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Original article

Hormonal and Biochemıcal Parameters Analysis of the Yeld Cows Blood

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Abstract

In the research was selected animals with various pathologies of the reproductive system in order to study the hormonal and biochemical status of the yeld (infertile) cows. Further, groups were formed in accordance with the disease type. The experimental groups included clinically healthy fertilized animals and animals with a physiological ovary cycle. In the study was The authors of this article measured the levels of sex steroids, adrenal and thyroid hormones, and also determined the indices of the main metabolic processes, the levels of macro- and micronutrients, and vitamin A in the blood serum. Excluding the group of cows with luteal cysts, the infertile animals demonstrated a noticeable decrease in progesterone between by 2.4-14.5 times. The yeld (infertile) cows without clinical changes in the genitals, cows with uterine subinvolution and ovarian hypofunction showed a markedly reduced (between by 1.6-4.1 times) testosterone level in the blood. A decrease in the estradiol concentration was noted in all the studied groups, some animals demonstrated the deviations in the cortisol and triiodothyronine levels from the indices in the healthy cow groups. The hormonal status of the infertile animals indicated probable violations in the regulation systems of the endocrine glands, the initial links of which are the hypothalamus and pituitary gland. The biochemical blood characteristics, in turn, indicate that yeld (infertile) cows suffer from changes in metabolic processes, a macro-and micronutrients deficiency, especially the selenium deficiency. To restore the reproductive function, the use of hormone therapy in combination with additional sources of macro- and micronutrients is highly recommended.

Keywords: Hormones, Reproductive System, Cows.

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INTRODUCTION

The main place in the regulation of vital processes belongs to the endocrine system. The endocrine system glands produce hormones, together with which and the nervous, immune and tissue systems, metabolic processes in the animal body are controlled (Sundrum, 2015). Hormones also play a particularly important role in ensuring the reproductive animals' function (Safonov, 2008a or b).

During one ovary cycle, cows have three increases and decreases in the estrogenic and androgenic hormones levels. The concentration of progesterone in the blood plasma is the highest in the luteal phase of the cycle and the lowest in the follicular phase. While the follicle growth, the production of 17β-estradiol also increases. The ovary cycle, characterized by fluctuations in the sex steroids levels, is accompanied by the changes in the corticosteroid and thyroid hormones synthesis, for example, the follicles' growth is accompanied by an increase in the concentration of these hormones, mostly evident in the case of cortisol.

Metabolic hormones, which include cortisol and triiodothyronine, in turn, have the ability to influence the development of dysfunctional ovarian conditions. Thyroid hormones affect the gonadotropins' synthesis, while creating conditions for the follicles' maturation and ovulation. In addition to a sufficient iodine intake into the body for the production of thyroid hormones, it is important to ensure an adequate selenium intake from the feed. According to the number of studies, it has been found that pathological conditions of the reproductive system develop against the background of an increased level of oxidative stress, aggravated by a lack of micronutrients included in a structure of antioxidant enzymes (Awadeh et al., 1998; Safonov et al., 2008, Safonov et al., 2014, Kireev et al., 2018).

An increase in cortisol concentration characterizes the cattle in the early lactation period, which is associated with a high-stress load; it allows to increase the activity of compensatory mechanisms aimed at catabolism of adipose tissue and to obtain fatty acids from it. Also, it helps to obtain amino acids from muscle tissue proteins in order to provide the gluconeogenesis process with the necessary metabolism components (Plemyashov, Moiseenko, 2010; Simonov et al., 2017). It is also taken into account during the determination of the animals' hormonal status that the hormone level is characterized by the high phenotypic variability and susceptibility to daily fluctuations. The unfavorable external factors can have a very strong influence on the hormone level (Mazurina, 2017, Bova et al., 2014).

