UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 4. P. 47-62

Received: 27.07.2025 Revised: 10.10.2025 Accepted: 27.11.2025

DOI: 10.31548/veterinary4.2025.47

UDC: 619:591.4:636.2 619:591.4:636.2

Quantitative changes in progesterone, estradiol and follicle-stimulating hormone in the blood serum of cows using hormonal preparations

Lesya Klymkovetska
PnD inVeterinary Sciences
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str. Kyiv, Ukraine
https://orcid.org/0009-0004-9847-0381

Valentin Karpovskyi
Doctor of Veterinary Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str. Kyiv, Ukraine
https://orcid.org/0000-0003-3858-0111

Ihor Hryshchuk*
PnD, Assistant
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str. Kyiv, Ukraine
https://orcid.org/0000-0003-2571-6876

Volodymyr Dukhnytskyi
Doctor of Veterinary Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str. Kyiv, Ukraine
https://orcid.org/0000-0002-9670-1244

Lesya Shuranova
PnD, Assistant
University of South Bohemia
37005, 1645/31a Branišovská Str., České Budějovice, Czech Republic
https://orcid.org/0000-0002-5611-0490

Suggested Citation:
Klymkovetska, L., Karpovskyi, V., Hryshchuk, I., Dukhnytskyi, V., & Shuranova, L. (2025). Quantitative changes in progesterone, estradiol and follicle-stimulating hormone in the blood serum of cows using hormonal preparations. *Ukrainian Journal of Veterinary Sciences*, 16(4), 47-62. doi: 10.31548/veterinary4.2025.47.

**Corresponding author*

Abstract. Most modern enterprises producing animal products actively use hormonal preparations for animals without a detailed study of their effects on the body. This study aimed to investigate the impact of hormonal preparations on the dynamics of sex hormone levels in cows. The experimental groups of cows were formed according to the principle of analogous groups of 5 animals in each. The cows in experimental group 1 were administered 5 mL of the drug "Surfagon" intramuscularly, and the cows in experimental group 2 were administered 2.5 mL of the drug "Fertagil". The levels of follicle-stimulating hormone, progesterone and oestradiol were determined in the blood serum of cows during lactation on days 2, 6, 10, 14, 18, 22, 26 and 30. It was found that on day 2 of the study, the level of follicle-stimulating hormone in the blood serum of cows in experimental group 1 increased by 41.18% ($P < 0.05$), and in cows in experimental group 2, by 52.94% ($P < 0.01$) compared to the control. On days 26 and 30 of the study, the level of follicle-stimulating hormone in the blood serum of cows in experimental group 1 increased by 44-53% ($P < 0.05$), and in the blood serum of cows in experimental group 2 by 56-60% ($P < 0.05$). The progesterone content in the blood serum of cows in experimental group 1 remained unchanged until day 22. Then it increased by 18.22% ($P < 0.01$) in the experimental group 1, and by 15.42% ($P < 0.05$) in the blood serum of cows in experimental group 2, compared to the control. The level of oestradiol in the blood serum of cows in experimental group 1 increased by 1.79% ($P < 0.01$) from day 2 of the study and by 10.06% ($P < 0.01$) by the end of the study compared to the control group, which should contribute to better fertilisation of cows. The results obtained may be helpful for veterinary specialists and milk producers in regulating the effect of hormonal drugs on the bodies of cows and predicting the dynamics of their concentration in the blood

Keywords: cattle breeding; sex hormones; hormonal background; reproductive capacity; immunoenzymatic analysis

Introduction

The development of dairy farming depends on many factors, the most important of which are: obtaining high-quality milk, dynamic changes in herd size, and organising the reproductive capacity of the dairy herd at an economically viable cost. Numerous programmes have been developed for managing enterprise data, and one of the most progressive is the use of hormonal preparations for successful artificial insemination of cows. At the same time, it is essential to consider natural changes in the hormonal background of animals, as interference with the physiologically normal functioning of the humoral system can have adverse consequences for their organism.

E.M. Sitko *et al.* (2023) found that the use of dairy herd management programmes generally depends on the skilful organisation of the enterprise's economy and herd reproduction management. To assess this, the researchers used a combination of data, including the length of the waiting period, the success rate of herd fertilisation, the cost of feeding a specific herd, the length of gestation, and the efficiency of milk production. The authors note the importance of maintaining a herd reproduction programme, which is an integral part of the milk production cycle. At the same time, the best strategy is to organise artificial insemination of cows,

which largely depends on the use of appropriate hormonal drugs.

When administering various hormonal drugs to cows, several factors must be considered. As stated by N.A. Khudhair *et al.* (2021) note that one of the adverse effects of oxidative stress is its activation of the hypothalamic-adrenocortical axis, which in turn causes an increase in corticosteroids in the blood plasma of animals. This results in sharp changes in cortisol concentration, especially during pregnancy in cows, leading to an imbalance in reproductive hormones. Therefore, before using hormonal drugs, it is necessary to consider the possible risks to the cows' bodies. This is especially true at the beginning of the milk production cycle for young heifers. At the same time, as the authors emphasised, it is essential to adhere to the timing for first calving, which is achieved through skilful control of the hormonal background in the cows' bodies. The researchers found a pattern of interdependence between metabolic and sex hormones. By stabilising the concentration of sex hormones and successful artificial insemination, a significant shift in metabolic processes is possible. And when milk producers violate the procedure for artificial insemination of cows, several negative consequences for their bodies arise.

