

周産期における乳牛と初生牛のリンパ球機能の検討

誌名	Japanese journal of veterinary science
ISSN	00215295
著者	石川, 潤
巻/号	49巻3号
掲載ページ	p. 469-475
発行年月	1987年6月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



Observation of Lymphocyte Function in Perinatal Cows and Neonatal Calves

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Received 7 July 1986/Accepted 3 March 1987)

ABSTRACT. The changes in lymphocyte transformation rate and proportion of E-rosette forming cells (ERFC), in addition with serum concentration of IgG and cortisol were examined on dairy cows in the perinatal period. The cows were bled at the various stages of pregnancy, parturition and post-parturient period. Also in calves, the lymphocyte transformation rate and proportion of ERFC were examined on the blood samples collected at weekly interval from birth to 5 weeks of age. The blastogenic response of lymphocytes to concanavalin A and pokeweed mitogen decreased beginning from the 3rd trimester and reached a minimum at parturition. The proportion of ERFC and serum IgG concentration also decreased with parturition. The high serum cortisol level at parturition may influence lymphocyte activity in cows. In neonatal calves, the number of ERFC and responsiveness of peripheral lymphocytes to mitogen corresponded to adult level immediately after birth.—**KEY WORDS:** blastogenesis, E-rosette formation, perinatal immunity

Jpn. J. Vet. Sci. 49(3): 469–475, 1987

It is well known that the incidence of bovine infectious diseases such as mastitis or periparturient infections is high during the perinatal period. Therefore, the immune competence of cows has been supposed to be depressed by the stress of pregnancy and parturition. There are some reports dealing with the function of bovine peripheral lymphocytes in the perinatal period [13, 21, 22]. Well *et al.* [22] reported a reduced $^3\text{H-TdR}$ uptake in PHA-stimulated lymphocytes from newly calved cows. Kashiwazaki *et al.* [13] found a marked decrease of incorporation of $^3\text{H-TdR}$ in lymphocytes from multiparous cows after calving.

On the other hand, neonatal calves are often diseased with diarrhea and pneumonia particularly on large suckling farms. They have also been studied on the immune responsiveness [10, 15, 20] or on the cellular immunity [1, 12, 16, 18]. Person *et al.* [18] studied the sensitivity of peripheral bovine lymphocytes obtained from newborn calves up to 3 months of age and demonstrated that blastogenic responses were comparable to that of lymphocytes from adult cows

although the variation was wide.

The proportion of peripheral T lymphocytes in neonatal calves is reported to be low, in general [1, 16]. However, there is also a report mentioning a considerable number of peripheral T lymphocytes in neonatal calves [12].

According to these previous studies, the immune responses of peripheral lymphocytes in perinatal cows and newborn calves were not fully discussed.

In this experiment, the function of peripheral lymphocytes was examined in perinatal cows and calves. The number of circulating T lymphocytes, their blastogenic activity and in addition, serum IgG and cortisol levels were measured in pregnant and post-parturient cows.

MATERIALS AND METHODS

Blood samples: Fifty-two Holstein cows from 2 to 10 years of age and 9 calves from the University farm were used for this study. Blood was collected from the cows at various stages of the gestational and post-

parturient period; initially in the 1st or 2nd trimester, subsequently in the 3rd trimester, soon after parturition (within 6 hr post-parturition) and then at weekly intervals up to 4 weeks post-parturition.

The calves were bled at weekly intervals from birth to 5 weeks of age. They were all kept in outdoor hutches and fed whole milk and hay twice a day.

During the observation period, diseases were not clinically detected in the cows or calves except for one case of hypocalcemia after parturition.

The blood samples were collected in plain and heparinized vacuum tubes (containing 14 IU/ml of heparin) by venipuncture. The red blood cells (RBC) and white blood cells (WBC) were counted with a blood cell counter (PC-604, Erma optical works., Japan) and the lymphocyte counts were estimated by WBC and hemogram. Lymphocyte preparation was carried out by use of Ficoll Paque (Pharmacia, Sweden) gradient immediately after sampling.

