

# The Influence of Steroid Hormones of the Functional System Mother - Fetus on the Leukocyte Differential Count and on the Development of Predisposition to Bronchopneumonia in Calves During Neonatal Period

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**Abstract**— The aim of the work was to establish the level of steroid hormones in the blood serum of deep-pregnant cows and calves obtained from them and to establish correlations between hormonal and immunological parameters of the mothers and newborns.

It was found that steroid hormones of the mother and the fetus take part in the development of fetoplacental insufficiency, weakening of the barrier function of the mucous membranes of the respiratory tract, stress-induced disruption of the functioning of the immune system, which contributes to the development of the predisposition to bronchopneumonia in young cattle.

**Keywords**—cows, calves, microbronchitis, bronchopneumonia, leukocyte differential count, hormonal status

## I. INTRODUCTION

In mammals, 10 endocrine glands are known that form the endocrine system and produce hormones [1]. Hormones affect all fundamental processes in the body: they determine the level of activity of DNA and protein synthesizing systems, cell growth and proliferation, tissue formation, adaptation and maintenance of metabolic homeostasis.

The relationship between the hormone content in the mother and embryo is formed by the endocrine function of both organisms. Additionally, a new powerful endocrine organ, the placenta, appears during pregnancy [2]. Differences in the content of hormones in the mother and the fetus are the result of the functioning of the fetoplacental barrier, which provides selective transport of substances. Thus, polypeptide hormones pass through it in small amounts, steroid hormones pass at different rates with high permeability of the placental barrier to androgens, estrogens,

progesterone, and glucocorticoids, although the glucocorticoids can be splitted by placenta enzymes [3].

The endocrine status of the fetus is important for the timely maturation of its internal organs, which should be fully prepared to stop the direct supply of oxygen and nutrients from the mother's body. However, at the time of birth, many organs of newborn calves are functionally immature. The hormonal status of animals is also not fully formed by the end of pregnancy and undergoes significant changes in the last weeks of the prenatal period and in early neonatal ontogenesis to ensure adaptation to the extrauterine environment [4].

In the formation of the organism's resistance to infectious and non-infectious diseases, an essential role belongs to the state of the endocrine system. In case of hormonal imbalance in calves, immunodeficiency states develop that significantly reduces their resistance to external stress factors and increases the risk of respiratory diseases [5].

In humane medicine, a large number of studies have been devoted to the studies of the hormonal status of mother and fetus. The hormonal status of farm animals, especially young animals, as well as the relationships of endocrine parameters in the mother – fetus system remain poorly understood. It is important to study the relationship between hormonal status and the functionality of the immune systems of the mother and fetus, to identify how they are associated with the development of respiratory infections. These data will contribute to the study of hormones as biomarkers of potential risk of diseases [6].

The aim of this work was to study the content of steroid hormones in the blood serum of deep-pregnant cows and calves obtained from them, to establish correlations between hormonal and immunological parameters of mother and newborn.

## II. EXPERIMENTAL

The research was performed in the winter, during the cattle's dry period. It included 33 dams and 33 their calves of the Red-motley Holstein breed, selected by random sampling. The health status of the calves was assessed daily by determining their body temperature, heart rate and respiratory movements, presence/absence of diarrhea, cough, nasal discharge, eye discharge, behavior changes, sucking reflex activity, and appetite.

Samples of venous blood for laboratory studies in dams were obtained on the 239th – 262nd days of gestation, in calves – on the 2nd day after birth.

Blood samples in all animals were obtained from the jugular vein puncture and were collected into sterile vacuum tubes with EDTA and without anticoagulant. After clotting for 1 h at room temperature, blood samples without anticoagulant were centrifuged (UC-1612, ULAB, China) at  $4000 \times g$  for 10 min at room temperature and sera were carefully harvested and stored at  $-20\text{ }^{\circ}\text{C}$  until biochemical analysis.

The concentration of estradiol, progesterone, dihydroepiandrosterone sulfate (DHEA-S), cortisol and aldosterone in the blood serum was determined by enzyme-linked immunosorbent assay (ELISA) on the analyzer a "Uniplan AIFR-01" (Russia) using commercial kits manufactured by "NVO Immunotek" (Russia) and "Diagnostic Biochem Canada Inc." (Canada).

