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**MACRO-MINERAL BLOOD PROFILE IN FIRST CALVING COWS  
AT VARIOUS PERIODS OF THE REPRODUCTIVE CYCLE AND DURING  
NORMAL OR INFERTILE ESTROUS CYCLES**

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**ABSTRACT.** Achieving a certain level of macronutrients in the blood of productive animals is necessary both for maintaining their health and for the normal passage of the stages of the reproductive cycle. The purpose of the experiment was to investigate the concentration of macro minerals (sodium-Na, potassium-K, calcium-Ca, inorganic phosphorus-IP, and magnesium-Mg) in the blood of cows before first calving and in certain postpartum periods and to determine the possibility of using the level of these macro elements to control the conditions that are necessary for the resumption of normal ovulatory cycles and the fertilization of cows with a decrease of the interval from calving to fertilization. The data on the concentration of macronutrients in the blood of first calving cows before fertilization presented in this study were obtained under conditions that are characterized by the presence of optimal levels of total protein, albumin, total cholesterol, and high-density lipoprotein cholesterol. Cows were observed from 9 months of pregnancy to 145 days after calving. The serum concentrations of Na, K, Ca, IP and Mg were determined by colorimetric methods. The obtained results indicate the statistically significant variability of the concentration of Na, Ca, IP and Mg in the blood serum of primiparous cows in luteal or follicular phases of normal and infertile estrous cycles, with the achievement of levels, which were determined in these animals before calving at the end of the normal cycle before fertilization. At the end of a normal cycle, a significant decrease in the concentration of K ( $P<0.01$ ) in cows was found in comparison with its level in these animals before calving or in a luteal phase of the ovulatory cycle.

*Keywords: primiparous cows, reproductive cycle, normal estrous cycles, blood macro minerals, markers of fullness cycles*

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### **Introduction**

Consumption of a certain amount of macro elements is necessary both for maintaining the health of productive animals and for improving their dairy productivity and reproduction (McClure, McMaster, 2008). Main macro elements: sodium (Na), potassium (K), calcium (Ca), inorganic phosphorus (IP), and magnesium (Mg) are structural components of the body's organs and tissues, cofactors of enzymes and hormones, an integral part of biological fluids and tissues. Important functions of macro minerals are participation in the structural support of the body (skeleton) and active influence on reproductive processes (Hurley, Doane, 1989; Tiwary et al., 2010).

The estrous cycle is a reflection of the functional state of the ovaries in females during the reproductive cycle. The early resumption of the normal ovulatory cycle in animals after delivery depends on internal and external factors. Despite the disclosure of the participation of some metabolites in the process of restoring ovulatory cycles in cows (Vasilenko, 2008; Francisco et al., 2009; Reist et al., 2009), there are practically no data on the content of certain macronutrients in the blood of first-calf cows under conditions resumption of the normal cycle, followed by fertilization.

The aim of this work was to study the possibility of using the profile of macronutrients in the blood serum of cows during the first lactation to monitor the conditions necessary for the resumption of the normal sexual cycle with fertilization.

## Materials and Methods

*Experimental animals.* Cows care procedures followed ethical principles of biological studies on animals, as there is a report of an independent committee of the Institute of Physiology, Komi Scientific Center of Bioethics. The studies were conducted on healthy first-calving cows of the Kholmogory breed at the farm of Komi Scientific Center, Ural Branch of the Russian Academy of Sciences (Syktyvkar, Russian Federation) in October - May 2010-2013. Animals were kept in proper conditions of feeding and care. The productivity of cows at the peak lactation was 14-20 liters per day and 3835-4573 kg of milk per lactation. Cows were monitored from 9 months of pregnancy to 145 days after calving.

