Research Article

Method for Restoring Functional Ovarian Activity in Cows

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Abstract | The article describes the study on the efficacy of recombinant interferons in the restoration of functional activity of the ovaries. The search for rational means of restoring gonadal hormonal function is relevant given the high prevalence of hypofunctional disorders in highly productive cows. Ovarian hypofunction was found to occur in 28.9-43.9% of cows examined and increase with higher milk productivity. In first-calf heifers, ovarian hypofunction was detected 1.3 to 2.0 times more frequently than in second-lactating cows and 1.3 to 2.8 times more frequently than in third- or later-lactating cows. Complex use of bovine recombinant interferons -α, -γ twice with a 24-hour interval at a dose of 5.0 ml and interferon-tau 8-9 days after the second injection of interferons -α, -γ twice with an interval of 24 hours at a dose of 10.0 and 5.0 ml is accompanied by an increase in therapeutic efficacy for cows with ovarian hypofunction by 10.0-20.0% (90.0%). After the complex use of bovine recombinant interferons -α, -γ and interferon-tau on days 18-20 after the treatment onset, the content of glucose increased by 55.5%, that of vitamin A - by 67.2%, vitamin C - by 14.1 %, serum bacterial activity - by 5.0%, serum lysozyme activity - by 13.3%, phagocytic activity of leukocytes - by 6.2%, estradiol - by 36.7%, progesterone - by 5.5 times, dehydroepiandrosterone sulfate - by 73.1 %. The dynamics of the blood anti-Müllerian hormone increase in cows on days 18-20th after the treatment onset slows down (25.2%) as follicles reach preovulatory condition. Apart from restoring functional ovarian activity, recombinant interferons facilitate the stabilization of energy metabolism, the weakening of lipid peroxidation and endogenous poisoning, and the activation of antioxidant protection.

Keywords | Cows, Cytokines, Hormones, Ovarian hypofunction, Recombinant interferons

INTRODUCTION

High-yielding dairy cattle farming is based on industrial maintenance and operating technologies, which affect reproductive potential. However, as shown by the reproduction function analysis of cows in agricultural enterprises of the Russian Federation, the level of yield does not exceed 70-75% (Reshetnikova et al., 2012;

Shabunin and Alekhin, 2015; Shabunin et al., 2020). Calf loss is associated with low cow fertility, the premature slaughter of cows, processing costs, and dairy production losses (Grigoryeva et al., 2014; Baba et al., 2017; Safonov et al., 2018).

Among leading causes of fertility impairment in dairy cows is postpartum ovarian hypofunction manifested by

depression of ovogenesis, folliculogenesis, delayed recovery of sexual cyclicity, and longer calving interval, the frequency of which is usually 25.0-78.5% in the herd (Gorpinchenko et al., 2008; Williams et al., 2008; Walsh et al., 2011; Crowe and Williams, 2012; Crowe et al., 2014).

Ovarian hypofunction is found in nearly one in three (35.4%) cows 40-60 days after calving (Petersson et al., 2008). The extent of functional gonad disorder propagation depends on the age of the cows. Thus, in first-calf heifers, it is registered almost twice as often as older animals (Beam and Butler, 1999; Gümen et al., 2004; Hammon et al., 2006). Global research on gynecological pathology in large cattle has shown a direct correlation between the rate of ovarian hypofunction and the level of milk productivity (Fleischer el al., 2001; Yániz et al., 2008; Juodžentytė and Žilaitis, 2018; Cremonesi et al., 2020).

The hypofunctional condition of the ovaries is regarded as a polyetiological pathology. Gonadotropic hormones play a decisive role in the development of ovarian dysfunction. However, such metabolic hormones as growth hormone, thyroxine, insulin, insulin-like growth factor (IGF-1), tumor necrosis factor, leptin, cortisol, and interferon, as well as interleukins and cytokines, also contribute to the development of this pathology (Gong et al., 2002; Diskin et al., 2003; Mihm and Bleach, 2003; Lebedev et al., 2005; Safonov, 2008).

Gonadoliberins, pituitary, and extrapituitary gonadotropins, estrogens, progestins are used separately or in combination to correct gonad hypofunction (Lamb et al., 2001; Lima et al., 2009; Dewey et al., 2010; Rudolph et al., 2011).

