PRE- AND POSTPARTUM BLOOD VALUES AND POSTPARTUM CELL CONTENT OF VAGINAL SMEARS OF FEMALE MOOSE AND DAIRY COWS[1]

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ABSTRACT: The postpartum period of female moose (*Alces alces*) and dairy cows was studied in the Komi Republic where short summers (2.5-3.5 months) and long winters (8.5-9.0 months) prevail. Cholesterol concentrations in blood of pregnant and postpartum female moose serum blood varied between 1.79-2.01 mmol/l. Levels in cow blood increased 1.8 times from pregnancy to postpartum, with a noticeable rise at estrus. Total serum protein of female moose blood before and after calving was 65-67 g/l and in the cows blood 73 g/l and higher. Albumin-globulin ratio of female moose blood decreased before calving and increased up to 1.34 after calving. During 3 postpartum months the cell composition of female moose vaginal smears taken from the most extreme section of the vagina shows the early desquamation of 13.7-22.5% of immature superficial cells. Nuclei without cytoplasm constituted 24.1-58.8% with composition change in the moose rutting period similar to the vaginal smear composition in cows in the period of their estrus activity peak. Serum progesterone level of prepartum moose averaged 1.89±0.50 ng/ml as compared to 1.02±0.50 ngml after calving.

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The postpartum period of female ruminants is characterized by involutionary processes in reproductive organs and intensification of lactation, associated with metabolic changes needed for subsequent pregnancy. Physiological changes during the first 3-5 months of the postpartum period determine whether pregnancy will subsequently occur. These conditions differ from those in cattle. Seasonal estrus is one adaptation to rigorous climate. Recent research on blood changes which take place in cattle reproductive organs includes some information that the interval between calving and the first estrus depends on the serum cholesterol levels (Banga *et al.* 1988, Lebengarts 1994, Kafidi *et al.* 1990) and on the albumin-globulin ratio (Rowlands *et al.* 1980). A special feature of the serum cholesterol dynamics in postpartum cows is the noticeable rise of its concentration at estrus (Kochanov *et al.* 1994). Similar increased blood cholesterol levels were observed in calves associated with estrus activity (Lebengarts 1994). It was assumed that this stimulation was due to estrogen influence on lipid exchange.

Changes of the inner section of the vaginal wall (near the cervix uteri) reflect ovary function. The processes of maturing, proliferation, and desquamation are the direct result of the functional activity change of this tissue, which takes place under the direct influence of female hormones (Arseneva 1977, Farstad 1984, Post 1985). Vaginal epithelial cells of different species

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during estrus and other stages of the reproductive cycle change their form and character, and may be destroyed (Arseneva 1977; Dore 1978 a,b). *In vivo* and *in vitro* experiments demonstrate epithelial cell changes after injections of progesterone or progesterone with estradiol. These injections have some influence on the nuclear mitotic activity of epithelial cells (Peckman *et al.* 1963, Roslakova 1969, Ivasenko 1978). Studies of the albumin-globulin ratio, cholesterol, progesterone, and cell content of smears taken from the outer vagina may reveal features of the female postpartum period.

We studied reproduction of dairy cattle and moose in northern conditions (Vasilenko and Borisenkov 1987; Kochanov *et al.* 1994; Vasilenko *et al.* 1995 a,b; Vasilenko and Rubtsova 1996). The investigations included blood composition changes and vaginal smears of moose and cattle in similar climate conditions. Disruption of ruminant reproductive function in this rigorous environment may reduce reproductive activity.

METHODS

Observations of female moose in different periods of reproductive function were made at the moose farm of Pechoro-Ilych Nature Reserve during 1987-1989. Seven adult cow moose were investigated during prepartum and 9 females during postpartum. Dairy cattle reproduction was studied at the experimental farm of the Komi Science Center (Syktyvkar) during 1985-1994. Blood samples were collected from the jugular vein of female moose and cattle 2-20 days before calving and 6-53 days postparturition. Cholesterol concentrations, summary protein levels, albumin-globulin ratio, and serum progesterone composition of blood were determined. Sixteen blood samples of female moose were used for the study of cholesterol content, 8 blood samples for serum protein levels and albumin-globulin ratio, and 14 samples were used for progesterone content. Changes in exfoliated epithelial cell types of the inner vaginal walls were investigated using vaginal smears as adapted for ruminants (Vasilenko and Borisenkov 1987). A polyethylene pipette developed for artificial insemination was introduced into the outer vagina near the cervix to obtain a smear. Samples were placed on plate glass and fixed in 70% ethanol. The painting of smears was carried out according to the Romanovsky-Himz method. The cell content of smears was determined by observing 200 cells with a Biolar microscope at magnification of 12 X 10.

