Occurrence of Blood Cells and Serum Proteins in Bovine Fetuses and Calves

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SUMMARY

Hematological values of peripheral blood were determined for bovine fetuses and calves of various ages. Erythrocyte values increased through gestation. Fetuses 100 days or older had total values within the ranges of those reported for normal adult cattle. Mature erythrocytes were not observed in embryos and only a few were observed in fetuses 40 days of age. Fetuses 250 days or older had only a few rubricytes (< 10/100 WBC). Leukocytes were first identified in the peripheral blood of a 45-day old fetus. Absolute leukocyte values increased through gestation and reached maximum values shortly before parturition. Granulocytes were first observed at 130 days of gestation and reached maximum values near parturition.

Total serum protein and gamma-globulin concentrations of colostrum-deprived calves were similar to serum protein and gamma-globulin concentrations of fetuses older than 265 days and were lower than values for the colostrum-fed calves. The immunoelectrophoretic pattern of 59-day old fetuses, the earliest age at which serum samples were obtained, demonstrated albumin, an α_1 globulin and a β globulin, possibly transferrin. Additional α and β globulins appeared in the older fetuses and by 175 days of gestation serum electrophoretic patterns of the fetuses were similar to patterns normally found with adult bovine serum except for the absence of the gamma-globulins in fetal serum. Immunoglobulin M was detected in 39 of 95 fetal serum samples by radial diffusion and in 13 of 95 samples by immunoelectrophoresis. Immunoglobulin G was detected in ten of 95 fetal serum samples by radial diffusion and in six of 95 samples by immunoelectrophoresis.

RÉSUMÉ

Les auteurs ont déterminé les valeurs hématologiques du sang périphérique chez des foetus bovins et chez des veaux de différentes âges. Les valeurs des érythrocytes augmentèrent au cours de la gestation. Les foetus de 100 ours ou plus présentaient des valeurs totales correspondant à celles déjà rapportées chez les bovins adultes sains. On n'a pas observé d'érythrocytes parvenus à maturité chez les embryons et on n'en a noté seulement quelques-uns, chez des foetus de 40 ours. Les foetus de 250 jours ou plus ne présentaient que quelques rubricytes (<10/100 leucocytes). C'est dans le sang périphérique d'un foetus de 45 jours qu'on a identifié, pour la première fois, les leucocytes. Les valeurs leucocytaires absolues augmentèrent durant la gestation et atteignirent leur sommet, peu de temps avant la parturition. L'observation des granulocytes débuta au 130ième jour de gestation et ils atteignirent leur sommet, peu avant la mise-bas.

Les concentrations en protéines sériques totales et en gamma-globuline chez les veaux privés de colostrum s'avérèrent semblables à celles de foetus de plus de 265 jours, mais inférieures à celles des veaux ayant reçu du colostrum. Le tableau de l'immuno-électrophorèse chez les foetus de 59 jours, le plus jeune âge où on préleva des échantillons de sérum, indiquait la présence d'albumine, d'une globuline α_1 et d'une β globuline, probablement de la transferrine. D'autres globulines α et β apparurent chez les foetus plus âgés et, après 175 jours de gestation, les tableaux de l'électrophorèse du sérum des foetus étaient semblables à ceux qu'on observe normalement dans

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du sérum de bovins adultes, sauf pour l'absence de gamma-globulines dans le sérum des foetus. On décela l'immuno-globuline M, par diffusion radiale, dans 39 d'un lot de 95 échantillons de sérum foetal et, par l'immuno-électrophorèse, dans 13 autres échantillons de ce lot. On détecta l'immuno-globulin G, par diffusion radiale, dans 13 de ce lot de 95 échantillons de sérum foetal et, par l'immuno-électrophorèse dans six autres échantillons du même lot.

INTRODUCTION

Hematological values for peripheral blood of calves and adult cattle have been reported (12). Winqvist (21) described tissue hematopoiesis in the bovine fetus and newborn calf; however, few, if any, reports are available on hematological values of peripheral blood for the bovine fetus.

The time sequence of appearance of serum proteins in ontogeny has been reported (6, 17) for a number of species; however, data on the development of serum proteins in the bovine fetus have not, to the authors' knowledge, been reported.