Gonadoliberins of natural and synthetic origin with gonadotropin-releasing hormone (GnRH) properties are widely used to manage hypofunctional states of the ovaries. A 250 µg dose of GnRH during the first 3-4 weeks after calving at the stage of a dominant follicle formation can cause a preovulatory release of luteinizing hormone (LH) and ovulation induction in 90.0% of animals (McDougall, 1994).

Recently, the use of gonadotropins in cows with ovarian hypofunction has become increasingly popular (Lobodin, 2010; Sedletskaya and Dyulger, 2012). Administration of Follimag/Folligon at a dose of 3 IU/kg of body weight to inhibit functional activity of the gonads ensures the sexual cycle restoration in 97.1% and subsequent fertilization in 88.4% of cows (Nezhdanov et al., 2003; Bogdanova, 2006). However, the reaction of granulosa cells of antral follicles to gonadotropic hormone action largely depends on the number of these follicles in the ovaries (Ireland et al., 2011; Scheetz et al., 2012).

One of the ways to restore the functional activity of the genital gonads is to use a prolonged form of progesterone (Progestamag) as a 2.5% solution via intravaginal devices: PRID (Abbat Laboratories, USA), CIDR-B (Easi Bread, New Zealand), ear implants Norgestomet (Crestar, Intervet, Netherlands) (Nanda et al., 1989; Jaskowski et al., 2000; Mwaanga and Janovski, 2000; Tebble et al., 2001; Zulu et al., 2003; Gumen and Wiltbank, 2005; Todoroki and Kaneko, 2006).

A promising direction for the normalization of ovarian functional activity is the use of tissue biostimulants to mobilize protective and adaptive responses of the organism (Selivanov and Dunikov, 2005; Kobozeva, 2007; Gorpichenko et al., 2016). Researchers are developing methods to restore cyclic gonad activity with platelet-enriched plasma, vitamin and mineral complexes, and vegetable supplements (Cremonesi et al., 2020; Affandhy et al., 2021).

Ovarian hypofunction comprises a significant share of gynecological diseases in breeding, slowing down the cycle of cattle reproduction and increasing the culling rate (Yániz et al., 2008; Sharapa, 2017; Salman et al., 2021). Therefore, research into reserves to restore functional ovarian activity in the postpartum period in the context of high milk productivity of animals is one of the urgent tasks.

The research aimed to study the effectiveness of using recombinant interferons to restore functional ovarian activity in cows in a hypofunctional state. The authors analyzed the changes in the morphological, biochemical, and immunological blood parameters of animals that received different recombinant interferons and their combinations. The effect of treatment on reproductive parameters was also evaluated.

A complex therapy with bovine recombinant α-, γ-interferons, and interferon-tau is suggested to be the most effective for the correction of gonade hypofunction.

MATERIALS AND METHODS

RESEARCH SAMPLING

The issue of postpartum ovarian hypofunction propagation was studied in 2019-2020 on the example of Simmental breed at Zhito LLC (Semilukskiy district, Voronezh region), Red-Motley breed at Agrotekh-Garant LLC (Rostoshinskiy and Ertilskiy district, Voronezh region), Black-Motley breed of Russian selection at SP Vyaznovatovka LLC (Nizhnedevitskiy district, Voronezh Region) and Black-Motley breed of German selection at CJSC Slavyanskoye (Verkhovskiy district, Oryol region).

Research devoted to studying the efficacy of using

recombinant interferons to treat postpartum ovarian hypofunction was carried out in 2020 on Black-Motley cows at SP Vyaznovatovka LLC (Nizhnedevitsky district, Voronezh region). The average annual milk yield of cows included in the experiment 70-90 days after calving is 6.0-7.0 thous. kg for the 2nd-4th calving with tethered keeping method. The groups consisted of cows with a completed postpartum uterine involution. The body condition score was about 3 points with no obvious evidence of metabolic disorders, diseases of the mammary gland, uterus, and extremities. All the studied animals were kept in similar conditions and received similar feeding that corresponded to the physiological state and productivity level. The animals were handled according to the principles of treating experimental animals of the EU directive (86/609/EEC) and Helsinki declaration.