*Study design.* Venous blood samples were taken from 15 primiparous cows, starting approximately 20 days before the expected calving date, early postpartum (2-9 days after calving) and during the resumption of the first estrus cycles (22-42 days postpartum); from 6 animals during normal estrus cycles (35-79 days postpartum) and at the beginning of pregnancy (14-56 days) and from 6 cows during infertile (without fertilization) estrus cycles (36-99 days postpartum).

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 continued for the next 3-5 months of lactation. 20-minute observations of the animals were carried out hourly from 07:00 h to 09:00 h in the mornings, and two or three random observations were performed from 3:30 h to 7:00 h pm. Estrus was determined in the presence of following signs in animals: an increase in motor activity, a decrease in daily productivity, and the expiration of cervicovaginal fluid from genital organs. The duration of the estrous cycle was fixed as the duration of the interval from one estrus (or heat) to the next. We examined cows in the first half of the cycle (6-13 days of the cycle, the luteal phase) and during estrus at the end of the cycle before ovulation (19-22 days of the cycle, the follicular phase). 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*

Individual blood samples were collected by puncture of the jugular vein into non-heparinized (20 ml) glass tubes. After blood clotting at 37°C for 30 minutes, the samples were stored at 4°C for 20 hours. Then the samples were centrifuged at 3000 g for 20 min at room temperature. The sample supernatant (blood serum) was stored at -20°C until analysis. Biochemical blood tests were performed in serum. Concentrations of Na, K, Ca, IP, and Mg was measured by colorimetric methods using Vital Diagnostics kits (St. Petersburg, Russian Federation).

### *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  $\pm$  standard error of the mean (M $\pm$ SEM).

## Results and discussion

The concentrations of Na and Ca were the lowest in cows in the early postpartum (2-9 days postpartum) and during the resumption of estrous activity (22-42 days after parturition) compared with the data in animals before first calving. During these periods, an increase in Mg content and retention of IP and K levels in cows were observed compared to the concentration of these elements in animals before calving (table 1).

The concentration of Na, Ca, and Mg in the serum of cows at the end of the normal estrous cycle (before fertilization) is maintained at the level determined before calving, with a noticeable decrease in K (table 2).

**Table 1. Concentration of macro minerals in serum of primiparous cows before calving, during early postpartum and in a period of a resumption of estrous cycles (M±SEM, n=15)**

Blood serum macro minerals	Study periods		
	Before calving (about 20 days before the expected date of calving)	Early postpartum (2-9 days after calving)	Resumption of estrous cyclicity (22-42 days after calving)
Na, mM	165.6±2.6	154.4±5.3	155.3±2.3**
K, mM	4.90±0.05	4.76±0.07	4.92±0.07
Ca, mM	2.64±0.03	2.50±0.03**	2.48±0.05 *
IP, mM	1.92±0.05	1.78±0.07	1.95±0.07
Mg, mM	1.13±0.02	1.25±0.02***	1.25±0.03 **

Note: \* P<0.05, \*\* P<0.01 and \*\*\*P<0.001 compared with results in cows before calving.

**Table 2. Concentrations of macro minerals measured in serum of primiparous cows before calving, during infertile and normal estrous cycles (with subsequent fertilization), and during first two months of a pregnancy (M±SEM)**

Blood serum macro minerals	Study periods				
	Before calving (about 20 days before expected calving data) (n=15)	The infertile estrous cycles (36-99 days postpartum) (n=6)	The normal estrous cycles with subsequent fertilization (35-79 days postpartum) (n=6)	Pregnancy (n=6)	
				up to 14 days	up to 32-56 days
Na, mM	165.6±2.6	161.3±3.4	164.7±1.2	154.9±1.7 <sup>3)</sup>	161.1±6.7
K, mM	4.90±0.05	5.08±0.05*	4.50±0.08 **, <sup>3)</sup>	4.87 ± 0.02 <sup>2)</sup>	5.04±0.12
Ca, mM	2.64±0.03	2.60±0.03	2.50±0.08	2.56±0.05	2.51±0.05
IP, mM	1.92±0.05	1.71±0.02	1.88±0.06 <sup>1)</sup>	1.63±0.03 <sup>2)</sup>	1.83±0.15
Mg, mM	1.13±0.02	1.28±0.05**	1.10±0.02	1.25±0.02 <sup>3)</sup>	1.15±0.04

Note: \*P<0.05, \*\*P<0.001 compared with cows before calving; <sup>1)</sup> P<0.05, <sup>2)</sup> P<0.01, <sup>3)</sup> P<0.001 compared with the previous period.

