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NUMBER OF LYMPHOCYTES, NEUTROPHILS IN THE BLOOD AND THEIR RATIO AS INDICATORS OF RESUMPTION OF NORMAL ESTROUS CYCLES IN COWS

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ABSTRACT. The processes of animal fertilization and fetal development can largely depend on the interaction of immune cells with the structures of the reproductive organs. The article summarizes the results of a study of the distribution of white blood cells in cows (n = 21) in infertile (n = 45) and normal (n = 38) sexual cycles. It is shown that the total number of leukocytes in blood of cows in a normal cycle with subsequent fruitful insemination, generally increased ($P<0.001$) against the background of increased number of lymphocytes ($P<0.01$), decrease in segmented neutrophils ($P<0.05$), increased ratio of lymphocytes to neutrophils ($P<0.01$) and marked decrease of hematocrit ($P<0.01$) in comparison with animals in fruitless cycles. Significant changes in the ratio of functionally specialized white blood cells (lymphocytes and neutrophils) in cows during the resumption of full cycles can contribute to the creation of conditions necessary for fertilization. For the white blood of animals in normal cycles for 3-6 days before fertilization, the manifestation of short-term lymphocytosis, the presence of a minimum number of segmentonuclear neutrophils and the maximum values of the ratio of lymphocytes to neutrophils are characteristic. The maximum values of the ratio of the number of lymphocytes to neutrophils in the blood of cows in the first months after calving can be used as one of the prognostic markers of increased efficiency of their fertilization during this period.

Keywords: cows, estrous cycles, lymphocytes/neutrophils ratio, markers of fullness cycles

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Introduction

Disclosure of the special features of the functioning of physiological systems, including a reproductive function in farm animals, can be attributed to the current problems of biology (Yaqub et al, 2013). The conditions for the formation of ovulatory estrous cycles in highly productive cows after calving are mainly characterized by the level of hormonal regulators (Gavrichenko, 2006; Wathes, Bourne et al, 2007) or on the content of certain metabolites in the blood (Reist et al., 2003; Bearden et al., 2004; Vasilenko, 2008; Vasilenko, Roshchevsky, 2008).

The interaction of immune cells with the structures of the reproductive organs can provide an opportunity for fertilization of animals and fetal development. It is known that lymphocytes participate in the growth and maturation of follicles in the ovaries (Murdoch, Cormick, 1991). The presence of receptors for sex hormones in eosinophils and neutrophils determines the activity of these cells in the tissues of the reproductive organs in females before ovulation (Tchernitchin et al, 1976; Wang et al., 2001). The selective participation of these cells in the function of the reproductive system of females during the estrous cycle is manifested in quantitative and qualitative changes in the cellular composition of blood in the main and peripheral vessels of the reproductive organs (Borisenkov, Mongalev, 2006; Mongalev, Rubcova, 2008).

Despite the effectiveness of using leukocyte count to study the relationship between immune and reproductive functions in heifers (Vasilenko et al., 2011), there is almost no data on the distribution of these cells in cows at certain postpartum intervals (Subandrio et al., 2000; Uston, Lee, 2003) or in the process of ovulatory estrous cycles resumption. Therefore, studies of the cellular composition of white blood in cows

during postpartum period can be further used to characterize the conditions necessary for the formation of normal estrous cycles.

The aim of this work is to investigate the features of the distribution of lymphocytes and neutrophils and the values of their ratio in the blood of cows during normal estrous cycles with subsequent fertilization.

Materials and Methods

Experimental animals

The conditions for monitoring cows corresponded to the ethical principles of biological studies in animals, according to a report of the independent committee of the Institute of Physiology of the Komi Science Center for Bioethics. The studies were carried out on healthy cows of the Kholmogory breed 1-6 calving (n = 21) at the farm of Komi Scientific Center, Ural Branch of the Russian Academy of Sciences (Syktyvkar, Russian Federation). Animals were kept in proper conditions of feeding and care. The dairy productivity of cows was 4000 kg of milk for the period of lactation. Cows were observed from 9 months of pregnancy to 145 days after calving.

Study design

Detection of estrus was performed by visual observation of signs of sexual heat in cows from the 15th to the 25th day after calving, and was performed during the next 3-5 months of lactation. 20-minute observations of the animals were conducted hourly from 07:00 to 09:00 in the morning, and two to three random observations were performed from 3:30 to 7:00. Estrus was determined in the presence of the following signs in animals: an increase in motor activity, a decrease in daily productivity, the expiration of secretions from the genital organs. The duration of the estrous cycle was fixed as the duration of the interval from one estrus (or heat) to the next. Cows were examined in the first half of the cycle (luteal phase, 1-5, 6-14 days of the cycle), at the beginning of the estrus and at the end of the cycle before ovulation (follicular phase, 16-18, 19-22 days of the cycle). Cows observed in normal estrus were inseminated for 12 hours by a specialist with artificial insemination experience. The diagnosis of pregnancy by palpation was carried out 45-60 days after insemination.

