., 2019).

Statistical analysis

All calculations were performed using Statistical Package for Social Science (SPSS), version 22 (SPSS Inc., USA). One-way analysis of variance (ANOVA) was performed to compare the data of control and experimental groups of rabbit does. The data in the tables were presented as mean ± standard deviation. The normality of the quantitative variables was tested with the Shapiro-Wilk test. Since all variables were not normally distributed, the Mann-Whitney test was used to compare quantitative variables. The significant differences between the treatments were confirmed by Tukey as a post-hoc test. P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

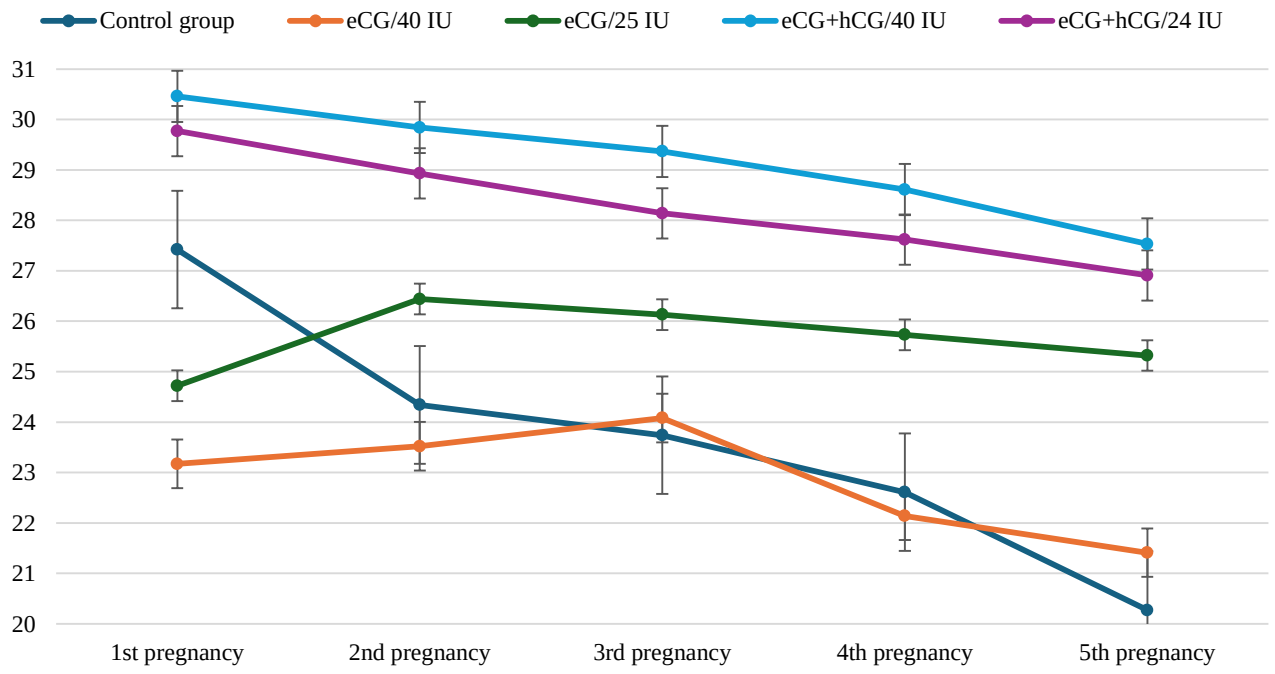
Changes in the dynamics of hormone levels of rabbits showed the specific effects of gonadotropins, compared to the GnRH analog ($p < 0.05$). The dynamics of FSH content in the blood serum of pregnant rabbit does during the experiment is shown in Graph 1. Thus, in the first pregnancy, the level of FSH was higher than the control indicators by 16.72 ± 0.38 IU/L and 17.46±0.42 IU/L in rabbits of experimental groups 1 and 2, respectively ($p < 0.05$). Subsequently, the level of FSH in animals of the first experimental group was characterized by slight upward fluctuations ($p < 0.05$). Positive dynamics of high activity of FSH in blood serum were observed in rabbits of experimental group 2.