To determine the leukocyte differential count, a drop of venous blood is thinly spread over a glass slide, air dried, and stained with a Romanowsky stain, the May-Grunewald-Giemsa technique. Two hundred cells are then counted, classified and determined their percentage ratio [7].

Statistical data processing was performed using the statistical software package STADIA 7.0 (InCo, Russia). Retrospectively, samples of adult and newborn animals were divided into 2 groups each: group D1 — dams whose calves had an uncomplicated bronchitis ( $n = 26$ ); group D2 - dams whose calves had bronchopneumonia ( $n = 7$ ); group C1 - calves with uncomplicated bronchitis ( $n = 26$ ); group C2 - calves with bronchopneumonia ( $n = 7$ ). The results are presented in the format "arithmetic mean  $\pm$  standard deviation", the medians of the indicators are given. The normality of the distribution was checked using the Kolmogorov's,  $\omega^2$  and  $\chi^2$  criteria. Medians were compared by Wilcoxon's W-test. Correlations were identified using the Spearman's test (rs). The zero hypothesis was rejected at  $P < 0.05$  [8]

## III. RESULTS AND DISCUSSION

It was found that the content of steroid hormones in the blood serum of deep-pregnant cows from the D1 and D2 groups did not significantly differ (Table I), although the median estradiol concentration in animals in the D2 group was almost half lower that of the same indicator in the D1 group.

The leukocyte differential counts of dams from both groups were characterized by neutrophilia and

monocytopenia (Table II). In group D2, an insignificant leukocytosis was detected. There were no differences between the leukocyte differential count indices in the D1 and D2 groups.

In dams, correlations were found between the content of progesterone and leukocytes (mainly lymphocytes), cortisol and monocytes (Table III). In group D1 correlations were found between the concentration of progesterone and the content of leukocytes and eosinophils, estradiol and band neutrophils, DHEA-S and monocytes, cortisol and band neutrophils. In the D2 group correlations were found between the concentration of estradiol and the content of lymphocytes, DHEA-S and band neutrophils, cortisol and leukocytes, aldosterone and segmented neutrophils and lymphocytes.

Also, correlations were found between the concentration of individual hormones in dams and leukocyte differential count of their offspring (Table IV).

TABLE I. HORMONAL STATUS OF DAMS IN GROUPS D1 AND D2

Hormones	D1 group	D2 group
Progesterone, nM/l	49.7 $\pm$ 31.7; Me=41.6	51.4 $\pm$ 32.6; Me=40.6
Estradiol, pM/l	189.0 $\pm$ 167.3; <b>Me=116.8</b>	140.0 $\pm$ 171.5; <b>Me=61.2</b>
DHEA-S, $\mu$ M/l	0.41 $\pm$ 0.36; Me=0.34	0.53 $\pm$ 0.44; Me=0.34
Cortisol, nM/l	90.5 $\pm$ 53.4; Me=78.3	113.0 $\pm$ 96.9; Me=72.1
Aldosterone, pg/ml	25.9 $\pm$ 4.2; Me=26.1	26.8 $\pm$ 4.3; Me=24.6

TABLE II. LEUKOCYTE DIFFERENTIAL COUNT OF PERIPHERAL BLOOD OF DAMS IN GROUPS D1 AND D2

Indicators	Normal indices	D1 group	D2 group
Leukocytes, $\times 10^9/l$	6–11	10.9 $\pm$ 3.6; Me=10.1	13.5 $\pm$ 5.6; Me=12.2; trend
Band neutrophils, %	1.5–2.5	5.2 $\pm$ 2.9; Me=5.0	4.1 $\pm$ 1.9; Me=4.0
Segmented neutrophils, %	17–25	27.5 $\pm$ 8.7; Me=30.0	25.4 $\pm$ 10.2; Me=31.0
Lymphocytes, %	60–70	61.9 $\pm$ 11.0; Me=61.0	63.6 $\pm$ 10.6; Me=62.0
Monocytes, %	1.5–3	0.7 $\pm$ 0.8; Me=1.0	1.0 $\pm$ 0.2; Me=1.0
Eosinophils, %	5–6	4.6 $\pm$ 3.3; Me=4.0	6.0 $\pm$ 3.1; Me=7.0

Notes: trend - differences from the D1 group at the level of the statistical trend ( $0.05 < P < 0.1$ ).

