Research Article

Ovarian Hypofunction and its Relationship to Serum Hormonal and Cytokine Profile in Cattle

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Abstract | This work examines how the postpartum suppression of ovulatory function in dairy cows is linked to their serum hormonal and cytokine profile. The concentrations of progesterone (P_4), estradiol-17β (E_2), testosterone (T) and dehydroepiandrosterone sulfate (DHEAS) in blood was determined via enzyme immunoassay. Serum levels of interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNFα), insulin-like factor (IGF-1) and anti-Müllerian hormone (AMH) were measured using Bovine Elisa kits (Cloud-Clone Corp., USA). The animals were divided into two groups: cows with a normal pattern of reproductive cyclicity resumption; cows with the suppressed function of the ovaries. It was found that cows with ovarian hypofunction had problems with the conversion of DHEAS into testosterone and estradiol-17β. There was no pre-ovulatory surge of estrogen in such cows. They also had lower hormone levels. The levels of DHEAS were lower by 22.8 to 25.0%. Meantime, the levels of estradiol-17β were lower by 27.3 to 55.6%, and the levels of testosterone were lower by 15.4 to 50.0%. Levels of TNF_α in cows with ovarian hypofunction were 1.43 to 2.07 times higher compared with controls. TNF_α stimulated the production of IL-1β, causing its serum concentrations to increase by 1.24 to 2.48 times. Meanwhile, the serum levels of IGF in cows with ovarian hypofunction were 1.58 to 2.65 times lower, indicating a decreased hormone-producing function of the gonads and a decline in insulin production. Serum AMH concentration in such cows was 1.20 to 1.92 times lower the first 2 months after calving, indicating that reproductive cycles were abnormal and that no mature follicles were present.

Keywords | Cows, Cytokines, Hormones, Postpartum, Suppression of ovulatory function.

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INTRODUCTION

The reproductive potential of cows heavily depends on the health of the uterus and ovary. Among many ovarian disorders, the most common ones occur postpartum, such as ovarian hypofunction (Crowe and Willams, 2012; Khurshid and Anjum, 2012; Dyulger, 2013; Mitina, 2018; Ventsova and Safonov, 2021), follicular and luteal cysts.

In cows, the loss of normal ovarian function can result in long-term or persistent infertility (Baba et al., 2017). The occurrence of ovarian disorders is a reason for culling. According to different estimates, between 7% and 15% of cows diagnosed with infertility due to ovarian dysfunction are culled annually (Sartori et al., 2006; Nelson et al., 2010; Kocharyan et al., 2012; Novikova et al., 2019).

In cows, the normal resumption of ovarian cyclicity occurs within 30-55 days after calving (Opsomer et al., 2000; Moreira et al., 2001; Stevenson, 2006; Nezhdanov et al., 2008; Chomaev et al., 2014). The prevalence of hypogonadism in postpartum female cows was reported between 23% and 50% (Gümen et al., 2004; Nezhdanov et al., 2014). Postpartum ovarian dysfunction in cows should be considered a hypothalamic-pituitary-gonadal (HPG) axis disorder associated with functional changes in the neuroendocrine system (Diskin, 2003; Chebel et al., 2006; Bachelot and Binart, 2007; Larsen et al., 2009; Sineva et al., 2019).

Among the causes of impaired ovarian function in high yielding cows after calving, researchers distinguish the negative energy balance, which results from the combination of three factors: the onset of lactation, the increased milk production, and, most importantly, the insufficient feed intake (Varenikov et al., 2014; Moroz, 2015). Energy deficiency during lactation is accompanied by an unfavourable shift in the metabolism of carbohydrates, fat and protein, and by metabolic, immune and endocrine homeostasis. The consequences are the disrupted production of luteinizing hormone, the key agent in the resumption of ovarian function that is secreted in the pituitary gland (Kessler et al., 2014; Santos et al., 2016; Roche et al., 2017; Lebedeva et al., 2018; Safonov et al., 2018; Vasilenko and Rusakov, 2018; Chernitskiy et al., 2019). The negative energy balance can also come with a substantial decrease in insulin-like growth factor, insulin, glucose and leptin, accompanied by an increase in ketones, unsaturated fatty acids, and β-hydroxybutyrate (Vanholder et al., 2006; Ortega et al., 2008; Silvestre et al., 2011).