E rosette assay : E rosette formation assay of lymphocytes was carried out according to the method of Paul *et al* [17]. Briefly, 100 μ l of lymphocyte suspension (5×10^6 /ml) in RPMI 1640 (Gibco, USA) containing 10% of fetal calf serum (FCS, Flow, USA) and 200 μ l of AET (2-aminoethylisothiuronium bromide, Sigma, USA) treated sheep erythrocytes (SRBC, 5% in RPMI 1640) were mixed and incubated at 37°C for 20 min. Then, the mixture was centrifuged at 200 g for 5 min and stored in the refrigerator. After overnight incubation at 4°C, a drop of brilliant cresyl blue was added to the suspension and the rosette forming cells (RFC) were counted with the haemocytometer. The data were expressed as mean values of two determinations.

Blastogenic response to mitogens: The blastogenic responses to concanavalin A (Con A, Gibco, USA) and pokeweed mitogen (PWM, Gibco, USA) were evalu-

ated by the glucose consumption test (GCT) [5, 11]. After 96 hours incubation with Con A (25 μ l/ml) or PWM (20 μ l per culture) at 37°C and 5% CO₂, the glucose concentration of the culture medium was determined by using a commercial kit (GL-V, Kyokuto, Japan). The lymphocyte blastogenic activity was expressed by the glucose consumption index (GCI) as follows:

$$\text{GCI} = \frac{(\text{G})_{\text{medium}} - (\text{G})_{\text{mitogen stimulated culture}}}{(\text{G})_{\text{medium}} - (\text{G})_{\text{control culture}}} \times 100$$

* (G) = glucose concentration

The culture conditions were as previously described [11]; cell numbers were adjusted to 5×10^6 /ml, and Con A (25 μ l/ml) or PWM (20 μ l per culture) was added to each culture, or PWM (20 μ l per culture) were added to each culture.

Serum IgG concentration: Serum IgG concentration was determined by single radial diffusion. Specific anti-bovine IgG was prepared as described previously [10].

Serum cortisol concentration: Serum concentration of cortisol was measured by radioimmunoassay (RIA) using commercial antiserum (Teikoku zoki, Japan).

RESULTS

Perinatal cows: The mean leucocyte and lymphocyte counts of the cows in the various stages of the perinatal period are shown in Table 1. The leucocyte counts increased sharply from 7,700/ μ l in the 3rd trimester to 13,800 μ l at parturition owing to neutrophilia and returned to the pre-parturient level soon after calving, where it remained stable. The mean lymphocyte count was constant, 3,070 to 4,180/ μ l, through the experimental period.

The changes of the mean RFC count are represented in Fig. 1. The mean percentage of RFC in the peripheral lymphocytes in the 1st or 2nd trimester, 3rd trimester, at par-

Table 1. Leucocyte and lymphocyte counts in the peripheral blood from pregnant and post-parturient cows

Period		Leucocyte count (μ l)	Lymphocyte count (μ l)
1st and 2nd trimester	(11) ^a	6,940 \pm 1,250 ^b	3,070 \pm 860 ^b
3rd trimester	(18)	7,700 \pm 1,220	3,730 \pm 950
parturition	(6)	13,800 \pm 3,940	4,120 \pm 770
1-2 weeks post-partum	(8)	6,840 \pm 1,820	4,180 \pm 650
4 weeks post-partum	(9)	8,270 \pm 800	3,230 \pm 620

a) Number of animals used.
b) Mean \pm S.D. of each group.