According to H.C. Evans *et al.* (2022), issues related to the organisation of dairy herd reproduction are linked to the functioning of the endocrine system. In particular, they are connected to hormone levels and their impact on both animal welfare and farm economics. The authors noted that hormone levels play a crucial role in regulating the homeostasis of cows, influencing their growth, development, and reproductive efficiency. The selected areas for improvement of these traits were based on the timely use of hormones, such as testosterone, estradiol, progesterone, and their synthetic analogues, including trenbolone acetate and zeranol, which can be strategically utilised in cattle breeding and meat production systems. At the same time, scientists have demonstrated that, despite significant advancements in the use of these drugs to regulate sex hormone levels in cows, the impact on the functional state of the cows' bodies, as well as on the human body that consumes products derived from such cows, remains uncertain.

After successful insemination of cows, the following critical stages are the period between three weeks before and after calving. M. Daudon *et al.* (2022) argued that it is during these periods that a sharp jump in metabolic processes and hormonal background occurs. The consumption of all necessary organic substances also increases actively, and metabolic adaptation systems are activated. In most cases, the energy requirements of the animal's body rise so much that they exceed the amount of energy compounds supplied by the feed. If a possible imbalance of nutrients in the animal's body is not prevented, a negative energy balance may develop. In this case, hormones that regulate homeostasis play an essential role. Therefore, according to the authors, correction of the hormonal background in cows, especially sex hormones, will help prevent the development of serious complications in the postpartum period.

In implementing a system for the successful development of cattle breeding in combination with reproductive technology development schemes, external risk factors may arise in addition to internal ones, as emphasised by W.S. Kim *et al.* (2023). These include, first and foremost, climate change. Sudden changes in temperature in the premises where animals are kept and, in the environment, can cause significant metabolic disorders in their bodies. As a result, stress develops in cows, which in turn provokes the release of cortisol, among other hormones. Therefore, the organisation of successful reproductive development in the herd

begins with stabilising the hormonal background of cows, taking into account the effects of external factors.

The use of various hormonal drugs to improve the reproductive capacity of cows in dairy and beef cattle farming is practically commonplace. At the same time, there is a wide variety of hormonal agents and methods for creating or extracting hormonal substances on the market. However, despite the active use of hormonal drugs and the success of cow fertilisation programmes, there are several problems. The introduction of hormones into an animal's body activates many mechanisms that are necessary to achieve the goal of their use, and, in turn, less attention is paid to the adverse effects on the hormonal balance of cows. After all, changes in hormone concentrations can cause disturbances in the animal's body homeostasis. At the same time, we must not forget about the risks to the human body that can be caused by consuming products obtained from such cows. Therefore, the study of changes in the hormonal background of sex hormones is a current and relevant issue today. The study aimed to establish the dynamics of sex hormone levels (follicle-stimulating hormone, progesterone and oestradiol) in the blood serum of Ukrainian black-and-white dairy cows after the administration of hormonal preparations before and during insemination.

Literature Review

Several factors can negatively impact the reproductive capacity of productive animals. The leading factors among them are environmental and technological factors, which, depending on their intensity of impact, can be both negative and conducive to the physiological reproduction of reproductive processes. As a result of their negative impact, animal productivity decreases, and pregnancy proceeds pathologically, which can lead to the birth of non-viable offspring. Therefore, to address several issues related to the reproductive capacity of animals, various schemes for using hormonal drugs to facilitate the successful insemination of cows have been implemented. However, problematic issues regarding the degree of influence of these substances on animal health remain open.

P.M. Fricke & M.C. Wiltbank (2022) investigated the effectiveness of the Ovsynch hormonal protocol for artificial insemination in cows. Thanks to the skilful use of appropriate hormone administration, the fertility rate of cows increased every year. The implementation of a multi-level hormonal background formation in animals contributed to the best preparation of cows for artificial insemination. However, it is not possible to clearly control changes in the hormonal background in cows, which leads only to a statistical effectiveness of fertilisation growth without taking into account the health status of the animals.

After studying the use of GnRH and PGF-2-alpha hormonal preparations, T. Afriani *et al.* (2025) established the dynamics of progesterone concentration in the blood of cows. According to their data, fluctuations in hormone concentrations were observed on the 11th day of the study, depending on the age of the animals. Older cows had higher progesterone levels than younger cows, indicating improved luteal phase function. However, artificial correction of the hormonal background in cows did not have a positive effect on the effectiveness of artificial insemination.

As confirmed by research conducted by Y.A. Amin *et al.* (2023), the use of the hormonal drug GnRH causes changes in sex hormones in cows. At the same time, an increase in the levels of progesterone, follicle-stimulating hormone and luteinising hormone was observed, as well as changes in testosterone and cortisol levels. The researchers also demonstrated changes in the concentration of minerals in the cows' bodies.

R. Mikuła *et al.* (2021) studied the condition of animals in the period after sharp hormonal changes. Spexin peptide hormone served as a marker in the study of changes in hormonal background, including progesterone and metabolites of sex hormones. Changes in hormonal levels and metabolic profiles in cows were observed between 21 days before calving and during the first 14 days of lactation. Along with changes in spexin peptide hormone levels, significant changes in hormone concentrations were observed, which affected metabolic processes in cows.

