The determination of the hormonal program efficiency in estrus synchronization of high-yield cows for sustainable agricultural development

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Abstract. At present, the improvement of methodological approaches and the search for more rational methods of hormonal processing for estrus synchronization in cows remains one of the urgent tasks of agricultural science and veterinary practice. As a result of the studies carried out, the influence of hormonal preparations on the dynamics of the corpus luteum lysis and the growth of follicles in the cows ovaries was determined using ultrasound diagnostics. As a result of ultrasound examination, it was found that the double use of Prosolvin promotes lysis of the corpus luteum. This is also evidenced by the low concentration of progesterone in the serum of cows. After the application of Gonadin ultrasound images show an active formation of follicles, the size of which gradually increases and reaches the maximum size before artificial insemination.

1. Introduction
At present, the development of dairy cattle breeding is a profitable direction, both from a livestock point of view and from an economic point of view, since dairy products are one of the sources of human nutrition [1, 2]. The success and development in this industry largely depends on the work of the animal insemination operator: identifying cows in heat, fruitful insemination and obtaining healthy offspring [3, 4]. But it is not always possible to predict the correct time of insemination or to identify estrous cows, and in some cases there may be a lack of workers on the farm [5]. Thus, the method of synchronizing estrous cows and heifers is gaining popularity. This method allows you to effectively solve reproduction tasks in a short time, namely, to manage the sexual cycle, clearly plan the time of calving, organize the fertilization of cows with possible minor pathologies, as well as facilitate the work of a livestock breeder and artificial insemination operator [6]. Estrus synchronization in cows and heifers allows solving the following tasks: to inseminate a large number of animals in a short time; to postpone the period of mass calving in dairy farming for economic purposes; organize the fertilization of animals in cases where the identification of estrus is difficult or impossible due to a number of production reasons; shorten the service period [7]. Thus, further improvement of methodological approaches and the search for more rational methods of hormonal treatment for estrus synchronization and therapy of cows remains one of the urgent tasks of agricultural science and veterinary practice [8].
The purpose of our work is to determine the dynamics of the corpus luteum induction and the growth of follicles in the ovaries of the cows under the estrus synchronization program, using ultrasound diagnostics.

2. Materials and methods
The production experiments were carried out on Holstein cows on the farm of LLC Agricultural enterprise “Donskoye” in the Kalachevsky district of the Volgograd region in the period 2020-2021. The animal welfare conditions in these farms meet zoohygienic, veterinary and sanitary requirements.

To determine the state of the ovaries in cows under the hormone program, an experimental group of 16 cows was formed. The selection of animals for the experimental group was carried out according to the principle of analogs, taking into account their age, physiological state, and clinical examination data.

The scheme for the estrus synchronization, developed by us, was used in the experimental group (Figure 1). The hormone program was started on Monday, the cows were injected intramuscularly with 2.5 ml of Gonadin (gonadotropin-releasing hormone) at a random stage of the estrus cycle, then a week later they were re-injected with Gonadin intramuscularly at a dose of 2.5 ml. The purpose of this injection is to induce ovulation of the dominant follicle (and thus start a new follicular wave) or to be injected when a new follicular wave has already formed spontaneously (therefore, the injection does not essentially affect the stage of the estrous cycle).

Seven days later, Prosolvin (prostaglandin) was injected intramuscularly at a dose of 1 ml, and after 24 hours the injection of Prosolvin was repeated to regress any corpus luteum present. The seven day interval was used to actually ovulate the follicle with the initial injection of GnRH, then the corpus luteum would grow large enough to respond to PGF2α.

Then, a day later, the cows received another 2.5 ml of intramuscular injection of Gonadin to ovulate the new dominant follicle. Nine days between two GnRH injections is enough for a new follicle to appear and grow to the appropriate size for a response to GnRH. Then, after 16 hours, artificial insemination was carried out and 2.5 ml of Gonadin were injected to increase the fertility of cows and prevent early embryonic mortality.

Ultrasound diagnostics of the corpus luteum and follicles in the ovaries of cows was carried out after lunchtime milking for 9 days, from the beginning of the hormone program to artificial insemination. The evaluation of estrus synchronization programs efficiency was carried out for the number of pregnant animals after 36 days, using rectal ultrasound.

For ultrasound examination of the ovaries, an Easy-Scan Curve ultrasound scanner from BCF Technology was used. Linear matrix converters with a frequency range of 5.0 to 7.5 MHz. Transrectal ultrasound investigation was performed by inserting an ultrasound transducer into the rectum. This made it possible to visualize and evaluate the architecture of the ovaries. At the beginning of each ultrasound examination, cows were fixed in headlocks. Excess feces were removed from the rectum to facilitate examination of the reproductive tract. The lubricated transducer was then placed in the palm and gently inserted into the rectum. The probe was pressed tightly against the ventral rectum and the examination was started. After taking pictures of the ovaries, the transducer was carefully removed.
from the rectum. The ultrasound results were interpreted in conjunction with farm records, herd data and visual observations.