**TABLE III. CORRELATION BETWEEN THE CONCENTRATION OF HORMONES AND LEUKOCYTE DIFFERENTIAL COUNT IN DAMS**

Hormones	D1 group	D2 group	Joint group
Progesterone	Leukocytes $r_s = -0.42$ ; $P=0.03$ Eosinophils $r_s=0.39$ ; $P=0.04$	–	Leukocytes $r_s = -0.38$ ; $P=0.02$ Lymphocytes $r_s = -0.38$ ; $P=0.02$
Estradiol	Band neutrophils $r_s = -0.43$ ; $P=0.02$	Lymphocytes $r_s = -0.79$ ; $P=0.02$	–
DHEA-S	Monocytes $r_s=0.37$ ; $P=0.04$	Band neutrophils $r_s=0.75$ ; $P=0.03$	–
Cortisol	Band neutrophils $r_s = -0.46$ ; $P=0.01$	Leukocytes $r_s = -0.79$ ; $P=0.02$	Monocytes $r_s=0.35$ ; $P=0.03$
Aldosterone	–	Segmented neutrophils $r_s=0.85$ ; $P=0.01$ Lymphocytes $r_s = -0.73$ ; $P=0.03$	–

**TABLE IV. CORRELATION BETWEEN THE CONCENTRATION OF HORMONES IN DAMS AND THE LEUKOCYTE DIFFERENTIAL COUNT OF CALVES OBTAINED FROM THEM**

Hormones	D1 – C1	D2 – C2	Joint groups
Progesterone	–	–	–
Estradiol	–	Lymphocytes $r_s = -0.79$ ; $P=0.02$	Monocytes $r_s=0.35$ ; $P=0.03$
DHEA-S	Band neutrophils $r_s=0.43$ ; $P=0.02$	Segmented neutrophils $r_s=0.93$ ; $P=0.005$ Lymphocytes $r_s = -0.75$ ; $P=0.03$	–
Cortisol	Band neutrophils $r_s = -0.44$ ; $P=0.02$ Monocytes $r_s=0.39$ ; $P=0.04$	Segmented neutrophils $r_s=0.82$ ; $P=0.02$	Segmented neutrophils $r_s=0.33$ ; $P=0.03$ Band neutrophils $r_s = -0.30$ ; $P=0.04$ Monocytes $r_s=0.33$ ; $P=0.03$
Aldosterone	Eosinophils $r_s = -0.28$ ; $P=0.04$	–	Eosinophils $r_s = -0.21$ ; $P=0.047$

In newborns with bronchopneumonia (group C2), no differences were found in the content of progesterone, estradiol, DHEA-C with the similar indicators in calves from the C1 group. In C2 group there were established the increase of the cortisol concentration at the level of the statistical trend and significant decrease of aldosterone concentration compared to calves from the group C1 (Table V).

The leukocyte differential count of newborn calves in both groups (Table VI) was characterized by slight leukocytosis. Moreover, in animals in the C1 group the level of lymphocytes increased, and in the C2 group – the level of neutrophils. Between groups C1 and C2, differences were found in the content of band neutrophils and lymphocytes. The increase (at the level of statistical trend) of segmented neutrophils content in calves from the T2 group was also registered compared to that in T1 group.

In joint group of newborn calves, correlations between the concentration of cortisol and the level of monocytes were revealed (Table VII).

**TABLE V. HORMONAL STATUS OF NEWBORN CALVES IN GROUPS C1 AND C2**

Hormones	C1 group	C2 group
Progesterone, nM/l	1.9±0.8; Me=1.7	1.9±0.7; Me=1.6
Estradiol, pM/l	405.9±351.8; Me=266.6	495.6±429.2; Me=305.0
DHEA-S, μM/l	0.18±0.14; Me=0.14	0.22±0.32; Me=0.10
Cortisol, nM/l	273.1±132.4; Me=251.4	416.1±308.5; Me=327.5 <sup>trend</sup>
Aldosterone, pg/ml	27.2±4.8; Me=26.4	23.5±4.5; Me=23.5*

Notes: \* – differences from group C1 are statistically significant ( $P < 0.05$ ); trend - differences from the C1 group at the level of the statistical trend ( $0.05 < P < 0.1$ ).