**Table 3. Concentrations of macro minerals measured in a serum of primiparous cows at a luteal (6-13 days of cycle) and follicular phases (19-22 days of cycle) of normal or infertile estrous cycles (M±SEM)**

Blood serum macro minerals	Periods of the estrous cycle (days)			
	Normal estrous cycles (with subsequent fertilization) (n=4-6)	21-22 days of estrous cycle (36-88 days from calving to study period)		Infertile estrous cycles (n=4-6)
	6-9 days of estrous cycle (29-76 days from calving to study period)			9-13 days of estrous cycle (39-121 days from calving to study period)
				19-21 days of estrous cycle (35-79 days from calving to study period)
Na, mM	150.1±2.1	164.7±1.2***	147.1±2.0	161.3±3.4**
K, mM	4.91±0.07	4.50±0.08 **	4.85±0.16	5.08±0.05 <sup>3)</sup>
Ca, mM	2.58±0.06	2.50±0.08	2.64±0.06	2.60±0.03
IP, mM	1.92±0.04	1.88±0.06	1.85±0.04	1.71±0.02 *, <sup>1)</sup>
Mg, mM	1.12±0.05	1.10±0.02	1.26±0.09	1.28±0.05 <sup>2)</sup>

Note: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 comp. with a luteal phase; <sup>1)</sup> P<0.05, <sup>2)</sup> P<0.01, <sup>3)</sup> P<0.001 compared with primiparous cows during follicular phases of normal or infertile estrous cycles.

A low concentration of K was determined at the end of normal estrous cycles with subsequent fertilization (4.50±0.08 mM), and its increase was noted up to the maximum value (5.04±0.12 mM) in animals at the beginning of pregnancy (32-56 days). The level of Na and IP decreases markedly, K (P<0.05),

and Mg ( $P < 0.05$ ) increase in animals at the beginning of pregnancy (up to 14 days) compared to data obtained during the fertilization period (table 2).

The level of Ca in the serum of cows during normal and infertile estrous cycles or up to 32-56 days of pregnancy reaches a level determined in the period before first calving (table 2).

It was found that the concentration of macro elements in cows varies during the passage of luteal or follicular phases of normal and infertile estrous cycles (table 3). The Na concentration was significantly higher during a follicular phase of normal or infertile cycles compared to its level in a luteal phase. The potassium level is markedly reduced in cows at the end of normal estrous cycles (21-22 days) with subsequent fertilization. In this case, a concentration of K was significantly lower than in animals during the same period of an infertile estrous cycle without fertilization.

An increase in K and Mg ( $P < 0.001$  and  $P < 0.01$ , respectively) and a decrease in IP in cows during a follicular phase (19-21 days) of the infertile cycle were found in comparison with the level of these macro minerals in animals in the follicular phase of a normal ovulatory cycle (table 3).

Experiments were carried out to study the profile of Na, K, Ca, -IP and Mg in the blood serum of cows before first calving and at various periods after calving with the aim to control conditions that determine the resumption of normal ovulatory cycles and the fertilization of cows with a decrease in the interval from calving to fertilization.

Early restoration of normal estrous cycles in cows in the postpartum period has positive influences on reproduction and dairy productivity (Westwood et al., 2002; Kawashima et al., 2006). If the processes of involution in the reproductive system of cows go without complications, then the signs of the first estrus cycle in the first calving animals were noted on 22-42 days after calving. The period after ovulation on average to 16 days of the cycle is characterized as a luteal phase or as a phase of development of the yellow body on the ovary. On the 17-18th day of the cycle, the follicular phase begins, during which the cow displays estrus (heat) before ovulation. Cycles in primiparous cows are repeated after 19-22 days and terminate after fertilization.