The postpartum period is crucial for both the physiological well-being of cows and the establishment of lactation processes. In their study, A. Sammad *et al.* (2022) concluded that timely insemination of cows had a positive effect on milk production technology. At the same time, emphasis was placed on the mandatory monitoring of hormone concentrations in cows during the postpartum period to reduce serious consequences for the body, namely the possible occurrence of a negative energy balance. The results of similar studies conducted by O.B. Pascottini *et al.* (2022) and R.P. Nevard *et al.* (2023) showed that the animal's body undergoes significant metabolic changes, particularly in various aspects of the body's immune defence, with the activation of adaptive mechanisms for the onset of the lactation period. This period is the most dangerous for the animal's health. If hormonal drugs are misused to improve the effectiveness of artificial insemination, they can lead to negative changes in energy balance.

Despite significant changes in cows' bodies that need to be taken into account when using hormonal drugs, there is a particular group of exogenous factors to which reproductive processes are sensitive. K. Miętkiewska *et al.* (2022) proved the importance of considering the impact of heat stress on the animal's body. According to the authors, sudden changes in climatic conditions pose a significant threat to the reproduction of cattle herds. It has been established that temperature fluctuations hurt the hormonal balance of cows. Therefore, taking this factor into account is also essential for effectively monitoring hormone levels in cows and improving the reproductive capacity of productive animals.

Materials and Methods

The study was conducted from 2022 to 2024 at Podilskyi Hospodar 2004 LLC in the village of Velyka Medvedivka, Shepetivka District, Khmelnytskyi Region, Ukraine on cows of the Ukrainian black-and-white dairy breed. All manipulations with cows were carried out in accordance with the basic principles of bioethics, in accordance with Article 26 of Law of Ukraine No. 3447-IV (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986); Directive 2010/63/EU (2010) and the General Ethical Principles of Animal Experiments adopted by the First National Congress on Bioethics (Law of Ukraine No. 249, 2012).

To conduct the study, three groups of animals were formed using the analogue method (one control group and two experimental groups), with five cows in each group. The cows in experimental group 1 were administered 5 mL of "Surfagone" intramuscularly 10-15 minutes before insemination. "Surfagon" stimulates the pituitary gland to release luteotropic hormone, which creates favourable conditions for fertilisation in the female genitals and stabilises the signs of oestrus in cows. Animals in experimental group 2 were administered 2.5 mL of "Fertagil" intramuscularly during artificial insemination. "Fertagil" contains gonadotropin-releasing hormone, which regulates the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH)

and stimulates the growth and development of follicles, induces ovulation and controls the function of the corpus luteum.

Subsequently, a study was conducted to determine the levels of FSH, progesterone and estradiol during lactation on days 2, 6, 10, 14, 18, 22, 26 and 30. The principle of the method for determining follicle-stimulating hormone was based on the use of a two-site immunoenzymatic assay (DetectX® Progesterone ELISA, United States). A conjugate (second anti-FSH antibodies labelled with peroxidase) and the blood serum samples under investigation were added to the wells of a plate with immobilised antigen (specific anti-FSH antibodies). To obtain blood serum, the samples were incubated at 37°C in a thermostat. The clotted blood was separated from the walls of the tube using a glass rod. The serum was poured into centrifuge tubes and centrifuged for 10 minutes at 3,000 rpm. The resulting serum was then collected with a pipette. FSH from the sample binds to the antigen on the surface of the well and the conjugate. Material that did not bind was removed by washing. Next, the activity of the enzyme bound to the surface of the well in the plate became visible upon addition of the substrate. It was measured at a wavelength of 450 nm, along with the intensity of the colour reaction, which is directly proportional to the amount of FSH in the samples studied in pmol/L.

Progesterone and estradiol were determined using a test system based on competitive immunoassay. The test samples and conjugate (peroxidase-labelled progesterone/estradiol) were added to the wells of a plate with immobilised antigen (specific anti-progesterone antibodies). Progesterone from the test sample competed with the conjugate for binding to the antigen on the surface of the well. After washing, the activity of the enzyme bound to the surface of the well of the plate was revealed by the addition of a substrate and measured at a wavelength of 450 nm. The intensity of the colour reaction is inversely proportional to the concentration of progesterone in the test sample in pmol/L.

Statistical analysis of the study's results on sex hormone content in cow blood was performed using Microsoft Excel, with an assessment of the reliability of the difference in indicators and a Student's t-test analysis at significance levels of $P < 0.05$, $P < 0.01$, and $P < 0.001$. Additionally, the mean values and standard deviations were calculated for each group and each study period. The normality of the data distribution was checked by analysing the variation characteristics. The results are presented as the mean ± standard error of the mean, which ensured the correctness of the comparison between groups.

Results and Discussion

After using the hormonal drug "Surfagon" in experimental group 1 and "Fertagil" in experimental group 2 before insemination, changes in the level of follicle-stimulating hormone in the blood serum were detected in cows. Changes in follicle-stimulating hormone were monitored for 30 days at 4-day intervals, reflecting changes in its synthesis process. The differences between the groups of animals that received hormonal drugs to stimulate fertilisation efficiency were compared with the control group (Table 1).