Graph 1. Dynamics of follicle-stimulating hormone in blood serum of pregnant rabbit does (n=12, lU/L).

During the study, there was a decrease in FSH levels in animals of experimental group 3, compared to control rabbits. In particular, in females during the first pregnancy, FSH levels were lower than the control indicators by 12.42 ± 0.28 IU/L ($p < 0.05$). Similar changes were observed in experimental group 4. While there was a tendency to increase FSH levels during the first pregnancy, in subsequent pregnancies (second and third), there was a tendency for FSH levels to decrease. Additionally, during the fourth and fifth pregnancies, FSH levels were lower than the control data by 13.12 ± 0.27 IU/L and 12.77 ± 0.24 IU/L, respectively ($p<0.05$).

It should be noted that in rabbits of the control group, the level of FSH during the five pregnancies was accompanied by minor fluctuations, and at the end of the experiment, it had a tendency to decrease. The dynamics of the content of luteinizing hormone (LH) in the blood serum of rabbits during the experiment is summarized in Graph 2.

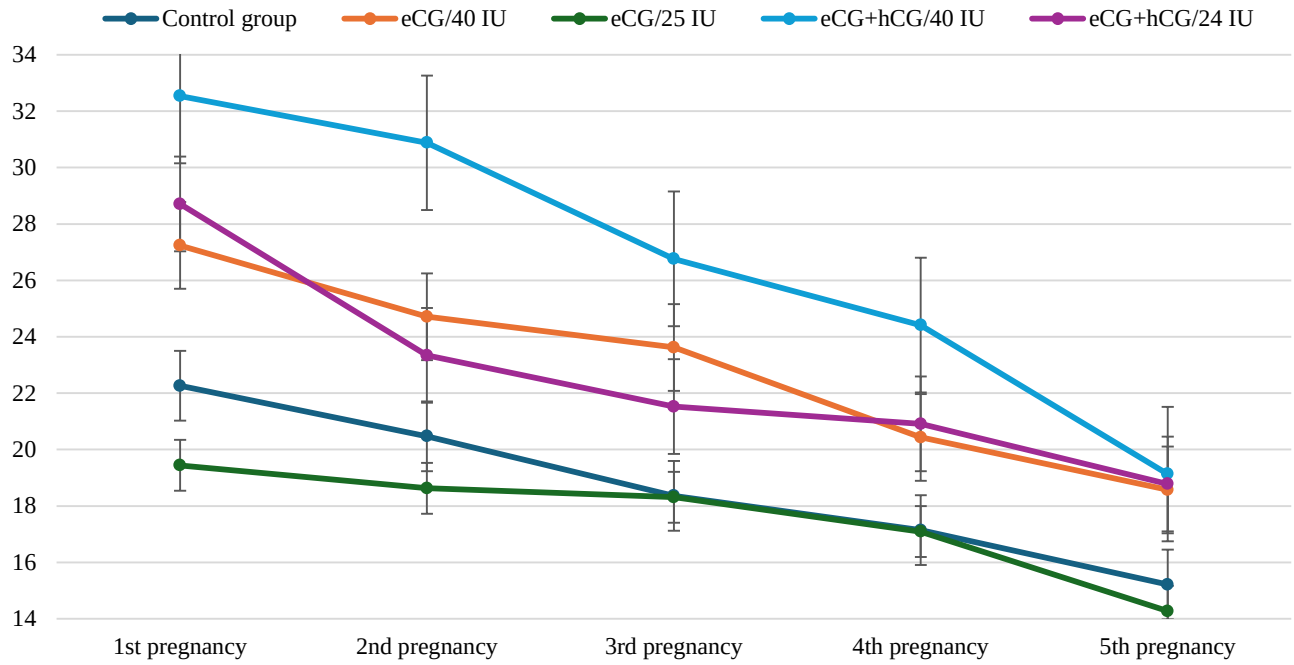


Graph 2. Dynamics of luteinizing hormone in blood serum of pregnant rabbit does (n=12, lU/L).