RESEARCH DESIGN

With an interval of 24 hours, the first-group cows (n=10) received two injections of bovine recombinant interferon-tau at a dose of 10 ml (first administration) and 5 ml (second administration) containing 10.000 IU of trophoblastic bovine recombinant cytokine type I (anti-luteolytic interferon-tau) (SPE ProBioTech LLC, Belarus).

The animals of the second group (n=10) were injected with bovine recombinant interferons -α, -γ intramuscularly twice with an interval of 24 hours at a dose of 5.0 ml/animal, 1 cm^3 of which contained at least 1.0x10^4 IU/cm^3 of the total antiviral activity of bovine recombinant interferon-α and -γ (SPE ProBioTech LLC, Belarus).

Third-group cows (n=10) were injected twice with an interval of 24 hours with bovine recombinant interferons -α, -γ at a dose of 5.0 ml/animal. After 8-9 days following the second interferon injection, interferon-tau was administered twice at a 24-hour interval at a dose of 10 ml and 5 ml.

The fourth group (n=10) was injected with sodium chloride saline solution at 10 mL doses twice at a 24-hour interval.

RESEARCH METHODS AND STATISTICAL ANALYSIS

Postpartum ovarian hypofunction was diagnosed based on clinical and echographic studies in accordance with the Methodical Guide for the Prevention of Infertility in High Yielding Dairy Cattle (Voronezh, 2010) and using Easy-Scan-3 scanner (Ireland) equipped with a linear transducer with a frequency of 7.5 MHz.

Clinical control over all animals included examining sexual cycle arousal, insemination, and fertilization stages using visual and ultrasound studies and transrectal palpation. The animals were examined for 70 days after the calving with a 7-day interval.

Blood samples for laboratory studies were taken from 5 cows in each group before administering the drug, as well as 8-9 and 18-20 days after the first injection. Blood for laboratory studies was collected in vacuum tubes with EDTA K-3 Conserving and Blood Serum Activator (PUTH Clot-Activator, China) before morning feeding following the principles of aceptics and anticeptics.

The content of progesterone (P$_4$), estradiol-17β (E$_2$), testosterone (T), dehydroepiandrosterone sulfate (DHEA-S), interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNFα), insulin-like growth factor (IGF-1), anti-Müllerian hormone (AMH), total protein and its fractions, glucose, total lipids, cholesterol, β-hydroxybutyrate, total immunoglobulins, serum bactericidal activity (SBA), serum lysozyme activity (SLA), circulating immune complexes (CICs), phagocytic activity of leukocytes (PAL), phagocytic number (PhN), phagocytic index (PhI), vitamins A, E, C, malondialdehyde (MDA), medium molecular peptides (MMP) was defined.

An ABX Micros 60 (HORIBA, Japan) hematological analyzer was used to analyze hemmorphological blood. Biochemical studies were performed on the Hitachi-902 (Hitachi, Japan) analyzer according to the Methodical Recommendations for the Use of Biochemical Methods for the Study of Animal Blood (Ministry of Agriculture, 2005). Protein fractions have been determined by electrophoresis in agar gel (Kushmarova, 1983), and total protein concentration has been determined by a kit from Vital Diagnostics (Vital Diagnostics, Russia). Total immunoglobulin amount was determined by zinc-sulfate technique according to McEven (1970), SBA was measured as per the method of Smirnova and Kuzmina (1966), SLA according to the method of Kagramonova and Ermolyeva (1960), phagocytic activity of leukocytes with Staph aureus antigen according to Gostev (1950), phagocytic index (PhI) and phagocytic number (PhN) according to Plyaschenko and Sidorov (1979), and malondialdehyde according to Buzlama *et al.* (1997).

The serum content of progesterone, 17β-estradiol, testosterone, and dehydroepiandrosterone sulfate was studied using reagents for enzyme immunoassays (CJSC NVO Immunotech, Russia). The serum concentration of IL-1β, TNFα, IGF-1, and AMH was determined using the IEA test systems specific to the species Bovine Elisa Kit (Cloud-Clone Corp, USA). The sensitivity of the TNFα test was below 3.1 pg/ml, IL-1 β - 6.5 pg/ml, IGF-1 - 0.65 ng/ml, AMH - 24.77 pg/ml.

Statistical processing of the obtained data was performed using Statistica 8.0 application (Stat-Soft, Inc, USA). The results were expressed as arithmetic mean and standard deviation (M±SEM). Student's t-test for independent

samplings was used to evaluate the significance of results. Differences were considered statistically significant at p<0.05.