The physiological changes in the steroid and thyroid hormones levels are accompanied by a change in the biochemical blood composition. This can be clearly noted for such indices as: total protein and its fractions, glucose, total lipids, cholesterol, calcium, phosphorus, and vitamins. For example, during the follicular phase of the ovary cycle, the indices of total protein, glucose, calcium and phosphorus, and vitamins A and E increase in the blood of cows against the background of a decrease

in cholesterol and vitamin C. In the luteal phase, the lowest level of total protein, glucose and phosphorus is noted as well, at a time when the level of cholesterol, and vitamin C becomes maximum. There is also a direct dependence of the animals' ability to fertilize on the albumins, sugar, phosphorus, vitamins A, E, and trace elements levels (Bisinotto et al., 2012; Michael et al., 2019).

This research was organized in order to establish the hormonal and biochemical status of dairy cows that were repeatedly unsuccessfully inseminated or suffered from various pathologies of the reproductive system.

MATHERIALS and METHODS

To conduct the experiment, the Black-and-White Dutch cows at the age of no earlier than 2 lactations were selected, of a similar milk productivity level, belong to one of the breeding farms of the Voronezh region. Therefore, for the purpose of the research, the following groups of animals were formed: 7 heads from yeld (infertile) cows without clinical changes in the reproductive organs, 7 heads from yeld (infertile) cows with uterine subinvolution, 10 heads from successfully fertilized cows, and 35 heads from yeld (infertile) cows with ovarian pathologies: with follicular cysts – 8 heads, with luteal cysts – 8 heads, with ovarian hypofunction – 9 heads, healthy non-pregnant cows – 9 heads.

The maintenance of animals is carried out according to a loose housing, feeding – from the feed table. The cows' diet is compiled according to generally accepted zootechnical methods for dairy cows, while taking their productivity into account.

The assessment of the clinical animals' condition was carried out while taking into account the anamnesis during the visual examination and the use of the transrectal palpation method and ultrasound scanner. The hormonal animals' status was determined by means of the laboratory blood plasma analysis, the ELISA test systems, and the Uniplan spark analyzer. The level of total protein was determined by the refractometric method, total lipids, glucose, calcium, inorganic phosphorus – by the "Hitachi-902" biochemical analyzer, trace elements – by the "Perkin Elmer-703" atomic absorption spectrometer, vitamin A-by the spectrophotometric method. Animals' blood was taken from the jugular vein in the morning hours before feeding in compliance with aseptic and antiseptic measures. The manipulations with animals were carried out according to the norms of humane treatment set out in the European Community Directive (86/609/EEC) and the Helsinki Declaration.

The obtained data were processed by mathematical analysis methods adopted in veterinary and biological studies, by the use of MS Excel and Statistica 6.0 Statsoft Inc. programs. The results in the tables are given as a mathematical expectation ± standard deviation.

RESULTS AND DISCUSSION

The research results of the hormone level in the blood of fertilized and yeld (infertile) cows are presented below (Table 1). The obtained data show that yeld (infertile) cows, characterized by repeated unsuccessful attempts to inseminate them, demonstrate reduced functional activity of the thyroid gland and ovaries.

Table 1. Hormones concentration in the blood of successfully fertilized and yeld (infertile) cows, nmol/l

Indices	Yeld cows		Fertile cows (N = 10)
	No clinical changes (N = 7)	Uterus subinvolution (N = 7)	
Progesterone	8.5 ± 3.64 *	7.3 ± 2.96 *	20.5 ± 3.85
Testosterone	8.7 ± 0.95 **	5.4 ± 1.48 **	14.1 ± 2.89
17β-Estradiol	0.76 ± 0.04 *	0.71 ± 0.03 *	0.85 ± 0.02
Cortisol	30.9 ± 7.35	27.0 ± 7.52	22.3 ± 3.25
Triiodothyronine (T3)	2.08 ± 0.72 *	2.08 ± 0.78 *	3.38 ± 1.34

* – $P < 0.05$, ** – $P < 0.01$, in comparison with fertilized cows

The progesterone level in the yeld (infertile) cows without any visible clinical pathologies are significantly reduced – by 2.4 times compared to pregnant animals, the testosterone level is 1.6 times lower, 17β-Estradiol – by 1.1 times, the triiodothyronine level – by 1.6 times. At the same time, animals have an increase in the adrenal hormone production – cortisol – by 38.6%. A higher cortisol level indicates the presence of stress factors affecting the cows' bodies, and at the same time it leads to the mobilization of compensatory mechanisms in it.