Table 1. Level of follicle-stimulating hormone in the blood serum of cows after administration of hormonal drugs, pmol/L (M ± m, n = 5)

Day of blood sampling	Control group	Experimental group 1	Research group 2
2	0.17 ± 0.02	0.24 ± 0.02	0.26 ± 0.01
6	0.15 ± 0.02	0.23 ± 0.01*8	0.25 ± 0.01**

Table 1. Continued

Day of blood sampling	Control group	Experimental group 1	Research group 2
10	0.13 ± 0.02	0.25 ± 0.02**	0.26 ± 0.02**
14	0.14 ± 0.02	0.23 ± 0.01	0.24 ± 0.01
18	0.08 ± 0.02	0.17 ± 0.02	0.18 ± 0.02**
22	0.13 ± 0.02	0.24 ± 0.01	0.25 ± 0.01**
26	0.15 ± 0.03	0.23 ± 0.02	0.24 ± 0.01
30	0.16 ± 0.03	0.23 ± 0.02	0.25 ± 0.01*

***Note**: *P < 0.05, **P < 0.01 compared to the control group*
***Source**: developed by the authors*

As can be seen from Table 1, on the second day of the study, an increase in FSH levels of 41.18% (P < 0.05) was observed in the blood of cows in experimental group 1, and an increase of 52.94% (P < 0.01) was observed in the blood of animals in experimental group 2 compared to the corresponding values in the control group. On the sixth day of the study, a higher concentration of FSH was observed. In the blood of cows that were administered "Sulfagone" intramuscularly, the FSH level increased by 53.33% (P < 0.05). In the blood of animals administered "Fertagil" intramuscularly, the concentration increased by 66.67% (P < 0.01) compared to the control. On the 10th day of the study, FSH levels in the blood of cows in experimental group 1 increased 1.92 times (P < 0.01), and in the blood of animals in experimental group 2, 2.0 times (P < 0.01) compared to the control group. Starting from the 14th day of the study, a gradual decrease in the differences in FSH levels was observed. In the blood serum of cows in experimental group 1, the FSH level increased by 64.29% (P < 0.05), and in the blood serum of animals in experimental group 2, it increased by 71.43% (P < 0.05). Despite a relatively sharp decline in hormone concentration on day 18 of the study, FSH levels in the blood serum of cows remained within acceptable limits. In the blood serum of cows that were administered "Sulfagone" intramuscularly, FSH levels increased 2.13 times (P < 0.01). In animals that received intramuscular injections of "Fertagil", the increase was 2.25 times (P < 0.01) compared to the corresponding values in the control group. On day 22 of the study, FSH levels in the blood serum of cows in experimental group 1 increased 1.85 times (P < 0.01), and in the blood serum of animals in experimental group 2, 1.92 times (P < 0.01) compared to the corresponding control.

In the following stages of the study, it was found that in the blood serum of cows administered "Sulfagone" intramuscularly, the FSH level increased by 44% on day 26 and by 53% on day 30 (P < 0.05). In contrast, the blood serum of animals that received intramuscular injections of "Fertagil" showed an increase of 56% and 60% (P < 0.05) compared to the control group. The analysis of the data revealed a general increase in the level of follicle-stimulating hormone, with a corresponding rise in its blood content in cows resulting from the additional use of hormonal drugs. The use of hormonal medications to increase the fertility of cows affected the concentration of progesterone. The experimental groups that received "Fertagil" and "Sulfagone" exhibited a gradual increase in the level of this hormone in their blood, as reflected in the biochemical analysis indicators. The characteristics of progesterone levels are shown in Table 2.

Table 2. Progesterone levels in the blood serum of cows after administration of hormonal drugs, pmol/L (M ± m, n = 5)

Day of blood sampling	Control group	Experimental group 1	Experimental group 2
2	0.35	0.36 ± 0.01	0.36 ± 0.01
6	0.34 ± 0.07	0.37 ± 0.01	0.35 ± 0.01
10	0.35 ± 0.01	0.36 ± 0.01	0.33 ± 0.01
14	0.33 ± 0.03	0.37 ± 0.01	0.32 ± 0.01
18	1.52 ± 0.1	1.65 ± 0.03	1.44 ± 0.05
22	2.14 ± 0.05	2.53 ± 0.07	2.47 ± 0.05
26	1.98 ± 0.07	2.32 ± 0.05	1.94 ± 0.09
30	2.82 ± 0.06	3.32 ± 0.08	3.43 ± 0.08**

***Note**: *P < 0.05, **P < 0.01 01 compared to the control group*
***Source**: developed by the authors*

Table 2 shows that from day 2 to day 18 of the study, only a tendency towards a change in the level of progesterone in the blood serum of cows was observed. Starting from day 22 of the study, it was found that the level of progesterone in the blood serum of cows in experimental group 1 increased by 18.22% (P < 0.01), and in animals in experimental group 2 by 15.42% (P < 0.05) compared to animals in the control group. On day 26 of the study, a significant increase in progesterone levels of 17.17% (P < 0.01) was observed in the blood serum of cows that were administered "Sulfagone" intramuscularly, compared to the control group. However, there were no statistically significant differences between the progesterone levels in the blood of cows administered "Fertagil" and the corresponding levels in the blood serum of cows in the control group. On day 30 of the study, the level of progesterone in the blood serum of cows in experimental group 1 increased by 17.73% (P < 0.01), and in the blood serum of cows in experimental group 2, by 21.63% (P < 0.01) compared to the corresponding values in animals in the control group. Changes in the level of the studied hormone are significantly manifested on the 22nd day of the study, with a notable increase observed in the experimental groups, reflecting the effect of these drugs on the processes of progesterone synthesis. The content of estradiol in the blood of cows, as determined by biochemical analysis of the control and experimental groups of animals during the study, exhibited a consistent pattern of increase in its level when using "Fertagil" and "Sulfagone". During the 30-day study period, the use of hormonal drugs stimulated higher levels of oestradiol, as shown in Table 3.