The present report provides hematological values of peripheral blood and serum protein analysis, particularly the immunoglobulins, that were determined during a study on the ontogenesis of the bovine immune response (14, 15). The fetuses were from apparently normal dams. Hematological values from fetuses of dams suspected of clinical illness and fetuses with bacterial or viral infections were excluded. Therefore, the values could be indicative of normal values and would provide a basis for comparison to values obtained in diseases of the bovine fetus and calf.

MATERIALS AND METHODS

Blood samples were obtained from three embryos (<40 days), 106 fetuses and 42 calves. Fifty-seven fetuses and three embryos were obtained from the Dairy Breeding Research Center, 36 were obtained from a local abattoir and 13 from the Animal Disease Laboratory. The ages of the fetuses obtained from the Dairy Breeding Research Center and the Animal Disease Laboratory were known from artificial insemination records. Ages of fetuses from the abattoir were estimated from a table based on the relationship of age to crown-rump length and weight measurements compiled by Tanabe[1]. The prenatal group included 50 males and 51 females. The sex of five fetuses, 40 days of age, and the three embryos was not determined. Seventy of the fetuses were Holstein, 19 Guernsey, eight Hereford, three Jersey, three Brown Swiss, and three Ayrshire.

The calves obtained from University herds were all males and included 14 Holstein, ten Ayrshire, seven Guernsey, six Brown Swiss, and five Jersey calves (16).

Blood samples were obtained from the jugular vein or femoral artery of calves and fetuses and from the umbilical cord of the embryos. The potassium-ammonium oxalate used as an anticoagulant was omitted from blood for differential counts (8). Blood smears for differential counts were prepared immediately, air-dried and stained with Wright's stain. In general, 100 white blood cells (WBC) were counted for each differential count. However, less than 100 WBC were counted on slides prepared from some of the younger fetuses due to the paucity of leukocytes. Leukocyte and erythrocyte counts were determined with an electronic cell counter[2]. Leukocyte counts of fetuses were corrected for nucleated red blood cells using the formula cited by Schalm (12).

The calf serum samples, for immunological studies, were generally obtained from blood collected during the first 36 hours after birth. Fetal serum samples, for immunological studies, were obtained from blood collected shortly after the fetus was removed from the uterus. Serum samples were not obtained from embryos or from fetuses less than 59 days of age. Total protein and gamma-globulin concentrations were determined for serum samples of all calves and fetuses older than 265 days. Total protein values were determined by the Biuret method (2). Two commercial serum standards with different concentrations of protein were used as controls[3]. The gamma-

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[2]Celloscope, Particle Data Inc., Elmhurst, Ill.

[3]Dade Reagents Inc., Miami, Fla.

globulin levels were determined by a commercially available turbidometric method, the Gamm-ulin test[4], which employs barium sulfate. Immunoelectrophoresis was performed according to the micromethod of Scheidegger (13). Radial diffusion assay for bovine immunoglobulin M (IgM) and immunoglobulin G (IgG) were performed according to the method of Fahey and McKelvey as modified by Klaus and Jones (11). The procedure for disc electrophoresis is outlined in the technical bulletin of Canal Industrial Corporation[5] and was performed as described by Davis and Ornstein (3). Commercially available human serum[6] and bovine serum fraction[7] were used as standards in serum protein analysis. Antisera specific for bovine IgM and IgG were prepared as previously described (14).

TABLE I. Erythrocyte Values of Fetuses and Neonates

Age (Days)	No. Animals	Erythrocyte Values[a] Mean	Max	Min
Embryos				
20 to 35	3	N.D.[b]	N.D.	N.D.
Fetuses				
40 to 50	9	2.0	2.3	1.7
55 to 65	9	2.5	3.0	2.0
70 to 80	9	4.1	5.0	3.1
85 to 100	8	4.9	5.9	3.9
104 to 120	12	5.1	6.1	3.7
125 to 135	9	5.6	6.9	4.5
140 to 155	8	5.5	6.7	4.4
160 to 170	9	5.9	7.4	4.0
175 to 190	7	5.7	6.9	4.1
205 to 220	7	5.0	6.7	3.7
235 to 250	10	5.9	8.1	4.8
265 to 275	8	6.7	8.4	5.0
Calves				
0 to 3	7	7.9	9.8	6.8
4 to 7	13	8.7	11.7	6.9
8 to 14	5	7.1	8.4	6.5
15 to 30	6	8.3	10.0	6.8