Only 3 animals among the yeld (infertile) cows with undetected clinical changes in the reproductive organs demonstrate the presence of an ovary cycle with the formation of a functioning yellow body. The progesterone level in these animals was 15.9±8.84 nmol/l, at a time when the progesterone level remained at a low level in animals with anovulatory cycles. The absence of ovulation and a low concentration of progesterone may indicate atresia in the follicles ovaries at an early stage of their maturation, which is probably due to a violation of regulation at the level of the hypothalamic-pituitary system (Shkuratova, Ryaposova, 2011). The anovulatory cycles in such animals may be corrected with the help of special stimulating or hormone replacement therapy.

The changes in the hormonal profile of animals similar to those previously considered are noted in the case of the chronic uterus subinvolution. The progesterone concentration in the blood of cows with pathology is 2.8 times lower than it may be in healthy pregnant animals, the testosterone is 2.6 times lower, 17β-Estradiol is 1.2 times lower, triiodothyronine is 1.6 times lower. There is a tendency to an increasing of the cortisol level by 1.2 times compared to the one in fertilized cows. The cyclic

activity of the sex glands was preserved only in 2 animals from the experimental group, in the rest the progesterone level in the blood during the observation period was 4.17±1.8 nmol/l, i.e. there was a disorder of the functional ovaries activity. Thus, chronic uterus subinvolution is accompanied by a disorder of the morphological structure functions of the sex glands involved in the hormonal regulation of the hypothalamic-hypophysial-gonad axis, as well as the absence of the ovulation of the mature follicle with the subsequent formation of the corpus luteum. Treatment of such animals should be aimed not only at restoring the uterus morphological structure and functions, but also at correcting the functional ovaries activity.

Table 2. Biochemical status of fertilized and yeld (infertile) cows, blood serum

Indices	Yeld cows		Fertile cows (N = 10)
	No clinical changes (N = 7)	No clinical changes (N = 7)	
Total protein, g/l	81.0 ± 1.55	84.9 ± 2.39 **	79.4 ± 1.24
Glucose, mmol/l	1.84 ± 0.14	1.54 ± 0.17	1.69 ± 0.26
Total lipids, g/l	4.03 ± 0.17 **	4.31 ± 0.28	4.28 ± 0.14
Cholesterol mmol/l	5.90 ± 0.42	6.02 ± 0.85	5.78 ± 0.26
Calcium, mmol/l	2.59 ± 0.03 **	2.52 ± 0.07 **	2.65 ± 0.04
Phosphorus, mmol/l	1.99 ± 0.07 **	1.96 ± 0.14 **	2.16 ± 0.11
Iodine (associated with protein), μg%	2.07 ± 0.18 **	2.62 ± 0.21 *	2.35 ± 0.15
Manganese, μg%	15.5 ± 0.69 **	16.8 ± 1.21 **	19.0 ± 0.63
Copper, μg%	78.1 ± 1.75 **	77.9 ± 2.35 **	85.3 ± 1.44
Selenium, mcg %%	15.4 ± 0.83	13.4 ± 1.20 **	16.2 ± 0.87
Vitamin A, μg%	71.5 ± 4.85 **	43.9 ± 11.2	53.6 ± 5.44