Table 3. Serum oestradiol levels in cows after administration of hormonal preparations, pmol/L (M ± m, n = 5)

Day of blood sampling	Control group	Experimental group 1	Experimental group 2
2	67.09	68.29 ± 0.11	67.48 ± 0.65
6	61.11 ± 0.46	67.26 ± 0.34	63.35 ± 0.37
10	61.45 ± 0.36	63.88 ± 0.52	63.70 ± 0.20
14	59.81 ± 0.62	63.71 ± 0.42	62.16 ± 0.34*
18	54.68 ± 0.75	62.32 ± 0.50***	54.68 ± 0.82

Table 3. Continued

Day of blood sampling	Control group	Experimental group 1	Experimental group 2
22	56.34 ± 0.25	57.80 ± 0.40	54.85 ± 1.06
26	58.64 ± 0.34	62.32 ± 0.52	62.23 ± 0.60**
30	45.30 ± 0.40	55.50 ± 0.68***	44.56 ± 1.26

***Note**: *P < 0.05, **P < 0.01 01 compared to the control group*
***Source**: developed by the authors*

As shown in Table 3, on day 2 of the study, the level of oestradiol in the blood serum of cows in experimental group 1 increased by 1.79% (P < 0.01) compared to the control group. In contrast, the level of oestradiol in the blood serum of cows in experimental group 2 did not show statistically significant differences compared to the corresponding control. On the sixth day of the study, the level of oestradiol in the blood serum of cows administered "Sulfagone" increased by 10.06% (P < 0.01). In the serum of animals administered "Fertagil", the concentration increased by 3.67% (P < 0.05) compared to the corresponding values in the control group. On day 10 of the study, a decrease in the level of oestradiol in the serum of cows was observed. At the same time, in the blood of cows in experimental group 1, the level of oestradiol increased by 3.95% (P < 0.05), and in the blood serum of animals in experimental group 2, by 3.66% (P < 0.05) compared to the control. On the 14th day of the study, the level of oestradiol in the blood serum of cows in experimental group 1 increased by 6.52% (P < 0.01), and in animals in experimental group 2, by 3.93% (P < 0.05) compared to the corresponding values in cows in the control group.

On days 18 and 22 of the study, the level of oestradiol in the blood serum of cows administered "Fertagil" intramuscularly, in both the control and experimental groups, did not show statistically significant differences. However, on day 18, the serum oestradiol levels in cows that received intramuscular injections of "Sulfagone" increased by 13.97% (P < 0.001), and on day 22 of the study, in the blood of cows in experimental group 1, it increased by 2.59% (P < 0.05) compared to the values in the control group. On day 26 of the study, an increase in the level of oestradiol in the blood serum of cows in the experimental groups was established. Thus, in the blood serum of cows in experimental group 1, the level of oestradiol increased by 6.28% (P < 0.01), and in the blood serum of cows in experimental group 2, by 6.12% (P < 0.01) compared to that in animals in the control group. On day 30 of the study, the estradiol level in the serum of cows that received intramuscular injections of "Fertagil" did not differ significantly from that in the control group. In contrast, in the blood serum of cows that were administered "Sulfagone" intramuscularly, the oestradiol level increased by 22.52% (P < 0.001). Thus, the study revealed a shorter time of "Fertahil"'s effect on stimulating oestradiol synthesis, which reflects the effectiveness of this drug.

Modern dairy farms always use reproductive hormones, and this is true for virtually every country in the world. In particular, according to M. Javed *et al.* (2023), the rapid development of artificial insemination and the popularisation of genetic material transport have led to a sharp increase in demand for this product worldwide. The use of pharmaceuticals is a necessary measure in modern conditions for the reproduction of cattle. In addition, with the increase in the world's population, the demand for high-quality and safe food products has grown, and the implementation of successful artificial insemination systems cannot

be achieved without the use of appropriate hormonal drugs. However, these substances also have adverse effects. If misused, they can lead to the loss of highly productive livestock, reduce the overall balance of hormones other than sex hormones, and generally cause homeostasis disorders. It is also worth noting that pharmaceuticals can remain in the bodies of animals in the form of residues, which can pose a danger to both animals and humans.

H.A. Sharawy *et al.* (2022) analysed a programme for the use of hormonal drugs called Ovsynch, assessing the effect of a synthetic analogue of sex hormones on reproductive performance and hormone levels. The experimental group of cows received intramuscular gonadotropin-releasing hormone at a dose of 10 μg according to the programme, and 7 days later, they received intramuscular synthetic analogue of prostaglandin F2α at a dose of 500 μg and intramuscular analogue of gonadotropin-releasing hormone at a dose of 10 μg, administered intramuscularly 48 hours after the administration of prostaglandin. An increase in the blood levels of progesterone and oestrogen was observed in the experimental group of cows compared to the control group. At the same time, cortisol levels decreased. When compared with the results obtained in this study, a similar trend was observed: with artificial stimulation of hormone synthesis processes, their total amount increased compared to the control group.