RESULTS AND DISCUSSION

The prevalence of hypofunctional ovarian conditions has been studied for cows with different levels of milk yield and other breeds (Table 1). It has been found that in cows of Simmental breed with milk productivity of 4.0 thous., the incidence of ovarian hypofunction averages 28.9%, including 44.1% in first-calf heifers, 27.0% in second lactation cows, and 18.6% in third or later lactation cows.

In cows of Red-Motley breed of domestic selection with milk productivity of 4.5 thous kg, an increase in the incidence of ovarian hypofunction was 30.6%, including up to 37.6% in first-lactating cows, up to 26.2 % in second-lactating cows, and up to 28.4% in third- or later lactating cows.

In Black-Motley cows of Russian selection with a productivity of 6.2 thous kg, there is up to 35.1% increase in hypofunctional ovarian disorder incidence, which is 1.2-1.3 times higher compared to less productive animals, including 43.9% among first-calf heifers, 32.4% in second-lactating cows, and 27.3% in third- or later lactating cows.

The highest frequency of ovarian hypofunction was found in German-bred Black-Motley cows with a milk production level of 9.2 thous kg. On average, ovarian hypofunction was diagnosed in 43.9% of the examined animals, including 52.8% in first-lactating cows, which is 1.5 times higher than second-lactation cows and 2.3 times higher than in third or later lactation cows.

During the study, gonad hypofunction was detected on average in 259 out of 755 cows examined, or 34.3%. This disease has the highest percentage of first-lactating cows – 44.8%, and the lowest in adult animals – 25.8-29.7%.

For example, ovarian hypofunction is diagnosed on average in 34.3% (28.9-43.9%) of cows examined and tends to increase with higher dairy productivity. In first-calf heifers, ovarian hypofunction was recorded in 37.6-52.8% of cases, which is 1.3-2.0 times higher than in second-lactating cows and 1.3-2.8 times higher than in third- or later lactating cows.

The study results on the clinical effectiveness of recombinant interferons in hypofunctional gonad states of cows are presented in Table 2. Thus, in the group of animals that were twice injected with saline, the restoration of sexual cyclicity was restored in 40.0% of animals, including 20.0% after 8-9 days and 20.0% after 18-20 days.

Table 1: Prevalence of hypofunctional ovarian disorders in cows.

Animals	Milk productivity, thous kg	Animals examined	Cows with detected ovarian hypofunction	
			Cows	%
Simmental breed				
Cows, total number, including	4.0	114	33	28.9
1st lactation		34	15	44.1
2nd lactation		37	10	27.0
3rd or later lactations		43	8	18.6
Red-Motley breed				
Cows, total number, including	4.5	271	83	30.6
1st lactation		85	32	37.6
2nd lactation		84	22	26.2
3rd or later lactations		102	29	28.4
Black-Motley breed (Russian selection)				
Cows, total number, including	6.2	222	78	35.1
1st lactation		82	36	43.9
2nd lactation		74	24	32.4
3rd or later lactations		66	18	27.3
Black-Motley breed (German selection)				
Cows, total number, including	9.2	148	65	43.9
1st lactation		89	47	52.8
2nd lactation		37	13	35.1
3rd or later lactations		22	5	22.7
Total				
Cows, total number, including	-	755	259	34.3
1st lactation		290	130	44.8
2nd lactation		232	69	29.7
3rd or later lactations		233	60	25.8

Table 2: Efficacy of using recombinant interferons for the restoration of the functional ovarian activity in cows (M±SEM).

Group	Number of cows	Restoration of the sexual cyclicity				Efficacy, %	Period from calving to fertilization	Fertilization rate
		In 8-9 d		In 18-20 d				
		cows	%	cows	%			
1. Interferon-tau	10	7	70.0	1	10.0	80.0	98.5±10.2	3.06±0.25
2. Interferons -α, -γ	10	5	50.0	2	20.0	70.0	110.2±12.2	3.29±0.13
3. Interferons -α, -γ + interferon-tau	10	5	50.0	4	40.0	90.0	93.5±8.3	2.81±0.12
4. Saline	10	2	20.0	2	20.0	40.0	123.5±12.4**	3.55±0.17**

*, P <0.05; **, P <0.01; ***, P <0.001 - compared to administration of saline (group 4).