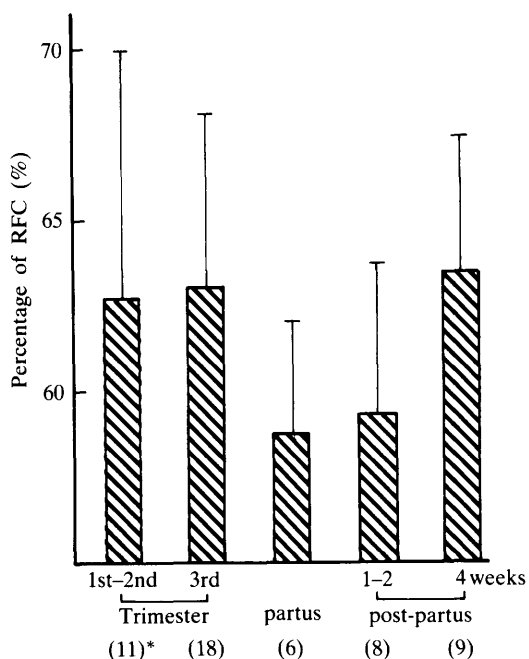


Fig. 1. The changes of rosette forming cell (RFC) in the bovine peripheral lymphocytes in the various stages of pregnant and post-parturient period. * = number of cows used.

turition, 1-2 weeks and 4 weeks post-parturition was 62.76, 62.99, 58.70, 59.29 and 63.53%, respectively. A slight decrease of the mean RFC percentage was observed at parturition.

Lymphocyte cultures from late pregnant and newly calved cows responded less to

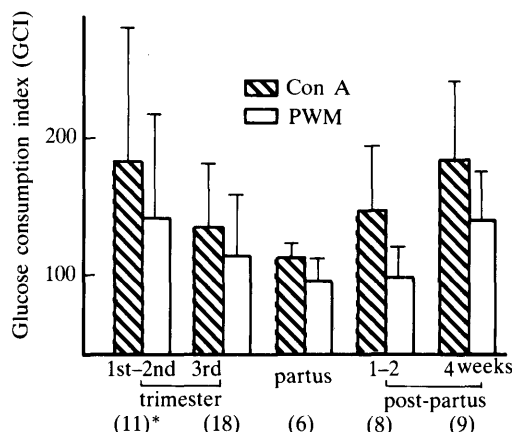


Fig. 2. The blastogenic responses of peripheral lymphocytes in the cows during the pregnant and post-parturient period.

* = number of cows used.

Con A than cultures from cows in early stage of gestation or during the non-pregnant period (Fig. 2). Glucose consumption index (GCI) in the parturient period was significantly low ($P > 0.05$), 112 ± 113 , compared with the GCI in the early gestational period, 183 ± 107 . After parturition, the CCT increased gradually toward the previous level. The changing pattern of the GCI with PWM was the same as with Con A although the responsiveness to PWM was less weak than to Con A.

The changes of serum concentration of IgG were shown in Fig. 3. The serum IgG levels dropped at a late stage of gestation and were lowest at parturition (12.58 mg/ml), significantly low compared with the non-pregnant level ($P > 0.05$). The levels of IgG began to increase toward non-pregnant level within a week after parturition and reached a normal level (21.06 mg/ml) at 3 weeks post-partum.

Serum concentration of cortisol below 1,000 pg/ml till 2 weeks before parturition increased rapidly to 5,500 pg/ml at parturition ($P > 0.05$) and then declined to the previous level within a week (Fig.4).

Neonatal calves: As shown in Table 2, leucocyte counts varied from birth to 5

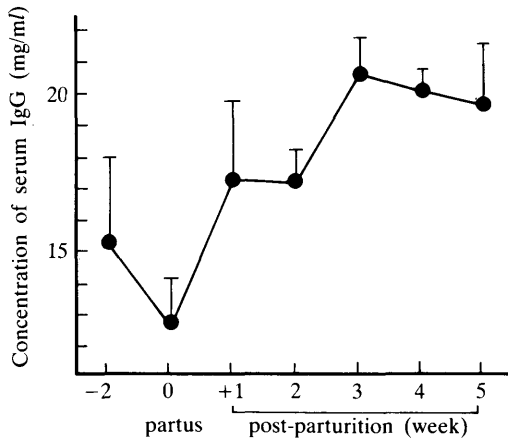


Fig. 3. The changes of serum concentration of IgG in 6 cows during the pregnant and post-parturient period.