* – P < 0.05, ** – P < 0.01, in comparison with fertilized cows

A comparative assessment of repeatedly inseminated infertile cows without pronounced clinical changes in the reproductive system shows a reduced level of lipids in the blood serum by 5.8% in comparison with springing animals (Table 2). They also demonstrate a slight decrease in the calcium and phosphorus levels, a decrease in the manganese level by 18.4%, copper level – by 8.4%, protein-bound iodine — by 13.5% and a higher concentration of vitamin A – by 34.4% higher than in fertilized cows. The incomplete satisfaction of the need for macro-and micronutrients acts as an additional etiological factor of reproductive disorders in yeld (infertile) animals (Nikolaev, Konopeltsev, 2019). While chronic uterus subinvolution, cows also have a decrease in the calcium and phosphorus level in the blood serum – by 4.9 and 9.3%, respectively, and a lack of trace elements: the concentration of manganese is reduced by 11.6% compared in comparison with springing animals, copper – by 8.7%, selenium – by 17.3%. There is a tendency to a reduced vitamin A level. The iodine level in such cows was 11.5% higher than in springing animals. The state of uterine subinvolution is accompanied by a high protein level in the blood serum, which was 6.9% higher than in the group of fertilized cows.

In the second research series, the hormonal status of infertile cows with various ovarian pathologies was analyzed in comparison with the indices of clinically healthy animals with cycles (Table 3).

Table 3. Hormones concentration in the blood of the yeld (infertile) cows with ovarian disease and healthy cows with cycle, nmol/l

Indices	Yeld (infertile) cows with ovarian disease			Healthy cows with cycle (N = 9)
	Follicular cysts (N = 8)	Luteal cysts (N = 8)	Hypofunction (N = 9)	
Progesterone	1.3 ± 0.46 **	17.4 ± 4.30	1.04 ± 0.25 **	15.1 ± 3.85
Testosterone	12.8 ± 0.67 *	10.1 ± 0.49	2.44 ± 0.40 **	9.9 ± 3.37
17β-Estradiol	0.71 ± 0.02 **	0.79 ± 0.05 *	0.67 ± 0.10 **	0.90 ± 0.12
Cortisol	64.4 ± 13.40 **	12.6 ± 2.78 **	29.0 ± 7.97 *	40.2 ± 11.20
Triiodothyronine (T3)	3.77 ± 1.19 **	2.13 ± 1.15	1.86 ± 0.70	2.25 ± 0.86

* – P < 0.05, ** – P < 0.01, in comparison with fertilized cows

Depending on the form of the ovarian disorder, the hormonal animal status was characterized by significant differences. While detecting follicular cysts in cows, a low progesterone level in the blood was found, therefore its level was 11.6 times lower than in healthy animals, and the level of 17β-Estradiol was also lowered by 25.3%. At the same time there was an increase in the testosterone concentration by 29.3%. Such a situation characterizes a violation of the enzymatic processes of estrogens and progesterone synthesis.

The highest concentration of triiodothyronine and cortisol in the blood is observed while the presence of follicular cysts. An increase in the functional activity of the adrenal glands leads to a 1.5 times higher hormone level than in healthy animals. It is assumed that this causes a blockade of the production and incretion of gonadotropin hormones of the thyroid gland and further leads to a violation of ovulation and cyst formation.

Animals with identified luteal cysts show a high progesterone level in comparison with other groups of cows with pathology, in addition, the hormone level is 15.3% higher than in cyclical cows. The amount of testosterone corresponds to the indices of healthy animals, the concentration of 17β-Estradiol is reduced by 14%, and the concentration of cortisol is significantly reduced – by 3.2 times. The present study ~~authors of the article~~ suppose that luteal cysts in this case are associated with the cyclic release asynchrony of luteinizing hormone while the regulatory function of the hypothalamic-pituitary system changes.

The hypofunctional state of the ovaries in cows is manifested by prolonged anaphrodisia, inability to fertilize, and low production of sex hormones. In the group of cows with hypofunction, the progesterone level was reduced by 14.5 times, testosterone level – by 4.1 times, and 17β-estradiol level

– by 34.3% in comparison with the animals having a healthy cycle. There was also a decrease in the triiodothyronine concentration. The results of measuring the cortisol level in the blood of cows of this group were uneven. In 5 animals, the reduced hormone secretion was detected with a blood concentration of 13.2±1.63 nmol/l and in 3 animals it was an increased one, with a concentration of 60.7±10.63 nmol/l. In the first case, cows may be under the influence of chronic distress, and in the second – in a state of the acute one.