Changes in levels of exfoliated separate cell types, cytolysis or presence of superficial epithelial cell nuclei without cytoplasm, and presence of leucocytes was determined. Exfoliated cell types and nuclei in vaginal smears were calculated as per cent of 200 cells. Sampling of 2 - 3 smears was made for each animal. Vaginal smears were taken from 5 adult moose and 2 16-month-old moose. A double injection of 0.5 mg of Estrophan, a synthetic analog of the prostaglandin $F2_a$, was injected into each 16-month-old moose to induce and synchronize estrus activity. Vaginal smears from 23 cattle were obtained from the 27th to the 145th day after calving.

RESULTS

Blood Indices

Protein levels in moose blood did not change in prepartum and postpartum periods and were 67±2 and 65±5 g/l, respectively (Table 1). The albumin-globulin ratio decreased before calving and increased to 1.34 after calving. The serum cholesterol ratio of cow moose before and after calving was 2.01±0.37 mmol/l and 1.79±0.36 mmol/l, respectively. Serum progesterone levels of female moose 17-58 days postparturition were 1.02±0.22 ng/ml (Table 2). The serum

Table 1. Cholesterol concentrations, serum protein levels, and albumin-globulin ratio in the blood of female moose in different study periods.

Study period	*n*	Cholesterol, mmol/l	*n*	Serum protein, g/l	Albumin-globulin ratio
The end of pregnancy, before calving	7	2.01±0.37	2	67±2	0.56±0.30
After calving	9	1.79±0.36	6	65±5	1.34±0.44

Table 2. Serum progesterone levels in female moose.

Study period	*n*	Progesterone, ng/ml
Before calving	3	1.89±0.50
17-th and 53rd day after calving	2	1.02±0.22
12-month-old females (in spring)	4	1.10±0.30
16-month-old females (before the first rutting period)	5	1.05±0.75

progesterone ratio in the postpartum moose was similar to 12- and 16-month-old females and was equal to 1.89±0.50 in moose 2-20 days prior to parturition.

Cell Composition of Vaginal Smears

Analyses of the cell composition of moose vaginal smears indicated that the maximum level of exfoliated epithelial cells (up to 98-100%) was observed in the moose smears until the 51st and 58th days postpartum (Table 3). Closer to the moose rutting period (99th day), the levels of superficial cells decreased to 90% and simultaneously the quantity of intermediate cells increased up to 10%. The presence of cytolysis in vaginal smears (the appearance of nuclei without cytoplasm) varied during the moose female postpartum period from 35-49% per 200 cells on the 7-17th days to 42-59% on the 51st and 58th days. On the 99th day of the female moose postpartum period the levels of superficial cell nuclei without cytoplasm decreased to 24%. A noticeable decrease in the cytolysis levels to 2.4-6.3% was observed in the female moose smears taken at the end of the estrus cycle in autumn.

We observed the same cytolysis values in dairy cow vaginal smears in the estrus period before ovulation. The degree of the vaginal epithelium maturation (quantity of superficial cells without nuclei) in the smears of the first group of dairy cows during the first estrus cycle (27-64 days postpartum) was 97% (Table 4). The number of proper superficial epithelium cells ranged from 1.5% to 11.0%. The proportion of nuclei without cytoplasm varied from 0.3% to 10.0%.

The superficial cell content of smears taken in the cows of the third group (44-145 days postpartum) did not differ from the content of this cell type in the smears of the cows of the first and second groups. We separated 2 subgroups of cows which had different amounts of superficial epithelium

Table 3. Cell content of moose female vaginal smears in different intervals after calving.