**TABLE VI. LEUKOCYTE DIFFERENTIAL COUNT OF PERIPHERAL BLOOD OF NEWBORN CALVES IN GROUPS C1 AND C2**

Indicators	Normal indices	C1 group	C2 group
Leukocytes, $\times 10^9/l$	5.0–9.3	12.6±4.3 Me=13.1	10.8±2.9 Me=10.8
Band neutrophils, %	12–15	11.3±3.8 Me=12.0	15.1±6.8 Me=17.0*
Segmented neutrophils, %	32–40	32.9±8.3 Me=32.0	39.7±10.7 Me=38.0 <sup>trend</sup>
Lymphocytes, %	40–50	55.2±8.4 Me=53.5	44.4±13.0 Me=43.0*
Monocytes, %	0–5	0.2±0.4 Me=0.0	0.1±0.4 Me=0.0
Eosinophils, %	0–3	0.5±0.8 Me=0.0	0.6±1.0 Me=0.0

Notes: \* – differences from group C1 are statistically significant ( $P < 0.05$ ); trend - differences from the C1 group at the level of the statistical trend ( $0.05 < P < 0.1$ ).

The correlation was found between the cortisol content and the level of monocytes in group C1, while in group C2 correlations were found between the concentration of estradiol and the number of lymphocytes, cortisol and eosinophils, aldosterone and segmented neutrophils and lymphocytes (Table VII). The strength of the correlation between the indicators in calves in the C2 group was higher than that in the C1 group.

In the mother – fetus system for the joint groups, correlations were found between the content of progesterone ( $r_s = -0.36$ ;  $P = 0.02$ ) and cortisol ( $r_s = 0.43$ ;  $P = 0.007$ ), in the group D1 – C1 only between the levels of progesterone in the mother and the fetus ( $r_s = -0.50$ ;  $P = 0.009$ ), and in the group D2 - C2 only between the estradiol content ( $r_s = 0.86$ ;  $P = 0.01$ ).

**TABLE VII. CORRELATION BETWEEN THE CONCENTRATION OF HORMONES AND LEUKOCYTE DIFFERENTIAL COUNT IN NEWBORN CALVES**

Hormones	C1	C2	Joint group
Progesterone	–	–	–
Estradiol	–	Lymphocytes $r_s = -0.71$ ; $P=0.04$	–
DHEA-S	–	–	–
Cortisol	Monocytes $r_s=0.39$ ; $P=0.04$	Eosinophils $r_s=0.59$ ; $P=0.04$	Monocytes $r_s=0.35$ ; $P=0.03$
Aldosterone	–	Segmented neutrophils $r_s = -0.76$ ; $P=0.03$ Lymphocytes $r_s=0.78$ ; $P=0.02$	–

The hormonal statuses of adult animals in groups D1 and D2 were generally similar. Noteworthy is the median estradiol level decreased by 47.6% in dams, whose calves were infected with bronchopneumonia compared to the same indicator in group D1 (Fig. 1) and the presence of a strong correlation between estradiol levels in the mother – fetus system in animals from groups D2 and C2 in the absence of such a connection between groups D1 and C1 and in the joint groups. 90% of the estrogens in the mother – fetus system are synthesized by the adrenal glands of the fetus, and its content in the mother's organism is one of the indicators of the normal development of the fetus and placenta [2]. A decrease in its concentration in cows in group D2 indicates fetoplacental insufficiency [9]. An negative correlation between the concentration of estradiol and the number of lymphocytes in cows from the D2 group, the concentration of estradiol in D2 cows and the relative number of lymphocytes in their calves (C2), and also between the concentration of estradiol and the lymphocyte content in calves from the group C2 can indicate on the immunosuppressive effect of this hormone on the lymphocytic system of the fetus in case of fetoplacental insufficiency.

