

Evaluation of the gonadotropic preparation effect on the hormonal status of the infertile cows

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Abstract. To study of the effect of the gonadotropic preparation on the hormonal status of the infer-tile cows, 47 red-and-white cows belonging to the breeding farm (Voronezh Region, Russian Federation), having an ovarian hypofunction, at the age from 3 to 8 years old and with the live weight of 480-600 kg, were included in the experiment. The animals in the experimental group (n=27) received a single dose of placental gonadotropin, i.e. Follimag at the 1000 I.U. dose. The animals not injected with the preparation (n=20) formed the negative control group. Cows' blood samples were taken before injection, 4, 8 and 14 days after it. The hormone concentration (progesterone, es-tradiol-17 β) was determined in blood serum by the immunoenzymometric analysis. Introducing the preparation led to the increase of the progesterone level in the blood of the experimental group cows on average of 2.85 times and estradiol-17 β increased by 23.2% as compared with the background, progesterone increased 1.95 times and estradiol-17 β by 9.0 % compared with the control on the 14th day of the experiment. It was established that the degree of the ovarian hormonepoietic reaction manifestation and its direction in prescribing the exogenous gonadotropins depends on the time of the preparation injection relative to natural waves of the follicles growth. Using the gonadotropic preparation provided a 21.6-time increase in the blood content of progesterone on the 14th day of the experiment and that of estradiol-17 β of 1.29 times, when injected at the beginning of the follicles growth wave. When administered at the follicular atresia stage, it led to an increase in the progesterone level of 2.1 times and that of estradiol-17 β of 1.29 times.

1 Introduction

Pathological alterations in the genital organs are the main cause of infertility in the cattle breeding stock, which leads to a lack of animal yield and reduction in the productive longevity of animals. High incidence of infertility provoking pathologies concerning reproductive organs is registered among the highly productive cows. Most often, sterility in cows appears during parturition and in the post-partum period [1, 2].

The most common causes of reproductive problems include the ovaries functional disorders in the form of their hypofunction, which account for about 50-60% or more of all the ovarian disorders [3, 4, 5]. External factors of the disease pathogenesis include inadequate rations and feeds of poor quality in terms of metabolic energy, plastic substances, vitamins and mineral elements, as well as organism intoxication, short-term exercises, year-round stall keeping and weak insolation. Parturition pathologies and problems with acute postpartum and chronic endometritis also increase likelihood of the hypofunction [4, 6, 7, 8, 9]. It was established that the ovarian hypofunction could appear against the background of elevated pro-inflammatory

cytokines and leukocytes levels [9, 10]. Disorders in protein and lipid metabolism, hepatitis, weakening of the thyroid gland endocrine and of the hypothalamus-pituitary system, as well as the ovarian stroma are leading to a decrease in fertility [6, 11, 12, 13].

Correction of infertility in cows, which appears due to the gonads hypofunction, could be introduced in zoo-technical and veterinary practices using various methods, means and their combinations. A variety of approaches is a consequence of a large number of the ovarian dysfunction pathoetiological factors. Currently, biotechnical methods for normalizing the copulation function based on the introduction of gonadotropic preparations are widely used [8, 14, 15]. These methods induce the growth of follicles and ovulation in the ovaries of animals.

Hormone therapy of the cow infertility was being practiced for a long time. An accumulated extensive base of scientific research on the use of gonadotropic preparations indicates an ambiguous response of the ovaries to administering the exogenous hormones in different animals, and the expected clinical effect is not always achievable. In connection with the foregoing, the task of improving biotechnology in controlling the cows'

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reproductive function through introduction of hormonal preparations is not reducing relevance of this problem for scientific community and for practical business.

We assume that the solution to the problems of ensuring the cows' fertility is impossible without studying the effect of exogenous gonadotropins on the ovaries hormone-producing function taking into account growth waves and follicular atresia in animals.

2 Materials and methods

The scientific research was carried out on the basis of the breeding farm located in the Pavlovsky district of the Voronezh Region, Russian Federation.

47 sterile cows with ovarian hypofunction aged 3-8 years old and weighing 480-600 kg of the red-and-white breed were selected for the experiment. Animals of the experimental group (n=27) were once injected with placental gonadotropin preparation, i.e. Follimag, of the 1000 I.U. dose. Animals of the control group (n=20) were not injected with the preparation and provided the negative control.

The ovaries functional state was determined by their surface structure, size and consistency. The absence of mature follicles and corpus luteum, decrease in size, smooth surface and hard consistency were considered to be characteristic signs of the hypo functional disorder. An additional sign was the uterine atony in cows. During the experiment, all animals were subjected to regular clinical observations and registration of the copulation cyclicity stages onset.