In females of experimental group 3, there was an increase in the LH level during the experiment ($p < 0.05$). The changes observed in rabbits of experimental group 4 were less pronounced in terms of LH level dynamics compared to the control data. In the first pregnancy, LH levels were higher in experimental group 4 rabbits by 29.77 ± 0.86 IU/L, and in the fifth pregnancy by 26.91 ± 0.73 IU/L ($p < 0.05$). For instance, in rabbits of experimental group 1 during the first pregnancy, LH levels were lower than those of the control group by 23.17 ± 0.71 IU/L ($p < 0.05$), with a tendency to decrease in the second and fourth pregnancies. However, an upward trend was observed in the third and fifth pregnancies. Similarly, in females of experimental group 2, LH levels were initially lower than the control data by 24.72 ± 0.74 IU/L ($p<0.05$) but showed an increasing trend in the second pregnancy. Subsequently, in the third, fourth, and fifth pregnancies, LH levels were significantly higher, by 26.13 ± 0.69 IU/L, 25.73 ± 0.72 IU/L, and 25.32 ± 0.70 IU/L, respectively ($p < 0.05$). Differences in LH levels between the first and fifth pregnancies were evident. Towards the end of the study, LH levels were likely lower than those of the first pregnancy by 20.27 ± 0.61 IU/L ($p<0.05$), as seen in Graph 2. A similar trend of LH level decrease, akin to FSH, was observed in rabbits of the control group throughout the entire study period.

The level of 17β-estradiol in the blood serum of rabbit does from different reproductive cycles exhibited dynamic changes during the experiment (Graph 3). The authors of the current study observed an increase in 17β-estradiol levels in the blood serum of rabbits in experimental groups 1, 3, and 4, whereas in animals of experimental group 2, the level of this hormone did not exceed the control data. In rabbits of experimental group 1, during the first pregnancy, the level of 17β-estradiol exceeded that of the control group by 27.24 ± 0.74 pmol/L, and in the fifth pregnancy by 18.57 ± 0.39 pmol/L, respectively ($p < 0.05$). Conversely, in animals of experimental group 2, the level of this hormone was lower during the first and second pregnancies by 19.44 ± 0.51 pmol/L and 18.63 ± 0.42 pmol/L, respectively ($p < 0.05$). In the

third and fourth pregnancies, it almost corresponded to the control data, while during the first pregnancy, it was reduced by 14.27 ± 0.24 pmol/L ($p < 0.05$).



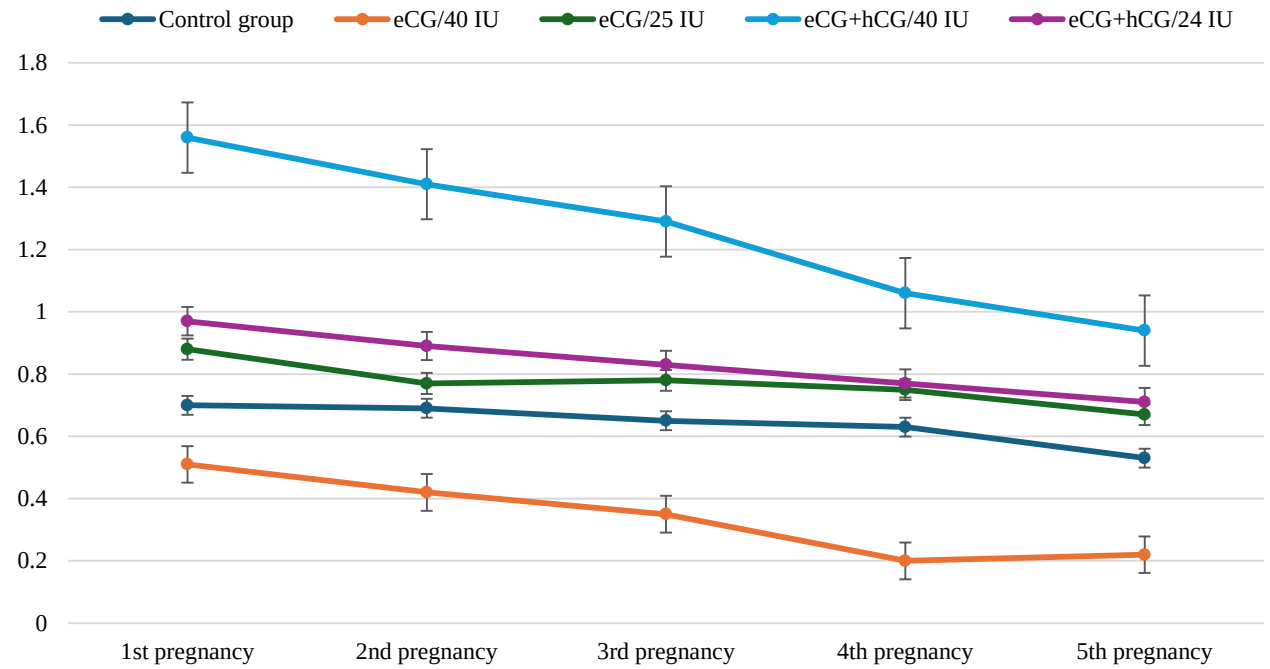
Graph 3. Dynamics of 17β-estradiol in pregnant rabbit does' blood serum (n=12, pmol/L).