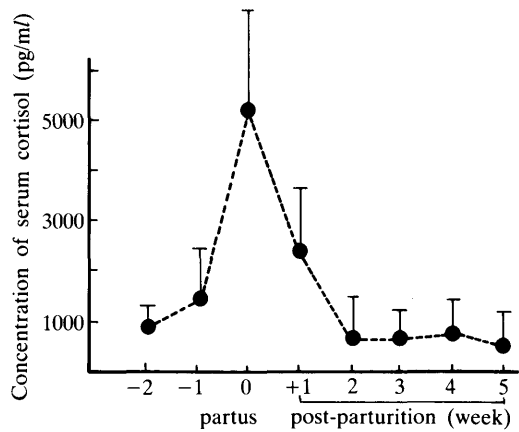


Fig. 4. The changes of serum concentration of cortisol in 13 cows during the pregnant and post-parturient period.

Table 2. Changes in bovine leucocytes, lymphocytes and T lymphocytes in 9 calves from birth to 5 weeks of age

Age (weeks)	Leucocyte count ($/\mu\text{l}$)	Lymphocyte count ($/\mu\text{l}$)	T lymphocytes (%)	T lymphocytes ($/\mu\text{l}$)
0	13,030 \pm 1,910	3,220 \pm 1,380	61.1 \pm 3.7	1,968 \pm 51
1	9,250 \pm 1,380	5,310 \pm 1,890	60.5 \pm 4.3	3,211 \pm 80
2	9,780 \pm 930	4,230 \pm 870	61.5 \pm 6.4	2,602 \pm 56
3	11,010 \pm 890	4,820 \pm 730	62.5 \pm 3.7	3,012 \pm 27
4	9,300 \pm 2,450	6,130 \pm 2,190	58.5 \pm 5.4	3,586 \pm 118
5	10,180 \pm 720	5,070 \pm 710	56.9 \pm 5.8	2,884 \pm 41

weeks of age and lymphocyte counts steadily increased with age. The percentage of T lymphocytes in the peripheral blood obtained within 6 hours after birth ranged from 54.7 to 63.4% with a mean of $61.1 \pm 3.7\%$. This level was kept thereafter with a slight decrease toward 5 weeks of age (Fig.5). The absolute number of T lymphocytes increased gradually with maturation as total lymphocyte counts increased (Table 2). The blastogenic responses of calf lymphocytes to Con A and PWM were represented in Fig.6. Immediately after birth, the mean GCI was 179 for Con A and 137 for PWM, almost the same level as in adult cows, although the individual variation was rather wide.

DISCUSSION

The reduction of the number of T lymphocytes and of the lymphocyte blastogenic response to Con A and PWM has been confirmed in human pregnancy [2,7,14].

The present study also suggests that bovine lymphocyte function is depressed during the perinatal period. The lymphocytes from cows in late pregnancy, at parturition and in post-parturient period showed lower proliferative responses as compared to those from cows in the non-pregnant or early pregnant stage.

Kashiwazaki et al. [13] reported that the blastogenic response to PHA, Con A and PWM was decreased after calving in multiparous cows. The same depression was

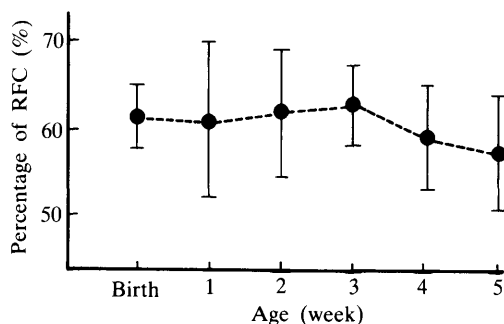


Fig. 5. The changes of rosette forming cell (RFC) count in the peripheral lymphocytes of 9 calves from birth to 5 weeks of age.

observed in sheep [3]. Well *et al.* [22] also demonstrated that lymphocytes from newly calved cows were less responsive to PHA than those from non-pregnant cows. The author suggested that stress and increased serum corticosteroid level might be related to this decreased blastogenic response in the parturient period.