Table 4. Biochemical status of the yeld (infertile) cows with ovarian disease and healthy cows with cycle

Indices	Yeld (infertile) cows with ovarian disease		Healthy cows with cycle (N = 9)
	Ovarian cysts (N = 16)	Hypofunction (N = 9)	
Total protein, g/l	81.0 ± 1.65	84.5 ± 2.02 **	80.2 ± 2.08
Glucose, mmol/l	1.70 ± 0.14	1.86 ± 0.17 *	1.62 ± 0.16
Total lipids, g/l	4.52 ± 0.27 **	3.08 ± 0.25 **	3.58 ± 0.13
Cholesterol mmol/l	6.03 ± 0.48 **	4.93 ± 0.40	5.09 ± 0.33
Calcium, mmol/l	2.65 ± 0.04	2.57 ± 0.02 *	2.61 ± 0.05
Phosphorus, mmol/l	2.23 ± 0.12	2.02 ± 0.21 **	2.19 ± 0.06
Iodine (associated with protein), µg%	2.03 ± 0.16 **	2.56 ± 0.19	2.69 ± 0.19
Manganese, µg%	17.1 ± 0.91 **	18.1 ± 0.85 **	15.6 ± 0.65
Copper, µg%	82.8 ± 3.39 *	81.4 ± 1.77 **	80.0 ± 1.87
Selenium, mcg %%	14.6 ± 0.68 **	12.7 ± 0.32 **	16.0 ± 0.73
Vitamin A, µg%	53.2 ± 4.07	49.9 ± 6.85	50.2 ± 5.21

* – P < 0.05, ** – P < 0.01, in comparison with fertilized cows

The biochemical status of animals with cystic ovarian changes is characterized by high concentrations of lipids and cholesterol, 37.8 and 18.5% higher, respectively, than in the group of animals with a healthy cycle, which may be explained by an imbalance of metabolic body processes. The macronutrient status did not differ from that of healthy cows, while there was a reduced selenium level by 8.8% and iodine level by 24.5% against the background of high functional activity of the thyroid gland in follicular cysts. The availability of manganese exceeded the healthy animals level by 9.6%, and the availability of copper – by 3.5%. Therefore, the hypofunctional ovarian disorder in cows is accompanied by a sufficiently high level of total protein and glucose, and at the same time a decrease in the supply of macronutrients. The most noticeable was a decrease in the selenium level– by 20.6%, at a time when the manganese level was 16.0% higher than in animals with a healthy cycle. Thus, pathological processes in the ovaries of cows occur against the background of changes in the biochemical profile of blood and the lack of individual macro- and micronutrients.

CONCLUSIONS

The failure of the steroidogenesis regulation in yeld (infertile) animals in most cases occurs due to the changes in the levels of progesterone, estrogen, and testosterone, which, through feedback, affect the hypothalamic-pituitary system responsible for controlling the hormone synthesis by the ovaries.

The study of the hormonal status of yeld (infertile) cows with various ovarian pathologies shows that the pathophysiological pathways of the formation of luteal cysts are associated (to a greater extent) with the hypothalamic-hypophysial-adrenal axis. The changes in the indices of sex hormones in this cows group were the smallest ones. In addition to the elements of this system, changes in the activity of the thyroid gland and the ovaries themselves are involved in the follicular cyst formation.

Changes in the biochemical profile of blood are associated with the development of pathologies of the reproductive organs and infertility in dairy cows. Shifts in the balance of lipid, protein, and carbohydrate metabolism are usual, there was an insufficient supply of mineral elements in all the groups (in comparison with healthy animals). A particularly strong deficiency was observed in providing the yeld (infertile) cows' bodies with selenium – its deficiency is inherent in all the considered groups and amounted to 4.9-20.6%.

In order to correct the cows' infertility, in all the cases, the specialized hormone therapy may be used in combination with providing them with a full-fledged diet and the addition of additive mineral elements sources.

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