The dynamics of changes in the level of 17β-estradiol (17β-E) in the blood serum of rabbit does after hormonal treatments revealed dose-dependent effects in experimental groups 3 and 4. In experimental group 3, the level of 17β-E increased by 32.54 ± 1.17 pmol/L in the first pregnancy compared to the control, while in the fifth pregnancy, it increased by only 19.13 ± 0.41 pmol/L ($p < 0.05$). Similarly, in experimental group 4, the level of 17β-E increased by 28.71 ± 0.77 pmol/L in the first pregnancy and by 18.78 ± 0.38 pmol/L in the fifth pregnancy ($p < 0.05$). Notably, significant changes in the level of 17β-E occurred in the control group, where the hormone level decreased steadily throughout the 1st-5th pregnancies. Specifically, in the fifth pregnancy, the level of 17β-E (22.26 ± 0.67 pmol/L) was significantly lower than that of the first pregnancy by 15.21 ± 0.27 pmol/L ($p < 0.05$). Generally, the values of the 17β-E level in the five pregnancies decreased constantly.

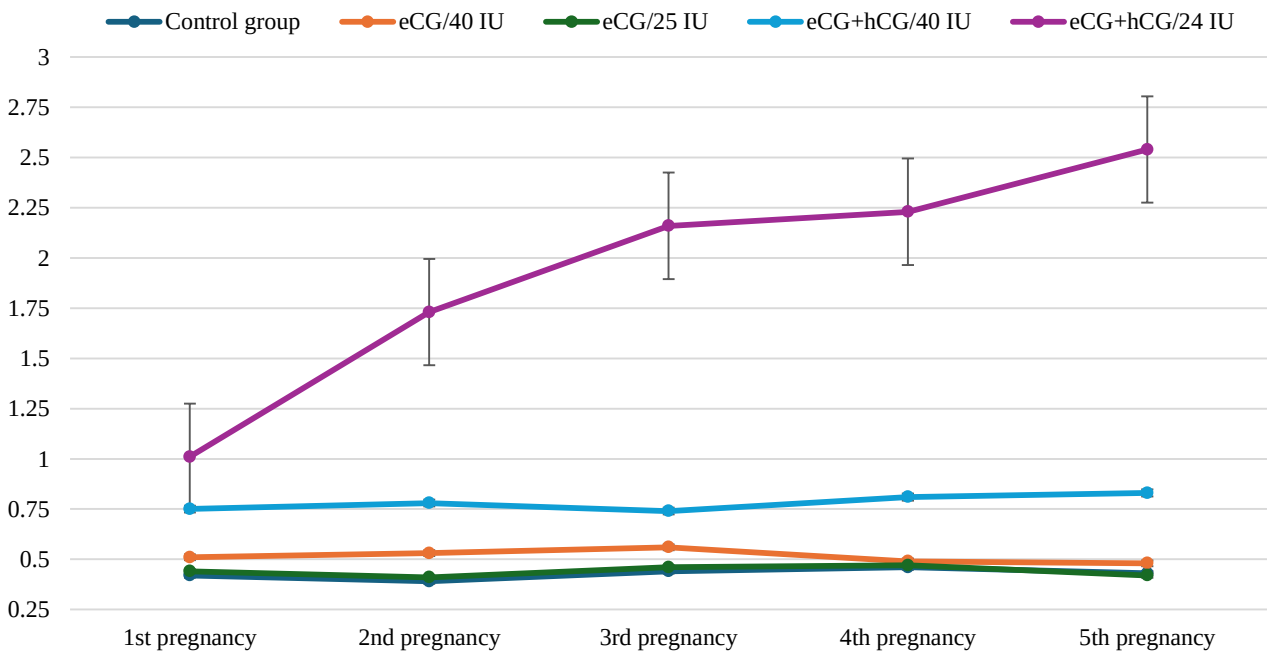
The dynamics of changes in the level of progesterone (P4) in the blood serum of rabbit does after hormonal treatments are illustrated in Graph 4. Females of experimental groups 2 and 4 exhibited a moderate effect on the level of P4 in blood serum, while rabbits of experimental group 3 showed a significant increase, and those of experimental group 1 showed a decrease in P4 levels compared to the control ($p < 0.05$). Specifically, in the first pregnancy, the level of progesterone was lower by 0.51 ± 0.02 nmol/L, in the second and third pregnancies by 0.42 ± 0.01 nmol/L and 0.35 ± 0.01 nmol/L, respectively, reaching its maximum decrease in the fourth and fifth pregnancies by 0.20 ± 0.01 nmol/L and 0.22 ± 0.01 nmol/L, respectively ($p < 0.05$). In females of the control group, only minor fluctuations in the level of P4 were detected during the experiment, but by the end of the study, this indicator was lower by 0.53 ± 0.01 nmol/L compared to the value of the first pregnancy ($p < 0.05$).