W. Barański *et al.* (2024) investigated the use of a gonadotropin-releasing hormone antagonist called Buserelin in cows to improve follicle-stimulating hormone release. Thanks to the controlled use of this pharmacological drug at a dose of 4 mcg, it was possible to increase the concentration of follicle-stimulating hormone in the blood of cows. As a result of this intervention, the ovulation process was successfully established. In the study, the effect of the administered drugs was assessed, and it was found that various changes in sex hormone levels occurred, depending on the pharmacological action of the administered drugs. This highlighted the need for a more detailed study of the effect of hormonal medicines on the body.

The popularity of prostaglandin and gonadotropin-releasing hormone preparations is due to their ability to activate reproductive capacity in animals. According to a study by A. Wicaksono *et al.* (2023), their use resulted in a reduction in the interval between calving and first insemination and an increase in the number of inseminations per cow. The use of these drugs also increased sex hormone levels. However, the authors emphasised that despite the positive results of the hormones used, the normal physiological reproductive capacity of cows was disrupted, and this required a more detailed study of the action of these agents and the appropriateness of their use. Considering the results of this work, different levels of hormones in the blood of the test animals were determined, which depended on the action of the administered hormonal drugs.

L.M. Vargas Ortiz *et al*. (2025) studied the effect of hormones applied in accordance with the J-Sinch and FTAI protocols. They found that the effectiveness of the hormonal drugs used was reflected in the level of sex hormones and affected the further functioning of the animals' bodies. At the same time, in cows from the experimental group treated according to the FTAI protocol, the levels of estradiol and progesterone in the blood increased. In contrast, the levels of follicle-stimulating hormone remained unchanged. In turn, D.Z. Bisinotto *et al.* (2024) investigated the effect of hormonal drugs on physiological processes in cows. It was established that the use of 17β-estradiol caused changes in the hormonal background in cows. At the same time, changes in hormone concentrations are more noticeable

when pharmacological drugs are used, as confirmed by studies.

As shown by the study by R. Sartori *et al*. (2024), the use of hormonal protocols based on progesterone and a combination of progesterone and estradiol causes changes in gonadotropic hormone concentration. The effectiveness of hormones was also assessed by fertility indicators, which depended on the concentration of sex hormones. The use of a combination of progesterone and oestradiol had a positive effect on the results of artificial insemination and ovulation induction. The use of progesterone alone in animal insemination protocols was less effective, which was caused by poorer stimulation of sex hormones in cows. W. Barański *et al*. (2024) evaluated the effect of the gonadotropin-releasing hormone agonist "Bruselin" on sex hormone levels in dairy cows. It was found that the use of this drug at a dose of 10-20 mcg caused an increase in the blood content of follicle-stimulating and luteinising hormones. The use of this hormone had a positive effect on the processes of the sexual cycle and the growth of follicles in the ovaries.

Analysis of the results of the current study and other scientific research indicates the effectiveness of hormonal drugs in dairy production. At the same time, for the best results, hormonal drugs are always used according to specific regimens. As a result, the duration of the effect of additional hormones on the body of cows varies from 5 to 40 days, depending on the specific technology used. As a result, the impact of these drugs on the body varies to a certain extent. The first time these hormones are used, the effectiveness of fertilisation will be pretty significant, as the level of sex hormones will be higher than in a regular sexual cycle. However, the next time, the effectiveness of fertilisation in cows that have been given the drugs will be lower because their bodies will become dependent on these injections. According to the results of biochemical blood analysis, this happens due to the intensive synthesis of sex hormones under the influence of artificial hormones. In general, such stimulation of the cows' bodies is not positive, as it disrupts the overall hormonal balance. Cases where the use of these drugs is necessary are the development of pathological conditions in the animal's body that make a normal sexual cycle impossible in production conditions. Therefore, it is advisable to use hormonal regimens in cases of fertility disorders, rather than in attempts to save materials on the farm.

Conclusions

This study focused on investigating the effect of hormonal drugs such as "Sulfagone" and "Fertagil" on sex hormone levels in cows. Changes in sex hormone levels in cows were observed when using two types of hormonal medications. It was determined that during the 30-day study, the level of hormones in the blood serum of cows varied depending on the drug used. When "Sulfagone" and "Fertagil" were administered to cows on the second day of the study, the level of follicle-stimulating hormone in the blood serum increased. Despite fluctuations in the level of this hormone, its concentration in the blood serum of cows remained higher than that of other hormones studied during the 30 days. When studying the level of progesterone in the blood serum of cows, it was found that there were no significant fluctuations in the concentration of this hormone from day 2 to day 18 of the study. However, starting from day 22, the level of progesterone in the blood serum of cows in experimental group 1 increased by 18.22% ($P < 0.01$), and in experimental group 2 by 15.42% ($P < 0.05$) compared to the corresponding control. In general, the serum of cows administered Sulfagone intramuscularly showed an increase in hormone levels compared to the control group until day 30 of the

study. At the same time, in the blood serum of cows treated with "Fertagil", hormone levels approached those in the control group by day 26 of the study, and from day 30 of the study, they increased again by 17.73% ($P < 0.01$). The level of estradiol decreased in the blood serum of cows in the control group during the 30-day study period, whereas in the experimental groups, it remained constant. When assessing the concentration of sex hormones in the blood of cows during the study period, it was found that the administration of the hormonal drug "Sulfagone" stabilised the concentration of progesterone and estradiol, in contrast to the use of the hormonal drug "Fertagil". In general, it has been demonstrated that the use of hormonal preparations results in an increase in hormone concentrations in the cows' bodies. In future studies, it is planned to evaluate the metabolic processes following the administration of these hormonal preparations to productive animals and to determine their effect on other body systems.