Tumour necrosis factor and interleukin (IL)-1β produced in adipose tissue play an important role in energy homeostasis (Vernon, 2005). Cytokines IL-1β, IL-6, and IL-8 in the blood and follicular fluid serve as immunological markers of ovarian failure (Lima et al., 2019).

The inhibition of hormone release in the pituitary gland could be associated with a decrease in ovarian secretory activity. Androgens and estrogens secreted from the ovaries are the key regulators of folliculogenesis and ovulation in mammals (Burke et al., 2000; Nezhdanov, 2003). Following a feedback loop, reductions in sex steroids from the ovaries diminish the functional activity of the hypothalamus and pituitary. This leads to a decrease in the secretion of pulsatile gonadotropin-releasing (GnRH) and luteinizing (LH) hormones.

This study aims to examine how the postpartum suppression of ovulatory function in dairy cows is linked to their serum hormonal and cytokine profile. The objectives of the study are: (1) to explore the dynamics of sex steroids (i.e., progesterone and estradiol-17β), testosterone, dehydroepiandrosterone sulfate; (2) to determine the concentration of each of the following hormones in blood: interleukin-1β (IL-1β), tumour necrosis factor (TNFα), insulin-like factor (IGF-1), and Anti-Müllerian hormone (AMH); and (3) based on hormonal and cytokine profile results, to investigate their role in the regulation of ovulatory function in dairy cows and to determine mechanisms of ovarian suppression. The results of the study will expand the modern understanding of pathogenetic mechanisms underlying the postpartum ovarian insufficiency in high yielding dairy cows and facilitate the development of effective correction methods.

MATERIALS AND METHODS

The study was conducted from February to October in 2020 on 16 Red Motley cows maintained at two Agrotekh-Garant LLCs, located in Zadonye (Ramonskiy district, Voronezh region; the total number of animals, 1200 animal units) and Rostoshin (Ertilskiy district, Voronezh region, Russia; total number of animals, 800 animal units) The animals used in the experiment were 2nd to 4th lactation cows, aged 4 to 6 years.

All cows under study were kept in individual tie stalls during the investigation. These were 2^{nd}-4^{th} calving cows producing 6 to 7 kg of milk on an annual basis.

During the 70-day postpartum period, the ovaries and uteri of cows were examined by transrectal palpation and ultrasonography at 7-day intervals to determine follicular populations, the time of ovulation, and the presence of a corpus luteum. Transrectal examinations were carried out using standard procedures in accordance with the Methodical Manual for the Prevention of Infertility in High Yielding Dairy Cattle (Voronezh). Ultrasonography was performed using an Easy-Scan-3 ultrasound scanner (BCF Technology, U.K.) equipped with a linear 7.5 MHz transducer. Based on the results of the examination, the animals were classified into two groups: cows with a normal pattern of reproductive cyclicity resumption (control group) and cows with the suppressed function of the ovaries (followed by irregular cycles).

Blood for laboratory analysis was obtained from all cows on days 6, 12, 19, 26, 33, 40, 47, 54, 61, and 68 after calving. Blood samples were collected from the tail vein into vacuum tubes containing a clot activator (PUTH Clot-Activator, China) in the morning before feeding. The samples were used to measure the concentration of each of the following hormones and cytokines: progesterone (P_4), estradiol-17β (E_2), testosterone (T), dehydroepiandrosterone sulfate (DHEAS), interleukin-1β (IL-1β), tumour necrosis factor (TNF_α), insulin-like factor (IGF-1), and Anti-Müllerian hormone (AMH). Serum levels of progesterone, estradiol-17β, testosterone, and DHEAS were measured by an enzyme-linked immunosorbent assay (ELISA) using the standard kits (NVO Immunotech, CJSC). Levels of IL-1β, TNF_α, IGF-1 and AMH in serum were determined by employing Bovine-specific ELISA test kits (Cloud-Clone Corp, USA). The sensitivity of assays was as follows: TNF_α assay, up to 3.1 pg/ml; IL-1β assay, up to 6.5 pg/ml; IGF-1 assay, up to 0.65 ng/ml; and AMH assay, up to 24.77 pg/ml. Statistical data analysis was performed using the Statistica 8.0 software (Stat-Soft, Inc, USA). Results are expressed as arithmetic means and standard deviations (M±SEM). The significance of differences between the samples was determined using the nonparametric Wilcoxon signed-rank test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

During the first 12 days after calving, all cows had a blood level of P_4 in the range between 0.86 and 0.90 nmol/L (Table 1). By day 19, some cows (group 1) experienced the spontaneous resumption of ovarian cyclicity, as evidenced by a 10.5% increase in P_4 concentration. On day 26, the level of P_4 in blood was found to become 9.1 times higher (8.18±0.09 nmol/L, $P<0.001$), signalling the onset of ovulation and formation of corpus luteum in the ovary. With corpus luteum formation, P_4 levels increased by 26.8 times ($P <0.001$), reaching 23.01±1.87 nmol/L on day 33.