Two-fold administration of bovine recombinant interferons -α, -γ contributed to the manifestation of sexual cyclicity on days 8-9th after the treatment onset in 50.0% of animals and day 18-20th 20.0% of animals. The period from calving to fertilization was also reduced by 13.3 days, and the fertilization rate declined by 0.26.

The administration of interferon-tau to cows restores sexual cyclicity and fertilization in 80.0% of cows on average in 98.5 ± 10.2 days with a fertilization rate equal to 3.06 ± 0.25.

The most effective was the combined use of recombinant interferons -α, -γ, and interferon-tau. Such therapy resulted in the restoration of sexual cyclicity on days 8-9th after the treatment onset in 50.0% of animals and days 18-20th – in 40.0 % of cows. Combined use of recombinant interferons -α, -γ and interferon-tau was effective in 90.0%, which was by 10.0% higher compared to interferon-tau monotherapy, 20.0% – compared to single use of recombinant interferons -α and -γ, and 50.0% – compared to saline administration.

The fertilization rate after the use of recombinant interferons -α, -γ, and interferon-tau was 93.5± 8.3 days. That is 5.0 days shorter than when applying monotherapy with interferon-tau, 16.7 days with recombinant interferons -α, -γ, and 30.0 days (P<0.001) with saline, at the fertilization rate decrease by 0.25, 0.48, and 0.74 (P<0.01), respectively.

Thus, using a combination of recombinant interferons -α, -γ and interferon-tau is 10.0-20.0% more effective. Duration of infertility reduced by 5.0-30.0 days, and a fertilization rate – by 0.25-0.74.

Clinical trial data for the use of recombinant interferons are confirmed by laboratory tests conducted on bovine blood before and after administration of the drug.

In comparison with initial data, cows injected with interferon-tau twice (group 1) on days 8-9 after administration of the preparations demonstrated the decreased concentration of total protein by 11.0% and albumin by 19.0% (Table 3).

This indicated the normalization of protein metabolism, as well as general immunoglobulins by 13.7%, medium molecular peptides by 27.3% (P<0.01), malondialdehyde by 11.8%, showing a decrease in endogenous load and intensity of lipid peroxidation processes.

At the same time, the content of β-globulins was higher by 29.8% (P<0.01), that of γ-globulins – by 12.3%, glucose by 31.3% (P<0.01), vitamin A by 53.8% (P<0.01), vitamin E by 8.5%, indicating a higher energy status and activation of the non-enzymatic link in antioxidant protection.

The blood concentration of estradiol in cows increased by 25.0% compared with the treatment onset, progesterone by 7.56 times, DHEA-S by 33.1%, indicating a wave of follicle growth and sexual cyclicity. After using recombinant interferon-tau, there was a decrease in the level of proinflammatory cytokines in the blood of cows, including TNFα by 19.6%, IL-1β by 25.6% (P<0.05). At that, IGF-1 increased by 1.65 times (P<0.01) and AMH by 1.69 times (P<0.05), which indicated the normalizing effect of recombinant interferon-tau.

In animals administered with recombinant interferons -α, -γ (group 2, 3) on days 8-9 after the treatment onset, the content of total protein decreased by 6.6%, albumin by 8.3%, β- hydroxybutyrate by 37.5% (P<0.001), nitric oxide by 36.1% (P<0.01), medium molecular peptides – by 36.4% (P<0.01). At the same time, the concentration of β-globulins was higher by 17.9% (P<0.05), glucose – by 12.6%, vitamin A – by 53.8% (P<0.01), vitamin E – by 10.6%, vitamin C – by 14.1%, estradiol – by 23.3%, progesterone – by 74.1% (P<0.001), and DHEA-S – by 23.9% (P<0.01). This indicated higher energy and antioxidant status of the animal organism after the administration of interferons -α, -γ and the beginning of their sexual cyclicity. Restored functional activity of the gonads occurred against the background of 14.6-22.1% lower TNF$_\alpha$ level (P<0.05) and 31.5-35.8% lower IL-1β level (P<0.01) due to increased synthesis of sex steroids. A more intense decrease of IL-1β level in the blood of cows is explained by a decrease in the stimulating effect f TNF$_\alpha$ on it.