In the first pregnancy, the level of progesterone exceeded the control value by 0.88 ± 0.04 nmol/L in rabbits of experimental group 2 and by 0.97 ± 0.05 nmol/L in experimental group 4 ($p < 0.05$). Similarly, in the second cycle, there was a tendency for an increase in progesterone levels in animals of experimental group 2, while in females of experimental group 4, it was significantly higher by 0.89 ± 0.04 nmol/L ($p < 0.05$). Further, in experimental group 2, an increase of 0.78 ± 0.04 nmol/L in the third pregnancy and 0.67 ± 0.03 nmol/L in the fifth pregnancy was observed ($p < 0.05$). Meanwhile, the level of progesterone was higher by 0.83 ± 0.04 nmol/L in the animals of experimental group 4 in the third pregnancy, and by 0.77 ± 0.04 nmol/L and 0.71 ± 0.03 nmol/L in the fourth and fifth pregnancies, respectively ($p < 0.05$). In experimental group 3, the level of progesterone exceeded the control data by 1.56 ± 0.07 nmol/L in the first pregnancy, and by 1.41 ± 0.07 nmol/L in the second pregnancy ($p < 0.05$). Subsequently, the growth weakened somewhat, with the level of progesterone being higher by 1.29 ± 0.06 nmol/L in the third pregnancy, 1.06 ± 0.05 nmol/L in the fourth, and 0.94 ± 0.04 nmol/L in the fifth pregnancy ($p < 0.05$). Considering these changes, the dynamics of testosterone levels in the blood serum of rabbits during the experiment were determined, as shown in Graph 5. In

experimental group 1, an increase in testosterone level was observed in the first, second, and third pregnancies, by 0.51 ± 0.03 ng/dL, 0.53 ± 0.04 ng/dL, and 0.56 ± 0.04 ng/dL, respectively ($p < 0.05$). However, in the fourth and fifth pregnancies, animals of this group showed only a small tendency to increase the studied indicator. Similar slight fluctuations in testosterone levels compared to the control values were found in the females of experimental group 2.