By day 40, P_4 fell by 5.4 times, down to 4.29±0.33 nmol/L, marking the end of the luteal phase of the ovarian cycle. By day 54, the P_4 concentration rose again, up to 22.4±2.12 nmol/L, indicating that a new cycle has begun.

In cases where the blood level of P_4 remained practically unchanged throughout the entire 54-day period, cows were considered to have an impaired ovulatory function with absent ovulation and absent corpus luteum. The activation of the hormone-synthesizing function of the ovaries was delayed and took place only on day 68, as evidenced by a 3.6 times increase in the blood P_4 level from baseline. Follicle production and ovulation processes in the control group were accompanied by an increase in estradiol-17β. In the first ovarian cycle, the serum concentration of E_2 grew from 0.25±0.01 to 0.39±0.03 nmol/L, a 56.0% increase (Table 1). In the second ovarian cycle, it went from 0.18±0.02 to 0.34±0.02 nmol/L, an 88.9% increase.

In the ovulatory dysfunction group, the E_2 level declined to 0.22±0.02 nmol/L from baseline by day 26, a 27.3% decrease, and then again decreased to 0.18±0.03 nmol/L by day 54. The E_2level returned to baseline by day 68. During the first 2 months after calving, there was no pre-ovulatory rise in estrogen production among cows with impaired ovarian function, and estrogen is a key factor in the ovulation of the dominant follicle.

Testosterone production in controls rose from 0.21±0.01 to 0.39±0.02 nmol/L, an 85.7% increase, by the time of the first ovulation and continued to wave up, reaching 0.50±0.03 nmol/L before the second ovulation (Table 1). In the ovarian dysfunction group, the serum concentration of T declined by contrast: during the first wave of follicle growth (days 19-26), it dropped by 27.6% to 50.0% ($P<0.01$); and at subsequent intervals, it fell by 15.4% to 46.2% ($P<0.001$). By day 68, however, it became 1.25 times higher ($P<0.05$), up to 0.40 ± 0.03 nmol/L. Given the fact that T is synthesized by theca cells in the follicle, such an increase may signal the onset of folliculogenesis.

Dehydroepiandrosterone and dehydroepiandrosterone sulfate are among the main precursors in the synthesis of biologically active sex steroids, namely testosterone and estrogen. In the control group (Table 1), by day 12, the serum concentration of DHEAS increased by 1.82 times compared to baseline. Follicle growth and ovulation came with a 9.5% decrease in DHEAS by day 19, followed by a 26.4% decrease by day 26. These changes indicate the increased production of testosterone and estradiol-17β and that more testosterone and estradiol-17β precursors have been consumed.

The dynamics of DHEAS in the ovulatory dysfunction group were similar to those in the control group. At the same time, something appeared to be blocking the conversion of this hormone to testosterone and estradiol-17β. It could be prolactin. Excessive prolactin inhibits the action of dehydrogenase and aromatase, the key enzymes catalyzing the conversion of DHEAS into active steroids. Serum levels of T and E_2 in cows with ovarian hypofunction were significantly lower when compared to cows with resumed ovarian function, especially during follicular growth (Table 1). As mentioned earlier, a steady tendency toward normalization of ovulatory function in this group was seen on day 68 (delayed resumption). It happened simultaneously with a 22.8% increase in DHEAS concentration from baseline ($P<0.05$). As for the cows with resumed cycles (Group 1), their 68-day serum levels of tes-

Table 1: Changes over time in the serum concentration of sex steroids in postpartum cows (nmol/L, M±SEM)