Acknowledgements

None.

Funding

The study was not funded.

Conflict of Interest

None.

References

[1] Afriani, T., Udin, Z., Hellyward, J., Purwati, E., Jaswandi, J., & Merdana, I.M. (2025). Serum progesterone levels post-cosynch treatment in pesisir cattle as a genetic resource in West Sumatra. *Advances in Animal and Veterinary Sciences,* 13(5), 959-965. doi: 10.17582/journal.aavs/2025/13.5.959.965.

[2] Amin, Y.A., Mahmoud, A.E.Z., Ali, R.A., Fouad, S.S., Shanab, O., Ibrahim, R.M., & Mohamed, R.H. (2023). Treatment of inactive ovaries of Holstein dairy cows by epidural injection of GnRH analogue (Receptal) and its impact on reproductive hormones, oxidant/antioxidant profile and micro- and macro-elements profile. *Animals,* 13(4), article number 653. doi: 10.3390/ani13040653.

[3] Barański, W., Nowicki, A., Crowe, M.A., Tobolski, D., & Zduńczyk, S. (2024). Effect of repeated low doses of gonadotropin-releasing hormone on the secretion of luteinising hormone and follicle-stimulating hormone, and ovarian function in dairy cows suffering from anovulation type I. *Animal Reproduction Science*, 270, article number 107602. doi: 10.1016/j.anireprosci.2024.107602.

[4] Bisinotto, D.Z., Mattos, A.C.D., Bonacim, P.M., Feltrin, I.R., da Silva, A.G., Poit, D.A.S., Laurindo Neto, A., Marques, H.S., Peres, R.F.G., & Pugliesi, G. (2024). Impact of 17β-estradiol administration at the moment of timed-AI in Nelore cows. *Theriogenology,* 224, 143-155. doi: 10.1016/j.theriogenology.2024.05.008.

[5] Daudon, M., Ramé, C., Estienne, A., Price, C., & Dupont, J. (2022). Impact of fibronectin type III domain-containing family in metabolic and hormonal changes during the peripartum period in dairy cows. *Frontiers in Veterinary Science,* 9, article number 960778. doi: 10.3389/fvets.2022.960778.

[6] Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes. (2010, September). Retrieved from https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng.

[7] European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. (1986, March). Retrieved from https://rm.coe.int/168007a67b.

[8] Evans, H.C., *et al*. (2022). Harnessing reproductive hormones in cattle with considerations for animal welfare and human health. *Journal of Animal Science,* 100(7), article number skac177. doi: 10.1093/jas/skac177.

[9] Fricke, P.M., & Wiltbank, M.C. (2022). Symposium review: The implications of spontaneous versus synchronised ovulations on the reproductive performance of lactating dairy cows. *Journal of Dairy Science,* 105(5), 4679-4689. doi: 10.3168/jds.2021-21431.

[10] Javed, M., Mazhar, M., Hayat, S., & Tahir, M.A.A. (2023). Effects of reproductive hormones in dairy farm animals. *MARKHOR (The Journal of Zoology)*, 4(02), 18-26. doi: 10.54393/mjz.v4i02.67.

[11] Khudhair, N.A., Abbas, H.R., & Alsalim, H.A. (2021). Relationship between enzymatic antioxidant activities and reproductive hormones in cows with retained placenta. *Archives of Razi Institute,* 76(5), 1537-1543. doi: 10.22092/ari. 2021.355553.1696.

[12] Kim, W.S., Ghassemi Nejad, J., & Lee, H.G. (2023). Impact of cold stress on physiological and metabolic changes in beef cattle. *Animals,* 13(6), article number 1073. doi: 10.3390/ani13061073.

[13] Law of Ukraine No. 249 "On the Procedure for Carrying Out Experiments and Experiments on Animals by Scientific Institutions". (2012, March). Retrieved from https://zakon.rada.gov.ua/laws/card/z0416-12.

[14] Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15.

[15] Miętkiewska, K., Kordowitzki, P., & Pareek, C.S. (2022). Effects of heat stress on bovine oocytes and early embryonic development. *Cells,* 11(24), article number 4073. doi: 10.3390/cells11244073.

[16] Mikuła, R., Pruszyńska-Oszmałek, E., Pszczola, M., Rząsińska, J., Sassek, M., Nowak, K.W., Nogowski, L., & Kołodziejski, P.A. (2021). Changes in metabolic and hormonal profiles during the transition period in dairy cattle – the role of spexin. *BMC Veterinary Research,* 17, article number 359. doi: 10.1186/s12917-021-03069-4.

[17] Nevard, R.P., Pant, S.D., Broster, J.C., Norman, S.T., & Stephen, C.P. (2023). Maternal behaviour in beef cattle: Physiology and assessment – a review. *Veterinary Sciences,* 10(1), article number 10. doi: 10.3390/vetsci10010010.