Progesterone formed in the placenta mainly enters the bloodstream of the mother and only in small amounts into the fetus bloodstream [2]. Indeed, blood progesterone levels in newborn calves in both groups were approximately 25 times lower than in their mothers (Figure). The negative correlation between blood concentrations of progesterone in cows from the D1 group and calves obtained from them and the absence of such connection in the D2 and C2 groups can also indicate fetoplacental insufficiency in animals, from which offspring predisposed to bronchopneumonia were obtained [10]. A negative correlation between the concentration of progesterone and the number of leukocytes in cows from the D1 group and the lack of a relationship between the content of maternal and own progesterone and the number of leukocytes in the fetus in the comparison groups D1 – C1 and D2 – C2 indicates the role of progesterone as a repressor of the mother's immune system during the gestation. In cows from group D2, this mechanism

of suppressing the immunological conflict in the mother – fetus system was obviously violated, which could be one of the reasons for the decrease in resistance to respiratory diseases in the offspring.

Positive correlations of the concentration of maternal DHEA-C with the levels of phagocytes in dams and calves indicate a stimulating effect of the hormone on the system of micro- and macrophages in the blood of animals. The lack of the connection between the concentration of calf's DHEA-S and leukocyte counts is probably due to the fact that the hormone content in newborn animals was insufficient for the manifestation of immunoregulatory effect.

The effect of cortisol on the immune system of adult animals was mainly associated with the repression of cells of the leukocyte fraction, which was especially evident in dams from group D2 (for this group, a strong negative correlation between the level of cortisol and the number of leukocytes was revealed). In newborn calves, positive correlations were found between the cortisol content and the number of monocytes (in group C1 and in joint group of newborn animals) and eosinophils (in group C2). It is likely that a short-term increase of the concentration of cortisol in the blood of calves caused by birth stress [11] could have a stimulating effect on the system of phagocytic blood cells in newborns. Activation of the phagocytic immunity is probably a manifestation of the defense mechanism against external pathogens in the early neonatal period, when the calf's lymphocytic system is still immature and is not capable of producing its own antibodies.

Aldosterone regulates the balance of Na<sup>+</sup>, K<sup>+</sup> and water in the body. When it is deficient, the water-salt balance is disturbed, the tone of blood vessels is weakened and circulatory failure occurs [12]. Aldosterone deficiency in calves from the C2 group, obviously, led to impaired water – salt metabolism and barrier function of the mucous membranes of the upper respiratory tract.

#### IV. CONCLUSION

Thus, the hormonal statuses of the mother and the fetus during pregnancy are interconnected and interdependent. Fetoplacental insufficiency, weakening of the barrier function of the mucous membranes of the respiratory tract, stress-induced dysfunction of the immune system occur with the participation of steroid hormones of the mother and fetus and contribute to the formation of a predisposition to the complicated bronchopulmonary diseases in young cattle.

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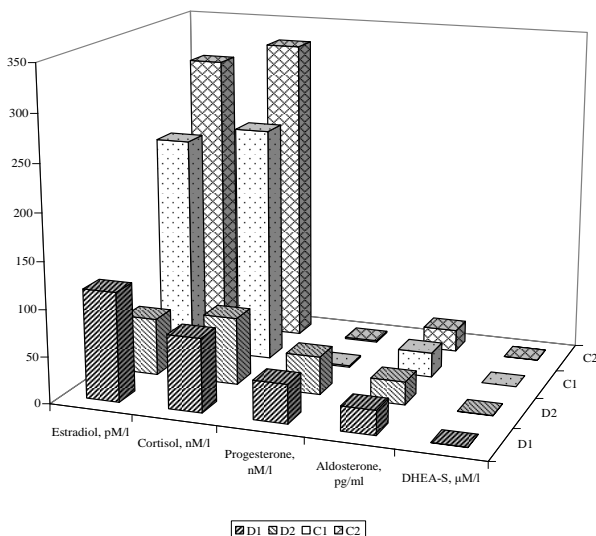


Fig. 1. The content of steroid hormones in the blood serum of dams and calves obtained from them

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