Table 3: Morphological, biochemical, and immunological blood indicators of cows 8-9 days after the administration of recombinant interferons (M±SEM).

Indicators	Before the administration (n=20)	Days 8-9 after the treatment onset			
		interferon-tau (n=5)	interferons -α, -γ (n=5)	interferons -α, -γ + interferon-tau (n=5)	saline (n=5)
Total protein, g/L	86.0±1.7	76.5±5.7	80.3±2.2	80.3±2.2	84.0±1.7
Albumins, %	51.7±1.6	44.3±1.3	47.4±0.9	47.4±0.9	50.7±1.6
α-globulins, %	12.8±0.6	13.0±0.3	13.6±0.1	13.6±0.1	11.8±0.6
β-globulins, %	16.8±0.6	21.8±0.9**	19.8±0.6*	19.8±0.6*	16.2±0.6
γ-globulins, %	18.7±0.8	21.0±0.6	19.1±0.5	19.1±0.5	17.7±0.8
Glucose mnol/L	1.6±0.1	2.1±0.2**	1.8±0.1	2.0±0.1	1.6±0.1
Total lipids, g/L	5.2±0.2	5.5±0.3	4.8±0.3	5.3±0.3	5.2±0.2
Cholesterol, mmol/L	6.1±0.3	5.8±0.6	5.1±0.5	5.5±0.5	6.1±0.3
β-hydroxybutyrate mmol/L	1.6±0.2	0.9±0.01***	1.0±0.01***	1.1±0.01***	1.6±0.2
Total Jg, g/L	37.1±1.0	32.0±3.0	33.2±0.3	33.2±0.3	30.1±1.0
CICs, g/L****	0.43±0.1	0.52±0.04	0.69±0.07	0.69±0.07	0.73±0.1
SBA, %	81.4±2.4	86.1±4.3	84.5±6.1	84.5±6.1	81.4±2.4
SLA, µg / ml	1.51±0.04	1.72±0.03	1.53±0.01	1.54±0.01	1.44±0.04
PAL, %	68.0±1.2	70.5±5.0	71.2±2.1	71.2±2.1	68.0±1.2
PhI, m.c./act. phagocyte	6.3±0.32	6.5±0.5	6.2±0.5	6.2±0.5	6.2±0.32
PhN, m.c./phagocyte	4.3±0.24	4.0±0.4	4.1±0.4	4.1±0.4	4.1±0.24
Vitamin A, µmol/L	1.3±0.01	2.0±0.2**	2.0±0.2**	2.0±0.2**	1.4±0.01
Vitamin E, µmol/L	14.1±0.5	15.3±0.6	15.6±0.6	15.6±0.6	13.1±0.5
Vitamin C, µmol/L	29.0±2.3	33.2±3.2	33.1±3.0	33.1±3.0	22.0±2.4
MMP, c.u.	1.1±0.1	0.8±0.04**	0.7±0.04**	0.7±0.04**	1.2±0.1
MDA, µmol/L	1.7±0.11	1.5±0.12	1.7±0.13	1.7±0.10	1.8±0.11
P_4, nmol/L	0.82±0.04	6.2±0.43***	10.8±0.6***	10.2±0.5***	2.2±0.43***
E_2, nmol/L	0.24±0.01	0.30±0.01*	0.37±0.02**	0.35±0.02**	0.30±0.01*
T, nmol/L	1.77±0.11	1.72±0.09	1.66±0.10	1.58±0.10	1.72±0.09
DHEA-S, nmol/L	0.151±0.01	0.201±0.01**	0.249±0.01**	0.234±0.01**	0.167±0.01
$TNF_α$, pg/ml	324.9±16.3	261.2±19.6	277.4±18.4	253.2±14.6*	316.7±19.3
IL-1β, pg/ml	35.5±2.2	26.4±1.5*	24.3±1.3**	22.8±1.5**	34.7±1.9
IGF-1, ng/ml	2.3±0.01	3.8±0.02**	3.6±0.02**	3.9±0.02**	2.4±0.02
AMH, pg/ml	45.7±2.8	77.6±5.5*	85.2±4.9**	96.7±6.1***	46.9±3.1

*, P <0.05; **, P <0.01; *** - P <0.001 - compared with the beginning of the experiment (before the administration of the preparations); ****CICs, circulating immune complexes; SBA, serum bactericidal activity; SLA, serum lysozyme activity; PAL, the phagocytic activity of leukocytes; PhI, phagocytic index; PhN, phagocytic number; MMP, medium molecular peptides; MDA, malondialdehyde; T, testosterone; DHEA-S, dehydroepiandrosterone sulfate; TNFα, tumor necrosis factor-alpha; IL-1β, interleukin-1β; IGF-1, insulin-like growth factor; AMH, anti-Müllerian hormone.