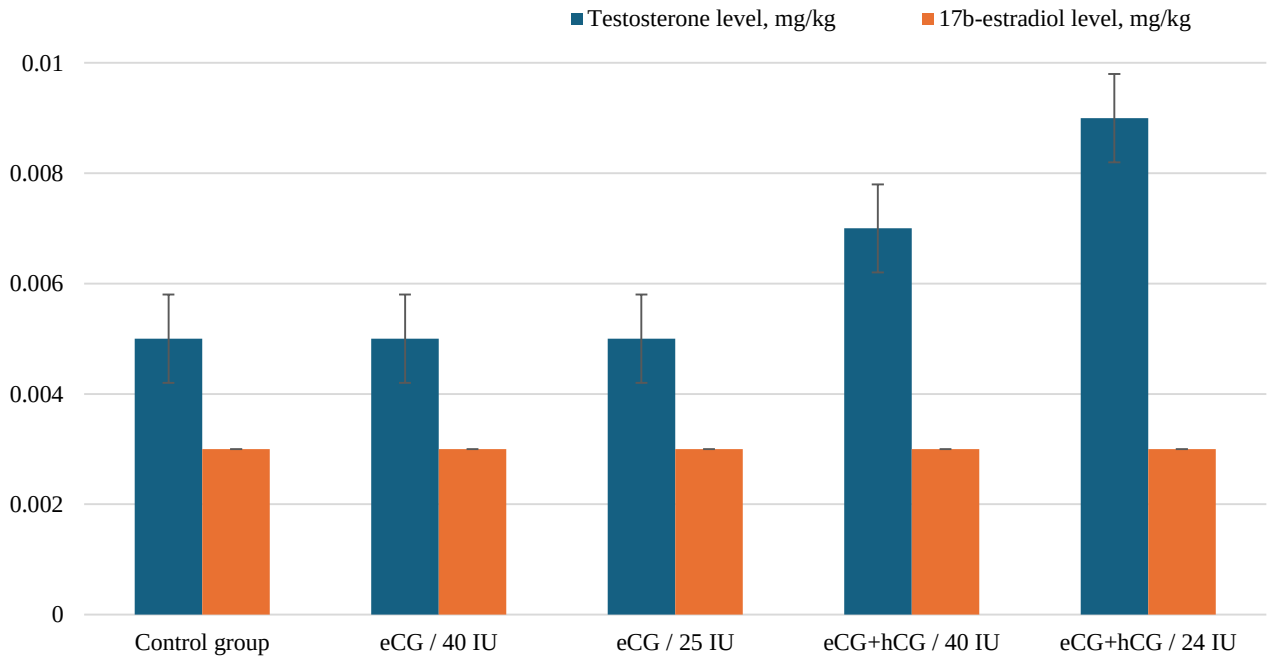
Graph 4. Dynamics of progesterone in the blood serum of pregnant rabbit does (n=12, nmol/L).



Graph 5. Dynamics of testosterone in blood serum of pregnant rabbit does (n=12, ng/dL).

A significant increase in androgenesis in the bodies of females in experimental group 3 was confirmed by a substantial rise in testosterone levels during the experiment, from 0.75 ± 0.09 ng/dL in the first pregnancy to 0.83 ± 0.11 ng/dL in the fifth pregnancy ($p < 0.05$). On the other hand, in the animals of experimental group 4, consistent development of hyperandrogenemia was observed, with testosterone levels in blood serum ranging from 1.01 ± 0.12 ng/dL in the first pregnancy to 2.54 ± 0.21 ng/dL in the fifth pregnancy, significantly higher than the control data ($p < 0.05$). The safety of the long-term use of hormonal means for the stimulation of ovulation in rabbits was evaluated by

assessing the content of sex hormones in the products of rabbit breeding, specifically rabbit meat, at the end of the experiment (during the 1st-5th pregnancies). The obtained data are presented in Graph 6. According to the data in Graph 6, the level of sex hormones (testosterone and 17β-estradiol) did not exceed the detection level. At the same time, there were no significant changes in the level of 17β-estradiol in the experimental groups, and the testosterone content showed an increasing trend, which confirms their safe content in rabbit products.



Graph 6. Sex hormone levels in the meat of rabbit does in the fifth pregnancy (n=4).