It is known that mineral substances enter an organism of productive animals (cows) exclusively from a fodder. Insufficient consumption of macro minerals influences a state of metabolic processes, in particular metabolism of Ca and IP, levels of vitamins and other substances in the blood, and may have a direct impact on a functional state of different physiological functions (Men'kova, 2003), including the reproductive function of ruminants (Small et al., 1997; Jeong et al., 2015; Shahzad et al., 2016).

High levels of Na were determined in cows before the first calving. The concentration of sodium in animals in a luteal phase of the estrous cycle was 147-150 mM, which is in agreement with the results obtained by other authors (Kenny et al., 2002; Leroy et al., 2004), and then increases to 161-165 mM at the end of normal or infertile cycles. There is evidence that the concentration of sodium in the blood serum and in the fluid of the uterus of cows is at the same level (Hugentobler et al., 2007). Potassium is a unique macronutrient for cows, as they consume large amounts of K with plant foods. In this study, the concentration of potassium in the blood of cows before and after calving, in contrast to sodium, remains at the same level. We have shown that the potassium concentration of first calving cows on the day of effective insemination with subsequent fertilization is reduced to 4.50 mM and increases to 5.08 mM in animals during the period of infertile cycles. These results for K levels are consistent with the literature data (Small, 1997; Mohebbi-Fani et al., 2007). The K concentration in the oviduct fluid is 4.46 mM (Grippio et al., 1992), and this level was determined in primiparous cows at the end of the normal estrous cycle with subsequent fertilization.

It has been shown that the concentration of potassium in the blood serum of a cow is reduced by 2.0-2.5 times compared to its level in the follicular fluid of the ovary, which is determined by the size of the follicle (Wise, 1987; Grippio et al., 1992). It is known that in women, on the 3-4th day after fertilization, the embryo passes from an environment with a relatively stable potassium content in the oviduct to the uterine fluid with a higher and changing potassium level (Casslen, Nilsson, 1984). Low concentrations of potassium in the blood of cows compared to its level in the oviduct and uterine fluid may indicate the presence of a mechanism for the active transfer of potassium to these structures (Hugentobler et al., 2007)

There is a direct relationship between the amount of Ca in the diet and the rate of fertilization of

cows (Hurley, Doane, 1989; Sharma, 2010-2011). A high level of Ca in the blood of cows was determined before the first calving. Its level was significantly reduced in animals in the early postpartum period and during the resumption of estrous cycles, which is probably associated with the increased expenditure of Ca for the milk production and a decrease in its assimilation from the gastrointestinal tract of cows during this period.

The concentration of Ca in first-calving cows during normal cycles with fertilization reaches the level determined in these animals at 9 months of pregnancy (2.50-2.64 mM). The concentration of Ca in the serum of cows is close to its level in the fluid of oviduct, i.e. 2.6 mM (Grippe et al., 1992). The achievement of a high Ca level in the blood of the primiparous cows during the resumption of normal ovulatory cycles is explained by the use of an adequate diet, stabilization of the concentration of this element in urine and milk, and, possibly, additional mobilization of Ca from bones and other tissues. The high values of Ca concentration in the blood of the first-calving cows are in good agreement with the results obtained by other investigators (Kume et al., 2003).

Metabolism Ca in cows is closely related to the metabolism of the IP. It was shown that early fertilization and reduction of the interval from calving to insemination of cows are observed at high concentrations of Ca and IP in the blood (Seifi et al., 2005). The level of phosphorus in the modern diet for cows exceeds the norm by 3-5 times. Our results confirm that the concentration of phosphorus in the blood of first-calving cows depends on the stage of the reproductive cycle, and is close to the indices of its level in other investigations (Small et al., 1997; Kume et al., 2003; Chorfi, Tremblay, 2002; Staric et al., 2001). The concentration of phosphorus in animals at the end of the ovulatory cycle is significantly higher than that in cows during the same period of the infertile cycle: 1.88 and 1.71 mM, respectively.