Blood analysis

Sampling of blood samples from the jugular vein into Eppendorf tubes, treated with heparin, was performed 3 hours after morning feeding. The hematocrit was determined using the MPW-310 centrifuge to determine the plasma level in the blood. The number of leukocytes counted in the Goryaev chamber. After staining blood smears according to Romanovsky-Giemsa (Vital Diagnostics, St. Petersburg, Russian Federation), a study of the subpopulation composition of leukocytes was performed by counting 300 cells on a light PZO microscope (Poland) with an immersion system (objective × 100, eyepiece × 12) (Men'shikov, 1987).

Statistical analysis

The statistical analysis was carried out using Statistica software for Windows (Basic). The significance of differences between the groups was assessed by *t*- test. All results are presented as the arithmetic mean ± standard error of the mean (M ± SEM).

Results

The composition of white blood in cows in normal estrous cycles was different from that in animals in infertile cycles (table 1): the total leukocyte count increased ($P<0.001$) due to an increase in lymphocyte count ($P<0.01$), decreased the number of segmented nuclear neutrophils ($P<0.05$) and higher values of the ratio of lymphocytes to neutrophils ($P<0.01$) were noted under conditions of marked decrease in hematocrit ($P<0.01$).

Table 1. ***Hematocrit, total leukocytes count, number of lymphocytes, segmented neutrophils and the ratio of lymphocytes to neutrophils in the blood of cows during normal or infertile estrous cycles*** **(**M ± SEM)

Indices	Normal estrous cycles (n = 38)	Infertile estrous cycles (n = 45)
Hematocrit, %	31.10 ± 0.55 27.80-34.49	33.20 ± 0.51** 31.47 -34.93
Total leukocyte count, 10^9/L	5.52 ± 0.17 5.03 – 6.01	5.14 ± 0.13*** 4.70 – 5.58
Lymphocytes, 10^9/L	3.43 ± 0.12 3.09 – 3.77	2.92 ± 0.11** 2.55 – 3.29
Segment-nuclear neutrophils, 10^9/L	0.85 ± 0.05 0.71 – 0.99	1.00 ± 0.05* 0.83 – 1.16
Lymphocyte-neutrophil ratio	4.13 ± 0.39 3.61 – 5.24	2.92 ± 0.22** 2.18 – 3.67

Notes: *P<0.05; **P<0.01; ***P< 0.001 compared with the normal estrous cycles. The numerator is M±SEM, denominator – the boundaries of the fluctuations of values.

The passage of a normal estrous cycle with subsequent fertilization in cows is characterized by an increase in lymphoid elements in the blood (table 2). An increase in the number of lymphocytes (P<0.05), a decrease in neutrophils (P<0.05) and a marked increase in the ratio of lymphocytes to neutrophils (P<0.001) were determined three to six days before ovulation (16-18 days of cycle).

Table 2. ***Total leukocytes count, the number of lymphocytes, segmented neutrophils and the ratio of lymphocytes to neutrophils in the blood of cows during normal estrous cycle*** **(**M ± SEM)

Indices	Periods of the estrous cycle (days)			
	1 – 5 (n = 10)	6 – 14 (n = 6)	16 – 18 (n = 9)	19 – 22 (n = 14)
Total leukocyte count, 10^9/L	4.79 ± 0.29	4.78 ± 0.31	5.95 ± 0.24	5.37 ± 0.34
Lymphocytes, 10^9/L	3.18 ± 0.15	3.21 ± 0.35	4.02 ± 0.28*	3.45 ± 0.25
Segmented neutrophils, 10^9/L	0.91 ± 0.11	0.89 ± 0.15	0.61 ± 0.02*	0.78 ± 0.08*
Lymphocyte / neutrophil ratio	3.36 ± 0.13	3.60 ± 0.25	6.48±0.20***	4.20 ± 0.17

Note: *P<0.05; **P<0.01; ***P< 0.001 compared with the data in the previous period of estrous cycle

Thus, the composition of white blood in cows in normal cycles 3-6 days before fertilization is characterized by the manifestation of short-term lymphocytosis, the minimum number of segmented nuclear neutrophils and the maximum values of the ratio of lymphocytes to neutrophils. the composition of white blood in cows in normal cycles 3-6 days before fertilization is characterized by the manifestation of short-term lymphocytosis, the minimum number of segmented nuclear neutrophils and the maximum values of the ratio of lymphocytes to neutrophils.