[18] Pascottini, O.B., Leroy, J.L., & Opsomer, G. (2022). Maladaptation during the transition period and fertility consequences in dairy cows. *Reproduction in Domestic Animals,* 57, 21-32. doi: 10.1111/rda. 14176.

[19] Sammad, A., Khan, M.Z., Abbas, Z., Hu, L., Ullah, Q., Wang, Y., & Wang, Y. (2022). Significant metabolic alterations influencing postpartum fertility in dairy cows. *Metabolites,* 12(1), article number 60. doi: 10.3390/metabo12010060.

[20] Sartori, R., Alves, R.L.O.R., & Lopes, A.L.M. (2024). Induction of puberty vs. induction of ovulation using steroid hormones in beef heifers: A comprehensive review. *Animal Reproduction*, 21(3), article number e20240072. doi: 10.1590/1984-3143-AR2024-0072.

[21] Sharawy, H.A., *et al*. (2022). Vaginal and uterine blood flow changes during Ovsynch and their impact on pregnancy outcomes in dairy cows. *BMC Veterinary Research,* 18(1), article number 350. doi: 10.1186/s12917-022-03444-9.

[22] Sitko, E.M., Di Croce, F.A., McNeel, A.K., Weigel, D.J., & Giordano, J.O. (2023). Effect of reproductive management programmes that prioritised artificial insemination at detected oestrus or timed artificial insemination on the economic performance of primiparous Holstein cows of different genetic merit for fertility. *Journal of Dairy Science,* 106(9), 6495-6514. doi: 10.3168/jds.2022-22674.

[23] Vargas Ortiz, L.M., Andrade Yucailla, V.C., García Díaz, J.R., Acosta Lozano, N.V., Aragadvay Yungán, R.G., & Lima Orozco, R. (2025). Effects of two hormonal protocols for FTAI on fertility of repeat cows. *International Journal of Molecular Sciences,* 26(12), article number 5499. doi: 10.3390/ijms26125499.

[24] Wicaksono, A., van den Borne, B.H.P., Steeneveld, W., van Werven, T., & Hogeveen, H. (2023). Hormone use for reproductive diseases and heat induction in relation to herd-level reproductive performance in Dutch dairy farms. *Preventive Veterinary Medicine,* 211, article number 105832. doi: 10.1016/j.prevetmed.2022.105832.

Кількісні зміни прогестерону, естрадіолу та фолікулостимулюючого гормону в сироватці крові корів за використання гормональних препаратів

Леся Климковецька
Кандидат ветеринарних наук
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0009-0004-9847-0381

Валентин Карповський
Доктор ветеринарних наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-3858-0111

Ігор Грищук
Доктор філософії, асистент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-2571-6876

Володимир Духницький
Доктор ветеринарних наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-9670-1244

Леся Шуранова
Доктор філософії, асистент
Південночеський університет
37005, вул. Бранішовська, 1645/31а, м. Чеські Будейовиці, Чеська Республіка
https://orcid.org/0000-0002-5611-0490

Анотація. Більшість сучасних підприємств із виробництва продуктів тваринного походження активно використовують гормональні препарати тваринам без детального вивчення особливостей їх впливу на організм. Метою дослідження було встановити, як гормональні препарати впливають на динаміку рівня статевих гормонів в організмі корів. Дослідні групи корів формувалися за принципом груп-аналогів по 5 тварин у кожній. Коровам дослідної групи 1 вводили внутрішньом'язово препарат «Сурфагон» у дозі 5 мл, а дослідної групи 2 – препарат «Фертагіл» у дозі 2,5 мл. У сироватці крові корів визначали рівень фолікулостимулюючого гормону, прогестерону та естрадіолу в період лактації на 2, 6, 10, 14, 18, 22, 26 та 30 добу. Встановлено, що на 2 добу дослідження у сироватці крові корів дослідної групи 1 рівень фолікулостимулюючого гормону зростав на 41,18 % ($P < 0,05$), а у корів дослідної групи 2 – на 52,94 % ($P < 0,01$) порівняно з контролем. На 26 та 30 добу дослідження рівень фолікулостимулюючого гормону у сироватці крові корів дослідної групи 1 зростав на 44–53 % ($P < 0,05$), а в сироватці крові корів дослідної групи 2 – на 56–60 % ($P < 0,05$). Вміст прогестерону в сироватці крові корів дослідної групи 1 не

змінювався до 22 доби, а в подальшому зростав на 18,22 % ($P < 0,01$), а в сироватці крові корів дослідної групи 2 – на 15,42 % ($P < 0,05$) порівняно з контролем. Рівень естрадіолу в сироватці крові корів дослідної групи 1, починаючи з 2 доби дослідження, підвищувався на 1,79 % ($P < 0,01$) і до кінця дослідження – на 10,06 % ($P < 0,01$) порівняно з контрольною групою, що має сприяти кращому заплідненню корів. Отримані результати можуть бути корисними для фахівців ветеринарної медицини та виробників молока щодо регуляції впливу гормональних препаратів на організм корів та можливості прогнозувати динаміку їх концентрації в крові

Ключові слова: скотарство; статеві гормони; гормональний фон; відтворюваність; імуноферментний аналіз