The concentration of AMH after two-fold administration of recombinant interferons -α, -γ increased by 1.86-2.12 times (P<0.01-0.001), which indicated the growth of follicles and the beginning of functional ovarian activity restoration.

In the blood of cows treated with saline (group 4) in 8-9 days, no significant differences in indicators of morphological and biochemical status were found in comparison with the beginning of the experiment.

In 18-20 days after the treatment onset (Table 4), the most pronounced changes were found in animals administered with recombinant interferons -α, -γ and interferon-tau. After the complex application of recombinant interferons on days 18-20 after the treatment onset, the content of β-globulins increased by 23.8% (P<0.01), glucose - by 55.5% (P<0.001), vitamin A - by 67.2% (P<0.01), vitamin

C - by 14.1%, SBA - by 5.0%, SLA - by 13.3%, PAL - by 6.2%, estradiol - by 36.7% (P<0.01), progesterone - by 5.5 times (P<0.001), DHEA-S - by 73.1% (P<0.01). At that, the concentration of β-hydroxybutyrate decreased by 2.0 times (P<0.001), CICs - by 9.3%, MDA - by 22.6% (P<0.05), MMP - by 36.0% (P<0.01). Changes in the indicators of the hormonal-biochemical status occurred against the background of a further decrease in the concentration of TNF_α and IL-1β by 1.49 (P<0.01) and 1.95 (P<0.001) times, respectively, compared with the initial study. It can be explained by the additional administration of interferon-tau, which triggered a cascade of anti-inflammatory responses.

An increase of IGF-1 in the blood of cows by 1.91 times occurred against the background of follicle growth in the ovaries and higher estrogen concentration. The dynamics of AMH in the blood of cows 18-20 days after the treatment onset changed (by 25.2%, P<0.01), which a preovulatory state of the follicles could explain.

Table 4: Morphological, biochemical, and immunological blood indicators in cows on day 18-20th after the administration of recombinant interferons (M±SEM).