To analyze hormone levels using the enzyme immunoassay (EIA), i.e. progesterone and estradiol-17 β , the venous blood was obtained from the animals before administering the preparation, then the procedure was repeated on the fourth, eighth and fourteenth days after administering the preparation. The whole blood was

centrifuged, and the resulting serum was frozen for storage at -15°C . EIA was performed using test systems manufactured by Chema-Medica and the Uniplan enzyme-linked immunosorbent reactions analyzer. Results were processed by mathematical methods accepted in scientific research.

3 Results

Data on hormone concentrations in blood of cows of the experimental group were analyzed taking into account the ratio between the time of animals' treatment with the preparation and the copulation cycle stages, i.e. follicles growth and atresia in the ovaries.

Action of Follimag, which contained follicle-stimulating hormones (FSH) and luteinizing hormones (LH), manifested itself already in 4 days after administration (Table 1). Against the background of the lack of these hormones, production of copulation steroids by the ovaries was being suppressed. The level of progesterone in the blood reliably ($P<0.05$) increased from 1.68 ± 0.269 to 4.83 ± 1.274 nmol/l or 2.88 times. The response reaction in the form of increased progesterone synthesis up to the level of 4.77-13.75 nmol/l at that time was observed in 54.5% of the experimental animals. After 8 days, the average level of progesterone in cows decreased by 26.7% to 3.54 ± 0.496 nmol/l (range from 1.78 to 7.06 nmol/l), although the response reaction to the preparation was already registered in 82.0% of the animals. After 14 days, the hormone concentration increased by 2.78 compared with the previous value and 5.85 times ($P<0.001$) compared with the initial value (up to 6.26-15.40 nmol/l). The response reaction to the preparation was registered in all treated cows; corpus luteum formation was registered in 75% of the animals.

Table 1. Follimag effect on the progesterone level (nmol/l) in the blood of cows with the ovarian hypofunction.

Indicators	Ovarian function state		
	Without accounting for copulation cyclicity	Follicles growth wave	Follicular atresia
Initial background	1.68 ± 0.269	0.51 ± 0.096	2.97 ± 0.458
After preparation administration	4 th day	4.83 ± 1.274	6.97 ± 1.751
	8 th day	3.54 ± 0.496	4.66 ± 0.825
	14 th day	9.83 ± 2.148	11.02 ± 2.129
			6.26

Analysis of the same results in comparison with the ovaries functional activity stage showed that progesterone production by the gonads increased sharply in the case of introducing placental gonadotropin against the background of follicles growth. In this case, the progesterone level increased 13.7 times in 4 days after the Follimag injection compared to the background ($P<0.001$). After eight days the increase compared to the background level was 9.1 ($P<0.001$), and after 14 days – 21.6 times ($P<0.001$).

Results of each animal individual observations in the experiment demonstrated that best results of the Follimag therapy were registered, when the preparation was administered on the first day of the starting follicle growth wave. The progesterone content in the blood at this time reached 7.06-15.40 nmol/l. With Follimag introduction on the second day of follicular activity, the hormone content rouse up to 6.18-9.90 nmol/l, when administered on the third day - up to 4.77-8.51 nmol/l. Thus, there appeared a tendency of the decreasing

effectiveness in the use of exogenous gonadotropins over time from the start of the follicle growth wave.

The lowest efficiency was shown by Follimag administration against the background of follicular atresia, the progesterone level altered by day 4 by 2.69%, and by day 8 – by 10.77%; the difference in results was

not significant. On the 14th day, the hormone content increased 2.1 times in comparison with the background level, which indicated ovulation with the formation of the corpus luteum. A similar result could be a consequence of gonadotropins endogenous synthesis activation in the animals.

Table 2. Follimag effect on the estradiol-17β level (nmol/l) in the blood of cows with the **ovarian hypofunction.**

Indicators		Ovarian function state		
		Without accounting for copulation cyclicity	Follicles growth wave	Follicular atresia
Initial background		0.69±0.032	0.63±0.056	0.77±0.028
After preparation administration	4 th day	0.66±0.027	0.66±0.057	0.67±0.022
	8 th day	0.85±0.091	0.87±0.079	0.84±0.176
	14 th day	0.85±0.103	0.81±0.136	0.99

In contrast to the progesterone content effect in the blood, preparation action on the estradiol-17β level started to be traced only on the 8th day after the animals' treatment (Table 2). By that time, the average increase in the concentration of the hormone was of 23.2%. Increase in the estradiol-17β content was more visible with the Follimag administration at the beginning of the follicles growth wave, i.e. 38.1%, compared with the background. When administered at the follicular atresia stage, the increase was 9.1%. After 14 days, the progesterone level under Follimag introduction at follicles growth range

was by 6.9% lower, and with introduction at the atresia stage, it was by 17.9% higher than that on the 8th day. Compared to the background indicator, increase in the estradiol-17β concentration by the 14th day occurred in 28.6% of both cases.