The concentration of Mg in the blood of primiparous cows before fertilization was determined at the level of 1.10 mM, which is significantly lower than in animals in cycles without fertilization (1.28 mM). The level of magnesium in the blood of cows was close to the data of other researchers (Sharma, 2010-2011; Hussain et al., 2001) It was shown that magnesium can influence the absorption of calcium and phosphorus (Sharma et al., 2004) and take part in enzymatic reactions associated with ATP. It is believed that the level of magnesium in the blood reflects its consumption, and not its way out of the body's depot (Whitaker, 2000). The assimilation of Mg in the body of cows can be determined by the level of its use for lactation and reproduction. Symptoms of hypomagnesia in cows are observed with a decrease in its concentration below 0.8 mM (Kabu et al., 2013). A significant decrease in the concentration of Mg in cows was detected in conditions of disturbance of ovulation (Das et al., 2009).

The data on the concentration of macronutrients in the blood of first calving cows before fertilization presented in this study were obtained under conditions that are characterized by the presence of optimal levels of total protein, albumin, total cholesterol, and high-density lipoprotein cholesterol (Vasilenko, Makarova, 2015).

### **Conclusion and Recommendations**

The obtained results indicate the statistically significant variability of the concentration of Na, Ca, IP and Mg in the blood serum of primiparous cows in luteal or follicular phases of normal and infertile estrous cycles, with the achievement of levels, which were determined in these animals before calving at the end of the normal cycle before fertilization.

This study presents the macro-mineral profile of the blood of primiparous cows and found that the conditions for their effective insemination followed by fertilization are characterized by the achievement of Na, Ca, IP, and Mg levels determined before the first calving and a noticeable decrease in K as compared to its level in these animals before the first calving or in a luteal phase of the cycle.

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### **Макро минеральный профиль крови у первотелок в разные периоды репродуктивного цикла и в ходе нормального или бесплодного полового цикла**

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Поддержание определенного уровня макроэлементов в крови продуктивных животных необходимо как для поддержания их здоровья, так и для нормального прохождения стадий репродуктивного цикла. Цель работы - исследовать содержание макроэлементов (Na, K, Ca, фосфора неорганического -IP и Mg) в крови коров перед первым отелом, в другие периоды репродуктивного цикла и определить возможность использования уровня этих элементов для контроля условий, необходимых для восстановления нормальных овуляторных циклов и оплодотворения животных в сокращенные сроки от отела до оплодотворения. Представленные в данном исследовании данные о концентрации макроэлементов в крови коров первого отела до оплодотворения были получены в условиях, которые характеризуются наличием оптимальных уровней общего белка, альбумина, общего холестерина и холестерина липопротеинов высокой плотности. Коров первого отела обследовали с девятого месяца стельности до 145 дней после отела. Концентрацию Na, K, Ca, IP и Mg в сыворотке крови определяли колориметрическими методами. Полученные результаты свидетельствуют о наличии статистически значимой вариабильности в содержании Na, Ca, IP и Mg у первотелок в ходе фолликулиновой или лютеиновой фаз нормальных или бесплодных половых циклов и достижения доотельных уровней этих элементов в конце нормального полового цикла перед оплодотворением. В конце нормального цикла отмечали заметное уменьшение концентрации калия ( $P < 0,01$ ) в крови животных по сравнению с его содержанием у первотелок до отела или в лютеиновую фазу полового цикла.

*Ключевые слова: первотёлки, макроэлементы крови, репродуктивный цикл, нормальный половой цикл, маркеры полноценных циклов*

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