Discussion

There is evidence that the total leukocytes count in the blood of cows increases in the postpartum period as compared with their number during the calving, and this fact is found in females of other animal species (Quiroz-Rocha et al., 2009). During this period, the leukocytes count can be used as an indicator that can characterize the functional state of the animal organism (Mateus et al., 2002). It is also known that

the immune system is associated with the functioning of the reproductive system (Trunova, 1984) and, lymphocytes, having receptors for sex hormones, take an active part in ovulation of the dominant follicle (Norman, Brannstrom, 1994).

It is believed that changes in the composition of blood leukocytes in cows during the estrous cycle are due to the alternation of activity or depression of the immune system (Kot et al., 1986). Based on the idea of the presence of selective leukocyte infiltration in tissues, one can assume an increase in the number of lymphocytes in cows at the end of the luteal phase of the cycle and an increase in neutrophils at the end of the follicular phase before ovulation. These fluctuations in lymphocyte and neutrophil counts in the blood of ruminant female can correspond to a period of mass atresia of follicles in the late luteal phase or the development of a dominant follicle with a high level of progesterone in the follicular fluid at the end of normal estrous cycles (Borisenkov, Mongalev, 2006).

During the ovulation period, physiological immunosuppression was determined in women, which was mainly due to the number of E-lymphocytes and increased phagocytic activity due to an increase in the absolute number of phagocytic neutrophils (Zhdanova, 2006). At 3-4 days before ovulation, the cows observed an increase in the ratio of lymphocytes to neutrophils on the background of a significant increase in the number of lymphocytes in the vessels of the reproductive organs (Mongalev, Borisenkov, 2016). It was found that the number of lymphoid cells in the blood of cows depends on the phase of the cycle: it increases in animals in a normal estrous cycle with fertilization compared to animals in the infertile cycle (Mongalev, Rubtcova, 2008).

Conclusion and recommendations

The results obtained in this study showed that the number of lymphocytes and neutrophils in the blood of cows can be determined by the features of the cycle (normal or infertile). Fluctuations in the lymphocyte-neutrophil ratio depending on the phase of the estrous cycle may indicate the presence of synchronism in the interaction of the immune and endocrine systems in the process of development of follicles and their ovulation in the ovary.

Thus, features of changes in the cellular composition of white blood in cows in the first months of lactation, which can be used to characterize the conditions necessary for the resumption of normal estrous cycles, are revealed. In cows at the end of the normal cycle with subsequent fertilization, a significant increase in the ratio of the number of lymphocytes to neutrophils, and an increase in the plasma content in the blood were determined.

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Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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Количество лимфоцитов, нейтрофилов в крови и их соотношение как индикаторы состояния возобновления полноценных половых циклов у коров

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Процессы оплодотворения животных и развития плода могут в значительной степени зависеть от взаимодействия иммунных клеток со структурами репродуктивных органов. В статье обобщены результаты исследования распределения лейкоцитов в крови коров (n = 21) в бесплодных (n = 45) и нормальных (n = 38) половых циклах. Показано, что общее количество лейкоцитов крови у коров, находившихся в полноценном цикле с последующим плодотворным осеменением, как правило, увеличено (P<0,001) на фоне повышенного количества лимфоцитов (P<0,01), снижения сегментоядерных нейтрофилов (P<0,05), увеличения соотношения лимфоцитов к нейтрофилам (P<0,01) и выраженного уменьшения гематокрита (P<0,01) по сравнению с показателями у животных в бесплодных циклах. Значительные изменения в соотношении функционально специализированных клеток белой крови (лимфоцитов и нейтрофилов) у коров в условиях возобновления полноценных циклов могут способствовать созданию условий, необходимых для оплодотворения. Для белой крови животных в полноценных циклах за 3-6 дней до оплодотворения характерным является проявление кратковременного лимфоцитоза, наличие минимального количества сегментоядерных нейтрофилов и максимальные величины соотношения лимфоцитов к нейтрофилам. Максимальные значения соотношения числа лимфоцитов к нейтрофилам в крови коров в первые месяцы после отёла могут быть использованы в качестве одного из прогностических маркеров повышенной эффективности их оплодотворения в этот период.

Ключевые слова: коровы, эстральные циклы, соотношение лимфоцитов к нейтрофилам, маркеры полноценных циклов

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