Immediately after parturition, RFC proportion had decreased by about 10% as compared to the non-pregnant level. The RFC proportion seemed to be normal in the 3rd trimester when the blastogenic activity of the lymphocytes was already considered to be depressed.

Serum IgG level began to decrease rapidly from the 3rd trimester and became lowest at parturition, suggesting the depression of cellular as well as humoral immunity.

Serum concentration of cortisol elevated sharply with parturition and decreased within 1–2 weeks after parturition. This steroid may be responsible for the depression of immune responses in the perinatal cows, since corticosteroids are known to be an inhibiting factor for lymphocyte activation [4, 8]. Further studies, however, are necessary to find the role of cortisol in immunosuppressive state in perinatal cows as recognized in many other immunosuppressive factors [14].

Binns [1] reported that RFC percentage

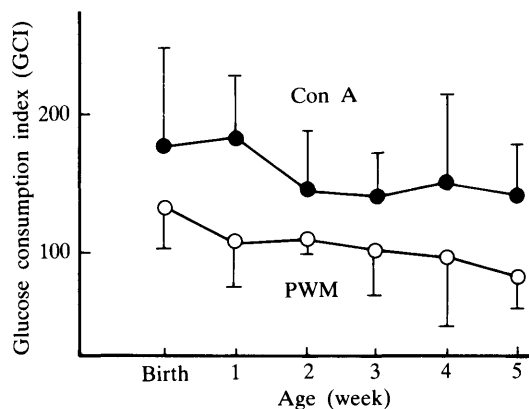


Fig. 6. The blastogenic responses of peripheral lymphocytes in 9 calves from birth to 5 weeks of age.

in the peripheral blood of newborn calves was low (23%) and increased gradually with age. Outteridge and Dufty [16] also reported the neonatal alteration of the RFC percentage in calves.

According to the present data, mean RFC percentage of neonatal calves are the same as the adult level soon after birth even though the individual variation is observed. The differences in data may be attributed to the treatment of SRBC, since AET treated SRBC were used for the E rosette assay in this study whereas non-treated SRBC were used in the above mentioned studies. Ishikawa [12] reported that RFC percentage of neonatal calves was $48.2 \pm 18.0\%$ at birth and soon reached adult level within a few weeks, using AET treated SRBC for rosetting assay.

The blastogenic activity of calves at birth was at the same level as that of adult cows, and thereafter slightly decreased till 5 weeks of age. The responses of the lymphocytes from calves to PWM were less obvious as compared to Con A.

Renshaw *et al.* [19] demonstrated that lymphocytes from 100 cases of bovine fetuses had significant blastogenic response to PWM after 90 days gestational age, those from 50% or more responded to Con A after 121 days'.

Person *et al.* [18] reported that blastogenic response of calf lymphocytes to PHA and Con A were found at various levels during the first 3 months of life. These may suggest that lymphocytes from neonatal calves have the ability to respond to mitogens, though individual variation was noted in the lymphocyte reactivity.

ACKNOWLEDGEMENTS. The author wishes to thank Mr. S. Kamimura, graduate student in this veterinary hospital, for his technical assistance and Dr. Miyazawa, the Department of Veterinary Obstetrics and Gynecology, Obihiro University of Agriculture and Veterinary Medicine, for RIA of the serum cortisol.

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要 約

周産期における乳牛と初生牛のリンパ球機能の検討：石川 潤（帯広畜産大学畜産学部家畜病院）——乳牛の周産期における免疫能を把握するため、分娩前後の血清 IgG 濃度およびコルチゾール濃度について、リンパ球幼若化能とTリンパ球数の変動を中心に検討した。また、仔牛についてもそのリンパ球幼若化能とTリンパ球数の推移を出生直後から5週齢まで経時的に観察した。分娩を契機としてリンパ球幼若化能、Tリンパ球数および血清 IgG濃度は低下し、回復は分娩後約3週間であった。一方、血清コルチゾール濃度は分娩時に有意に高くリンパ球機能低下に何らかの影響を与えているものと考えられ、仔牛の出生時、リンパ球幼若化能およびTリンパ球数はすでに成牛の水準に近いことが示唆された。