Thus, the gonadotropic preparation effect on the state of animal ovaries morphological and functional structures state with their hypofunction was more indicated in alteration of the progesterone synthesis, and, to a lesser extent, in the estradiol-17β. Alterations in the hormones ratio index are presented in Table 3.

Table 3. Alteration in the progesterone and estradiol-17β ratio indices during administration of Follimag to infertile **COWS.**

Indicators		Ovarian function state		
		Without accounting for copulation cyclicity	Follicles growth wave	Follicular atresia
Initial background		2.44	0.81	3.86
After preparation administration	4 th day	7.32	10.56	4.55
	8 th day	4.16	5.36	3.92
	14 th day	11.56	13.60	6.32

All presented options of the Follimag introduction demonstrated evident alteration in the progesterone and estradiol-17β ratio index towards its increase. Most significant alterations could be observed with introducing the preparation at the follicles growth stage. On the 4th day, in comparison with the background, the index increased 13.0 times, on the 8th day, it was 6.6 times higher, and on the 14th day, it was 16.8 times higher. Using the preparation against the background of follicular atresia led to the index increase on the 4th day of 1.2 times, on the 8th day, the index approached the original, and on the 14th day, the index increased 1.6 times. Without taking into account copulation cyclicity, increase in the progesterone to estradiol-17β ratio was

3.0, 1.7 and 4.7 in the corresponding periods. That once again confirmed high effectiveness of using the gonadotropic preparation to correct the ovarian hypofunction during the follicles growth wave.

Data provided in Table 4 show that the introduction of the Follimag preparation contributes to an increase in the progesterone level in 4 days after administration to animals of 2.45 times in comparison with the control indicator, and after 14 days – 1.95 times. The estradiol content in the blood, on the contrary, decreased in the experimental animals after 4 days by 9.6% compared to the control group, and after 14 days it was higher by 9.0%.

Table 4. Sex steroids content (nmol/l) in the blood of the cows during Follimag administration.

Indicator		Hormones		
		Progesterone	Estradiol-17β	Progesterone / estradiol
Initial background		1.68±0.269	0.69±0.032	2.44
In 4 days	Control	1.97±1.511	0.73±0.043	2.70
	Experiment	4.83±1.274	0.66±0.027	7.32
In 14 days	Control	5.04±1.610	0.78±0.053	6.46
	Experiment	9.83±2.148	0.85±0.103	11.56

Increase in the progesterone and estradiol ratio index was also noted. However, the indicator exceeded the control group value 2.7 times on the 4th day, 1.8 times on the 14th in the cows of the experimental group after Follimag introduction.

4 Discussion

Increase in the progesterone and estradiol ratio index was also noted. However, the indicator exceeded 2.7 times the control group value on the 4th day, 1.8 times on the 14th in the cows of the experimental group after Follimag introduction. It is known that FSH and LH secreted adenohypophysis control follicles growth in the ovaries, their maturation and ovulation. Their release cyclicly coincides with the follicles growth waves [16]. In most cows, 2 or 3 follicles growth waves are observed during the copulation cycle, less often - 1 or 4 [17, 18]. If three peaks of the gonadotropic hormones secretion and follicles growth waves are present in the copulation cycle, then they cover the period before ovulation, the 4th–6th and 11th–13th days after ovulation and are interconnected with action of the steroid hormones positive and negative feedback [19].

FSH stimulates the follicles growth and differentiation and promotes the steroid hormones synthesis. The LH role lies in ensuring maturation of follicles, ovulation and ovule maturation; androgens and prostaglandins are synthesized under the hormone action [19]. Weakening of the ovarian response reaction to gonadotropins under the corticosteroids effect in addition to the LH and FSH reduced production by the adenohypophysis leads to violation of the follicles maturation. High cortisol levels associated with stressful exposure are able to suppress the LH wave-like production [20, 21]. Based on this, it could be concluded that administering the gonadotropin preparation simulates the endogenous FSH and LH secretion waves activating copulation hormones synthesis in the ovaries, follicle morphogenesis and indirectly affecting hormonopoiesis in the anterior pituitary gland.

Due to the natural wave character of the gonadotropins secretion, synchronization of the exogenous gonadotropins administration synchronization in order to correct ovarian hypofunction with endogenous release of FSH and LH becomes of utmost importance. The emerging synergism of exogenous and endogenous hormones leads to effective restoration of the copulation cyclicly.

5 Conclusion

Administration of Follimag to infertile cows with the ovarian hypofunction leads to an increase in the progesterone concentration in blood after 4 days of 2.45 times in comparison with the control indicator, and after 14 days - 1.95 times. The estradiol concentration during the preparation administration decreased in the experimental group after 4 days by 9.6% compared with the control indicators, and after 14 days, it was higher by 9.0%.

The conducted study shows that for the most effective treatment of the ovaries hypofunctional state it is necessary to synchronize the gonadotropic preparation effect with the follicles growth wave. The term of drug administration should be optimally timed with the first or second day of the new follicles growth wave.

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