Experimental animal	Period of sampling	Superficial cell content, % (n=200 cells)	Superficial cell proper	Nuclei and shadow of nuclei (cytolysis)	Appearance of leucocytes
1	7-th day	98.4	19.0	35.0	-
2	17-th day	100.0	19.8	49.0	++
3	51-st day	99.6	13.7	58.8	-
4	58-th day	97.6	22.5	42.1	-
5	99-th day	89.8	15.7	24.0	+
6	The end of rut, a month after estrophan treatment	100.0	32.1	6.3	-
7	The end of rut, a month after estrophan treatment	100.0	3.9	2.4	-

cells with nuclei (proper superficial cells). In the first subgroup of 7, the quantity of this type was equivalent to the quantity in the cows in the first and second groups. In the second subgroup of 4 cows, the amount of these cells increased more than 5 times in comparison with their quantity in the smears of the cows of the first and second groups. We observed similar changes in the quantity of nuclei without cytoplasm: the amount of these nuclei in the smears of the first subgroup of 7 cows was equal to their amount in the first group of cows. This index increased 6 times from the number of nuclei without cytoplasm in the smears of the first subgroup of cows and 25 times more than in cow smears taken during the fertilization period.

The increase in number of proper superficial epithelial cells and nuclei without cytoplasm in the smears of the third group of cows provides evidence that the process of early epithelial cell desquamation occurs in the vaginal wall. It is possible that these results are indicative of the inflammation process or its consequence in the reproductive organs of the third group of dairy cows.

We investigated 3 cow moose on the 51st, 58th, and 99th days after calving. The animals exhibited excited behavior ("behavior estrus") and 2 cows showed vaginal mucus secretion on the 51st and 58th days, respectively. Cell composition of cow smears during "behaviour estrus" (Table 3, animals 3, 4, 5) showed distinct nuclei of superficial epithelial cells and cytolysis levels equivalent to those from cow moose on the 7th and the 17th days of the early postpartum period.

We observed estrus cycles with no normal epithelial cell reconstruction in the vagina. This suggested that insufficient development of superficial epithelial cells of the vaginal wall took place which lead to early desquamation of superficial formed cells with nuclei. This occurred in 13.7-22.5% of observations. The values of superficial formed cells with nuclei was equal to 0.8-7.5% of smears taken during the fertilization period of dairy cows (Table 4, group 2). Leucocytes were observed in some smears immediately after calving, during "behaviour estrus", and just prior to the rutting period.

Table 4. Cell content of dairy cow vaginal smears in different intervals after calving. Values are mean ± standard deviation (range).

Days post-partum	*n*	Duration of interval between calving and sampling	Superficial cell content, % total quantity of cells	Superficial cell proper	Nuclei and shadow of nuclei (cytolysis)	Other cells inter-mediate	Leuco-cytes
27-64	8	27-64	97.4±3.2 (90.0-99.7)	6.4±3.8 (1.5-10.9)	4.4±3.4 (0.3-9.8)	1.5±1.6 (0.1-4.6)	-
49-67	4	49-67	98.0±2.0 (95.2-100.0)	4.7±3.7 (0.8-7.5)	1.0±0.4 (0.5-1.3)	2.3±1.8 (0.3-4.5)	+
44-145	11	44-145	98.7±1.4 (94.7-99.6)	5.6±4.2(7)[1] (0.5-13.2)	4.4±9.0(8)[1] (0.5-10.1)	1.1±1.2[1] (0.5-4.6)	-
				24.2±8.7(4)[2] (17.7-33.8)	25.5±9.5(3)[2] (16.6-35.3)		-

[1]First subgroup, n=7
[2]Second subgroup, n=4

DISCUSSION

Serum cholesterol and blood protein levels did not change before and after calving in moose. Cholesterol level of cow moose serum was 1.8 times less than in dairy cattle during the periods under study. Serum progesterone levels of cow moose on the 17th and 58th days after calving was 2-2.5 times higher than before fertilisation and 3-16 times higher than in lactating female reindeer in this period (Pavlov *et al.* 1980). The first 3 postpartum months were characterised by absence of estrus activity. The appearance of overt behaviour characteristic of estrus activity and the existence of vaginal mucus in some females were not connected with the corresponding cytological changes in the vagina. The most pronounced change of the moose vaginal cell composition on the 7th and 58th days after calving was the high level of nuclei without cytoplasm; 35-58 times higher than in dairy cattle before fertilisation. The increase after calving in moose may be caused by high activity of the corpora lutea. The seasonal character of female moose reproduction may be related to increased activity of the corpora lutea 2-3 months after calving, which would increase progesterone levels in blood. However the progesterone ratio in blood of moose females was 1 ng/ml, similar to values found in wild muskoxen during peak estrus activity (Rowell and Flood 1988).

The vaginal smear analysis suggested low serum estrogen level in the pregnant cows (Kochoviy 1987) indicating that cow moose have the lowest estrogen activity during postpartum because of the seasonal inhibition of follicular growth in the ovary.

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