Day after calving	Sex steroids							
	P_4		DHEAS		T		E_2	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
6	0.86± 0.01	0.90± 0.02	12.1± 0.8	17.3± 1.0**	0.21± 0.01	0.23± 0.02	0.25± 0.01	0.27± 0.01
12	0.90± 0.02	0.87± 0.02	22.0± 1.4	18.5± 1.1*	0.24± 0.02	0.22± 0.02	0.28± 0.02	0.28± 0.01
19	0.95± 0.07	0.87± 0.02	19.9± 1.2	19.0± 1.4	0.29± 0.02	0.21± 0.01**	0.28± 0.03	0.26± 0.01
26	8.18± 0.09	0.91± 0.02***	16.2± 1.0	15.0± 0.8	0.28± 0.01	0.14± 0.01**	0.39± 0.03	0.22± 0.02**
33	23.01± 1.87	0.84± 0.01***	19.5± 1.2	21.4± 1.1	0.39± 0.02	0.21± 0.02***	0.32± 0.03	0.24± 0.02*
40	4.29± 0.33	0.83± 0.02***	17.7± 1.1	20.8± 1.3	0.33± 0.03	0.25± 0.01*	0.18± 0.02	0.25± 0.03
47	10.7± 0.92	0.85± 0.02***	26.9± 1.1	22.3± 1.2*	0.39± 0.02	0.33± 0.02*	0.34± 0.02	0.22± 0.01***
54	22.4± 2.12	0.84± 0.02***	32.3± 1.8	26.0± 1.5**	0.50± 0.03	0.37± 0.02**	0.30± 0.01	0.18± 0.01**
61	14.3± 0.92	1.64± 0.11***	28.3± 1.3	27.6± 1.6	0.42± 0.03	0.45± 0.03	0.23± 0.01	0.24± 0.01
68	9.72± 0,51	3.07± 0.25**	22.8± 1.2	28.0± 1.4*	0.32± 0.02	0.40± 0.03*	0.19± 0.02	0.27± 0.03**

* - P <0.05; ** - P <0.01; *** - P <0.001 indicates statistically significant difference when compared to Group I.

Table 2: Blood levels of cytokines in postpartum cows (M±SEM)

State of ovaries	Days of postpartum period									
	6	12	19	26	33	40	47	54	61	68
IL-1β, pg/ml										
Group I	45.6± 2.9	40.3± 3.1	32.1± 2.4	20.6± 1.5	24.6± 1.9	18.7± 1.3	9.7± 0.62	12.6± 0.9	10.3± 0.7	7.1± 0.5
Group II	60.1± 4.1**	50.9± 3.9*	45.3± 4.1	39.7± 3.5***	30.6± 2.2*	20.6± 1.6	24.1± 1.9***	18.6± 1.4**	15.9± 1.2*	10.6± 0.8*
TNF_α, pg/ml										
Group I	331.8 ±19.2	257.3± 15.2	221.1± 14.5	219.5± 12.9	189.4± 11.6	239.1± 14.3	229.3± 11.4	204.8± 15.9	214.1± 16.7	201.7± 14.2
Group II	421.5 ±23.1*	397.3± 20.4***	381.5± 23.4**	321.6± 19.8**	392.8± 22.1**	364.7± 26.4**	357.1± 29.7**	332.8± 23.6*	307.4± 24.1*	284.2± 19.7*
IGF-1, ng/ml										
Group I	2.9± 0.21	3.0± 0.26	4.5± 0.31	6.1± 0.45	5.1± 0.39	4.9± 0.33	7.2± 0.51	5.5± 0.39	4.9± 0.31	5.3± 0.40
Group II	1.2± 0.09***	1.9± 0.13**	2.0± 0.16***	2.3± 0.14***	1.8± 0.15***	3.1± 0.21**	2.9± 0.19***	3.6± 0.23**	4.2± 0.34	4.4± 0.37
AMH, pg/ml										
Group I	45.6± 3.1	62.7± 4.2	83.6± 6.6	134.7± 9.2	64.2± 4.3	102.7± 7.9	151.9± 11.2	74.1± 5.7	129.4± 11.3	122.7± 10.4
Group II	23.8± 1.5***	36.4± 2.1***	43.7± 2.8***	66.9± 4.5***	50.7± 3.4	71.6± 5.6**	89.7± 6.8**	80.3± 6.5	95.3± 7.1	102.4± 8.8

* - P <0.05; ** - P <0.01; *** - P <0.001 indicates statistically significant difference when compared to Group I.

-tosterone and estradiol-17β were higher by 25.0% (P<0.05) and 42.0% (P <0.01) from baseline, respectively. During the first ovarian cycle, the DHEAS-to-estradiol-17β ratio was 73.6, whereas, during the second ovarian cycle, it was 107.7.