DISCUSSION

An important aspect of the manifestation of the reproductive ability of animals is the full course of folliculogenesis in the process of ovulation (Arias-Alvarez et al., 2010; Barker et al., 2012; Burow et al., 2019). To improve ovulation and induce superovulation in various species of animals, the use of gonadotropins has been proposed (Cole, 2012; De Rensis and López-Gatius, 2014; Li et al., 2021). Particularly, a large number of studies are devoted to the induction of ovulation in rabbits using serum and/or chorionic gonadotropins, as well as a gonadotropin-releasing hormone analog (Dal Bosco et al., 2011; Sun et al., 2017; El-Ratel et al., 2020). Studies have insicated a large number of complications that can arise from incorrect dosage or long-term administration (Brouillet et al., 2012; Albu et al., 2014; Chai et al., 2017). Therefore, this study is devoted to determining the hormonal balance in rabbits during pregnancy. The obtained data indicate the safety of the proposed protocols of ovulatory stimulation of rabbit does, which corresponds to the results obtained by Sirotkin et al. (2014). It is confirmed by the level of hormones in their blood and meat, which is consistent with the data of other researchers (Arias-Alvarez et al., 2013; Rebaz et al., 2019; Abdel-Khalek et al., 2022).

With the introduction of serum gonadotropin (eCG) in both dosages, an increase in the level of FSH was noted in rabbits during the five pregnancies. These data are consistent with those obtained by other authors (González-Mariscal et al., 2007; Rosell et al., 2020). On the contrary, the combined use of serum gonadotropin with chorionic gonadotropin (hCG) caused negative dynamics of the level of this hormone, in particular its decrease, especially at doses of 40 IU. A decrease in the level of FSH can lead to a decrease in the production of offspring from rabbits and low efficiency of artificial insemination (Hashem and Aboul-Ezz, 2018).

At the same time, the level of luteinizing hormone (LH) in the animals of experimental group 1 was lower than that of the control group. Therefore, a higher dosage of eCG negatively affects the dynamics of LH. On the contrary, in rabbits of experimental group 2, the level of this hormone was characterized by an increase during the experiment. The obtained results are correlated with the data established by other researchers (Hassanein et al., 2021). The combined use of gonadotropins (eCG + hCG) in both dosages contributed to a significant increase in the level of LH in the blood serum of rabbits, thus during pregnancy, they showed the full functioning of the corpora lutea pregnancy (Quintela et al., 2001; Viudes-de-Castro et al., 2019).

The level of estrogens under the influence of ovulation inducers also underwent dynamic changes. Rabbits of experimental groups 1 and 4 had a moderate increase in the level of 17β-estradiol in blood serum. Similar results are obtained by other researchers (Zhang et al., 2017; Jolivet et al., 2022). It is worth noting that the introduction of eCG at a dose of 25 IU had no effect on the level of estradiol, and the combined use of gonadotropins (eCG + hCG) at a dose of 40 IU caused its excessive synthesis as shown by Mebes et al. (2015).

Progesterone (P4) as a leading hormonal factor during pregnancy, had a salient value in the performed experiments (Abd-Elkareem, 2017; Kowalewski et al., 2020). Thus, a high dosage of eCG (40 IU) contributed to a significant decrease in the level of P4 in rabbits, while a lower dose (25 IU) increased its content compared to the data of the control group. The same results are shown in several studies (Peiró et al., 2010). Good results were also obtained with the combined administration of gonadotropins (eCG + hCG) at a dose of 24 IU during the experiment, as rabbits had a higher level of it, compared to the control. However, a high dose of 40 IU caused a permanent state of hyperprogesteronemia, which had negative consequences on the health of rabbits and their offspring (Bréard et al., 1998; Hoffman et al., 2009).

Contradictory data have been obtained regarding the level of testosterone in experimental animals. In the rabbits of experimental group 2, no significant changes were found, compared to the control group. However, with a higher dosage of eCG in experimental group 1, there was a slight increase in testosterone levels during the first, second, and third pregnancies. The combined administration of gonadotropins (eCG + hCG) caused a state of hyperandrogenism in the animals, which was especially pronounced in experimental group 4. These findings indicate the negative consequences of long-term administration of gonadotropins, as shown earlier (Garcia-Garcia et al., 2009; Rebaz et al., 2019).