To determine the effectiveness of Prosolvin hormonal preparation, we performed an analysis of serum for the concentration of progesterone in 6 selected animals that were under the estrus synchronization program. Blood samples were collected in clean micro-centrifuge tube from subcaudal vena after artificial insemination of the cows. The serum was separated by centrifugation at 4 °C with a speed of 3000 rpm for 10 min and was stored at a temperature of -20 °C for a hormonal assay. Progesterone was determined by radioimmunological analysis.

3. Results and discussion

As a result of 7 day ultrasound examination of synchronized cows, a large corpus luteum with a lacuna in the middle was observed on ultrasound images, which gradually decreased in size. Luteal tissue manifested itself as distinctly echogenic areas in the ovarian stroma. The granularity of the luteal tissue is visible (cells are enveloped in septa with blood vessels that feed and carry out the role of a carrier of hormones formed here). The lacuna was most often located in the center of the corpus luteum and filled with luteal fluid. Sometimes the lacuna was located on the edge of the corpus luteum, but the connection with the corpus luteum is always visible and the wall is lined with luteal cells. Unlike the vesicular follicle, which is not associated with the corpus luteum, the lacuna is combined with it into one format. The lacuna is usually less than 1/3 of the corpus luteum size.

After the intramuscular injection of Prosolvin in a dose of 1 ml done twice with an interval of 24 hours, a rapid regression of the corpus luteum was observed, while the lacuna disappeared and follicles began to form (Figure 2).

![Figure 2. Ultrasound images. Dynamics of lysis of the corpus luteum in the ovaries.](image)

Several small follicles and a small corpus luteum are visible. Such cows do not show estrus. But after the application of Prosolvin, prostaglandins F2a are released by the uterus, which act on the corpus luteum, destroying it. The production of progesterone stops and, as a consequence, the progesterone block is removed, leading to the formation of a new follicular wave and the formation of a dominant follicle.

After the administration of Gonadin at a dose of 2.5 ml, active follicular growth was observed in the ovaries. The growing follicle releases hormones that bring the cow in estrus. Visually, the follicle is visible as a cavity filled with follicular fluid with a thin wall (the closer the ovulation, the thinner), more often protruding above the surface of the ovary. When ovulation approaches, the inclusion of white (hyperechoic zones) crystals can be observed in the follicle wall (Figure 3).
Figure 3. Ultrasound images. The dynamics of the growth of follicles in the ovaries.

As a result of the research of cows’ blood serum samples, we received the following data (Table 1).

Table 1. The content of progesterone in the blood serum of cows during estrus synchronization.

| No. | No. of animal | Milk yield, liters per day | Weight, kg | The day after calving | Progesterone ng/ml |
|-----|---------------|---------------------------|------------|-----------------------|-------------------|
| 1   | 4771          | 25.6                      | 639        | 54                    | 0.41              |
| 2   | 1801272       | 48.7                      | 642        | 53                    | 0.25              |
| 3   | 1703276       | 51.4                      | 641        | 55                    | 0.38              |
| 4   | 88850         | 53.6                      | 642        | 56                    | 0.35              |
| 5   | 93484         | 41.0                      | 639        | 58                    | 0.21              |
| 6   | 1801356       | 38.6                      | 640        | 56                    | 1.0               |

Thus, it can be seen from the data in Table 1 that 72 hours after the injection of Prosolvin, the level of progesterone in the blood of cows reaches low levels, which is due to the massive lysis of the corpus luteum in the ovaries under the action of prostaglandin.

As a result, the following data were obtained for determining the efficiency of the estrus synchronization program, which are shown in Table 2.

Table 2. Evaluation of the efficiency of estrus synchronization programs.

| Indicators | Synchronization scheme |
|------------|------------------------|
| Number of selected animals | cows | 16 |
| Number of cows with clinically expressed estrus | cows | 14 |
| Percentage of animals that came in estrus | cows | 87.5% |
| Number of pregnant cows | cows | 13 |
| Percentage of the first insemination | cows | 81.25% |

According to the information given in the table, it was found that the efficiency of the synchronization program is 81.25%. This is due to the fact that cows were injected with 2.5 ml of Gonadin before artificial insemination, to increase fertility and prevent early embryonic mortality.

4. Conclusion

After analyzing the ultrasound scan, it can be concluded that the double use of Prosolvin promotes lysis of the corpus luteum. This is also evidenced by the low concentration of progesterone in the serum of cows. Before the use of prostaglandin, the corpus luteum had a size of 2.4 cm, but after the intramuscular injection of Prosolvin, the corpus luteum regressed, its size began to decrease to 1.2 cm, then completely disappeared. After the application of Gonadin, an increase in the level of luteinizing
hormone in the blood of animals begins, which contributes to folliculogenesis, ovulation of follicles and the manifestation of estrus.

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