Indicators	Before the administration (n=20)	18-20 days after the treatment onset			
		interferon-tau (n=5)	interferons -α, -γ (n=5)	interferons -α, -γ + interferon-tau (n=5)	saline (n=5)
Total protein, g/L	86.0±1.7	75.5±5.7	78.3±2.2	70.3±2.2*	84.0±1.7
Albumins, %	51.7±1.6	47.3±1.3	48.4±0.9	52.4±0.9	50.7±1.6
α-globulins, %	12.8±0.6	13.0±0.3	13.6±0.1	13.6±0.1	11.8±0.6
β-globulins, %	16.8±0.6	20.8±0.9	19.8±0.6	20.8±0.6**	16.2±0.6
γ-globulins, %	18.7±0.8	21.0±0.6	19.1±0.5	22.1±0.5*	17.7±0.8
Glucose, mmol/L	1.61±0.11	2.33±0.20	1.91±0.12	2.55±0.13***	1.63±0.11
Total lipids, g/L	5.2±0.2	5.5±0.3	4.8±0.3	5.1±0.3	5.2±0.2
Cholesterol, mmol/L	6.1±0.3	5.8±0.6	5.1±0.5	5.5±0.5	6.1±0.3
β-hydroxybutyrate, mmol	1.6±0.2	0.9±0.01***	1.0±0.01	0.8±0.01***	1.6±0.2
Total Jg, g/L	37.1±1.0	34.0±3.0	33.2±0.3	35.2±0.3	30.1±1.0
CICs, g/L****	0.43±0.1	0.52±0.04	0.49±0.07	0.39±0.07	0.73±0.1
SBA, %	81.4±2.4	86.1±4.3	84.5±6.1	85.5±6.1	81.4±2.4
SLA, µg/ml	1.5±0.04	1.7±0.03	1.5±0.01	1.7±0.01	1.4±0.04
PAL, %	68.0±1.2	70.5±5.0	71.2±2.1	72.2±2.1	68.0±1.2
PhI, m.c./act.phagocyte	6.3±0.32	6.5±0.5	6.2±0.5	6.2±0.5	6.2±0.32
PhN, m.c./phagocyte	4.3±0.24	4.0±0.4	4.1±0.4	4.1±0.4	4.1±0.24
Vitamin A, µmol/L	1.31±0.01	2.08±0.18**	2.11±0.15	2.19±0.12**	1.44±0.11
Vitamin E, µmol/L	14.1±0.5	15.3±0.6	15.6±0.6	15.6±0.6	13.1±0.5
Vitamin C, µmol/L	29.0±2.3	33.2±3.2	33.1±3.0	33.1±3.0	22.0±2.4
MMP, c.u.	1.11±0.11	0.82±0.04	0.77±0.04**	0.71±0.04**	1.21±0.11
MDA, µmol/L	1.77±0.11	1.54±0.13	1.68±0.11	1.37±0.09*	1.81±0.11
P_4, nmol/L	0.82±0.04	6.0±0.43***	10.8±0.6***	12.1±0.7***	2.2±0.43***
E_2, nmol/L	0.24±0.01	0.29±0.01*	0.37±0.02**	0.41±0.02**	0.30±0.01*
T, nmol/L	1.77±0.11	1.69±0.09	1.66±0.10	1.75±0.10	1.72±0.09
DHEA-S, nmol/L	0.151±0.01	0.192±0.01**	0.249±0.01**	0.289±0.02**	0.167±0.01
TNF_α, pg/ml	324.9±16.3	253.1±16.2*	264.9±19.6*	216.8±15.5**	312.2±22.3
IL-1β, pg/ml	35.5±2.2	25.5±1.3*	23.7±1.7*	18.2±1.2***	34.1±2.1
IGF-1, ng/ml	2.3±0.01	3.9±0.02**	3.5±0.02	4.4±0.02***	2.5±0.02
AMH, pg/ml	45.7±2.8	88.7±4.9*	92.7±5.1**	72.3±6.5**	47.3±2.9

*, P <0.05; **, P <0.01; ***, P <0.001 - compared with the beginning of the experiment (before the administration of the preparations); ****CICs, circulating immune complexes; SBA, serum bactericidal activity; SLA, serum lysozyme activity; PAL, the phagocytic activity of leukocytes; PhI, phagocytic index; PhN, phagocytic number; MMP, medium molecular peptides; MDA, malondialdehyde; T, testosterone; DHEA-S, dehydroepiandrosterone sulfate; TNFα, tumor necrosis factor-alpha; IL-1β, interleukin-1β; IGF-1, insulin-like growth factor; AMH, anti-Müllerian hormone.

CONCLUSIONS AND RECOMMENDATIONS

Thus, ovarian hypofunction is diagnosed on average in 28.9 to 43.9% of the cows examined and tends to increase with an increase in milk production. In first-calf heifers, ovarian hypofunction is recorded 1.3-2.0 times more often than cows of the second lactation and 1.3-2.8 times than in cows of third or later lactation.

The complex use of bovine recombinant interferons -α, -γ and interferon-tau is accompanied by higher therapy efficacy for cows with a hypofunctional ovarian state by 10.0-20.0%. Duration of infertility shortened by 5.0-30.0 days, and fertilization rate declined by 0.25-0.74.

Restored functional activity of the gonads with recombinant interferons 7-8 days after the therapy began indicated higher energy and antioxidant status of the animal organism and the beginning of their sexual cyclicity.

After 18-20 days from the treatment onset, changes in hormonal-biochemical status indicated stabilization of energy metabolism, weakening of lipid peroxidation and endogenous intoxication, and activation of antioxidant protection against the background of a restored ovulatory gonadal function. At this time, a further decrease in the content of proinflammatory cytokines (TNF_α and IL-1β) was noted due to extra administration of recombinant interferon-tau. The level of AMH increased along with the concentration of IGF-1 in the follicular growth of the ovaries and an increase in estrogen concentration.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

CONFLICT OF INTERESTS

The authors have declared no conflict of interest.

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