The obtaining results are consistent with the data reported by El-Ratel et al. (2020). They found that the introduction of eCG with the injection of hCG or GnRH analogs before AI can synchronize estrus/ovulation to improve embryo production *in vivo*. In addition, fertility outcomes may be improved in rabbit does in which ovulation is induced by a single dose of eCG or hCG on day 5 after AI. As can be inferred, it is better to use hormonal ovulation inducers once or twice to avoid disturbances in the hormonal background and the health of rabbits. It should be noted that the obtained data on the content of sex hormones in rabbit meat correspond to the results obtained by Rebaz et al. (2019) in animals without hormonal treatment. Therefore, the proposed ovulatory stimulation protocols are safe for the consumer of rabbit breeding products.

The search for measures to replace hormonal inducers of ovulation and stimulation of estrus in rabbits by other means is an urgent problem of modern animal husbandry (El-Desoky et al., 2022). Alternative ways to stimulate ovulation in rabbit does. For example, it has been experimentally demonstrated that 24-hour temporary weaning can be an alternative non-hormonal method for oestrus synchronization during lactating in rabbit does of the second reproductive cycle, which was inseminated in the early postpartum period (Arias-Alvarez et al., 2010). controlling the lighting conditions of premises housing rabbit does presents an alternative approach to reducing the number of hormonal treatments. Thus, the study by Quintela et al. (2001) has revealed that a controlled lighting regime can be used to synchronize oestrus in lactating females instead of applying eCG treatment. Other researchers have shown the absence of reliable changes at different light intensities and a negative effect on body weight between the time of the first fertilization and the second period of parturition (Sun et al., 2017). Therefore, a promising direction for improving the reproductive health of rabbit does is the combination of hormones with metal nanoparticles that have antioxidant properties (Koshevoy et al., 2022; Naumenko et al., 2023). Moreover, it is promising to test the addition of herbal remedies, encapsulated hormonal inducers, etc. to the diets of rabbits (El-Desoky et al., 2021).

CONCLUSION

Different means of ovulation induction in rabbits, depending on the dosage, have a versatile effect on the level of hormones in blood serum but do not result in their accumulation in animal meat. Thus, products obtained from rabbits with long-term administration of hormonal drugs to stimulate ovulation are safe for the consumer. On the other hand, the obtained results indcated the presence of a negative effect of serum (eCG) and chorionic gonadotropins (hCG) on the hormonal balance of experimental females. The use of eCG at a dose of 40 IU contributed to an increase in the level of follicle-stimulating hormone (FSH), 17β-estradiol (E2), however, in this group of animals, a slight decrease in the level of luteinizing hormone (LH) and progesterone (P4) was noted. A lower dose of eCG (25 IU) contributed to an increase in FSH, LH, and P4 levels. The combined use of gonadotropins (eCG+hCG) was characterized by a negative effect on the hormonal background of rabbits including a high dose (40 IU) caused hyperprogesteronemia in experimental animals against the background of an increase in the level of testosterone and a decrease in FSH, and a dosage of 24 IU caused hyperandrogenia. It is worth noting that the long-term use of a gonadotropin-releasing hormone analog to stimulate ovulation is also not without negative changes – a significant decrease in the level of LH and a decrease in E2 was found in animals. Further research will be aimed at evaluating metabolic processes in rabbit does and developing ways to correct negative hormone dynamics.

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Authors' contributions

Svitlana Naumenko, Vsevolod Koshevoy, and Yuliya Tverdokhlib developed the research design and substantiated its methodology. Yuliya Tverdokhlib organized and conducted experimental studies, and Kateryna Sinyagovska and Ludmila Kovaleva took part in the selection of blood and meat samples of rabbits. Vsevolod Koshevoy and Gennadiy Hryshchuk carried out statistical processing of the research results. Olga Miroshnikova, Yuliya Tverdohlib and Svitlana Naumenko analyzed the obtained data, and wrote draft of manuscript. All authors took part in discussing the results, checking the analysed data, writing the article and agreed on the final version of manuscript for submission.

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All the authors of the ancient manuscript unanimously state that there is no conflict of interest.

Availability of data and materials

The authors of this study are ready to send all data supporting the findings of the research upon reasonable request.

Ethical considerations

The authors of this article, while performing the work and preparing the manuscript, complied with the requirements of current regulations to prevent ethical violations, including plagiarism, double posting and/or submission and redundancy, fabrication or falsification of data.

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