The inhibition of ovarian function in recently calved cows manifests in the decreased production of sex hormones (androgens and estrogens), which, in turn, affects the growth and differentiation of antral follicles and ovulation. Therefore, the postpartum ovarian insufficiency in high-yielding dairy cows can be described as intra-ovarian homeostatic insufficiency, developed under lactation-induced stress (Sineva et al., 2019).

Tumour necrosis factor-alpha (TNF_{α}) is a multifunctional proinflammatory cytokine synthesized by monocytes and macrophages. Cows with reduced ovulatory function displayed a 1.48 times decrease in the baseline TNF_{α} concentration 2 months after calving (Table 2). In contrast, cows that resumed ovulatory function showed a more pronounced decline in TNF_{α} concentration with a 1.65 times decrease from baseline. Namely, TNF_{α} concentrations in this group were 1.47-2.07 (P <0.01) and 1.43-1.63 (P <0.01) times lower than in the ovarian hypofunction group at the end of the first and second cycles, respectively.

A drop in the serum level of TNF_{α} in cows with resumed ovulatory activity occurred with an increase in progesterone production. As for cows with ovarian hypofunction, the serum TNF_{α} concentration in this group reached its minimum value once the resumption of the ovarian function occurred (day 68).

In the ovarian hypofunction group, there were 1.24 to 2.48 times higher concentrations (P <0.05-0.001) of IL-1β throughout the examined period when compared to the group (Table 2). The minimum IL-1β concentration (10.6-18.6 pg/ml) was observed at day 68. Two months after calving, cows with the impaired ovulatory function displayed a 1.49 times higher level (P <0.05) of IL-1β than controls. In the control group, the serum IL-1β concentration declined steadily, and the minimum values (7.1 pg/ml and 12.6 pg/ml) were observed earlier, at days 47 and 54, respectively.

TNF_{α} stimulated IL-1β production. The inhibition of ovulatory function is related to pro-inflammatory processes in the reproductive organs. These processes affect the functioning of gonads.

The serum level of insulin-like growth factor (IGF-1) in controls increased by 1.83 times during the first 2 months after calving (Table 2) and became 1.58 to 2.65 times higher (P <0.001) than in cows with impaired ovulatory function. It happened because hormones such as insulin, androgens, and estrogens stimulated the secretion of IGF-1 in the liver. Therefore, a lower concentration of IGF-1 in the blood of cows with impaired ovulatory function may indicate the presence of hypogonadism, on the one hand, and a decrease in insulin production, on the other hand.

During the first 68 days after calving, cows with ovarian hypofunction had 1.20 to 1.92 times lower levels of AMH (P <0.01-0.001, Table 2). The controls displayed a 2.95 times increase in AMH levels from baseline at day 26 and a 3.33 times increase in AMH levels from baseline at day 47. Subsequently, there was a dramatic decline in AMH concentration in this group, such as it practically reached the baseline. Perhaps, this was due to the development of dominant follicles, increasing aromatase activity, and an upswing in estradiol.

In cows with reduced ovulatory function, AMH concentration increased by 4.3 times from baseline and peaked at day 68, probably due to the resumption of the functional activity of the ovaries. A decreased AMH concentration in cows with ovarian hypofunction during the first 2 months postpartum also indicates that reproductive cycles were inadequate and that no mature follicles were present.

CONCLUSIONS

During the first 2 months postpartum, cows with suppressed ovulatory function exhibited no pre-ovulatory rise in estrogen production and their testosterone levels varied between 24 nmol/L and 50 nmol/L. The occurrence of ovulatory suppression is related to pro-inflammatory processes in the reproductive organs. Cows with suppressed ovulatory function thus had higher TNFα and IL-1β levels compared with cows that had resumed ovarian cycling activity. Meanwhile, the levels of IGF-1 in the blood of cows with suppressed ovulatory function were 1.58 to 2.65 times lower than in the blood of cows with resumed ovulatory activity. Lower levels of IGF-1 indicate a decrease in the hormone-synthesizing function of the gonads and a decline in insulin production. Finally, cows with suppressed ovulatory function presented 1.20 to 1.92 times lower concentrations of AMH in blood. The functional activity of gonads in cows with ovarian hypofunction resumed within the period between day 61 and day 68, as evidenced by a 4.3 times increase in AMH concentration by day 68 postpartum.

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CONFLICT OF INTERESTS

The authors had no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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