[a]Mean, maximum (Max) and minimum (Min) values x 10^6/mm^3
[b]Not done

RESULTS

Mean erythrocyte values of peripheral blood are listed by prenatal age and postnatal age (Table I). Prior to 40 days of gestation all cells were rubricytes in various stages of maturation. Mature erythrocytes were first observed at 40 days of gestation Erythrocyte counts increased during the prenatal period and appeared to reach maximum values during the early postnatal period. Fetuses 250 days or older had few rubricytes (< 10/100 WBC).

Absolute leukocyte values are listed (Table II). Leukocytes were first identified in the peripheral blood of a fetus at 45 days of gestation. Leukocyte values increased throughout gestation and reached a maximum shortly before parturition (265 to 275 day fetuses). Agranulocytes were the only leukocytes present in peripheral blood until 130 days of gestation, at which time a few granulocytes appeared. In fetuses less than 130 days of age, more than 50% of the lymphoid cells were blast cells, the remaining cells were small lymphocytes. Granulocyte values for fetuses over 265 days of gestation were similar to zero to three-day neonatal calf values. More than 90% of the granulocytes present through all periods of gestation appeared to be the mature form of the particular granulocyte. Undifferentiated cells, resembling myelocytes, were found in approximately 20% of the fetuses of various ages. Basophils were found in the blood of three fetuses. Two of the three fetuses were experimentally aborted by removal of the corpus luteum (18).

Total protein and gamma-globulin values are shown in Table III. Total protein and gamma-globulin values of the colostrum-fed calves were higher than the values for the other two groups. The colostrum-deprived calves had values that were similar to the fetuses. All colostrum-deprived calves and fetuses had gamma-globulin as determined by the turbidometric assay with Gamm-ulin.

Serum proteins were identified by their electrophoretic mobility and their position relative to the commercial standards. The immunoelectrophoretic pattern of the 59-day old fetuses demonstrated the presence of albumin, an α_1 globulin and β globulin, presumably transferrin. By 65 days of gestation, albumin, an α_1 globulin, α_2 macroglobulin and β globulin (transferrin) were present. Additional α and β globulins ap-

[4]Omni Tech., Santa Monica, Calif.
[5]Canal Industrial Corp., Rockville, Md.
[6]Dade Reagents Inc., Miami, Fla.
[7]Mann Research Lab., New York, N.Y.

TABLE II. Leukocyte Values[a] of Fetuses and Calves

Age (Days)	No. Animals	Leukocyte Values			Differential Values[b]				
		Mean	Max	Min	Lymph	Neut	Mono	Eos	Baso
Embryos									
20 to 35	3	0	0	0	0	0	0	0	0
Fetuses									
40 to 50	9	100	200	0	100	0	0	0	0
55 to 65	9	190	320	100	100	0	0	0	0
70 to 80	9	227	375	63	100	0	0	0	0
85 to 100	8	692	1,270	333	100	0	0	0	0
104 to 120	12	1,065	1,739	833	100	0	0	0	0
125 to 135	9	2,085	4,947	714	98.5	0.2	0.8	0.5	0
140 to 155	8	2,617	5,000	1,286	98.0	1.7	0	0.3	0
160 to 170	9	3,770	6,957	1,250	95.5	2.5	0.5	1.5	0
175 to 190	7	6,550	10,000	3,125	89.0	5.0	3.0	2.5	0.5
205 to 220	7	7,330	10,833	4,425	79.5	16.5	2.5	2.5	0
235 to 250	10	6,872	10,172	3,333	81.5	12.0	3.0	3.5	0
265 to 275	8	9,740	12,870	6,500	60.0	34.0	3.0	3.0	0
Calves									
0 to 3	7	9,530	17,900	2,000	56.0	35.0	7.5	1.5	0
4 to 7	13	9,300	14,400	5,700	65.0	29.5	3.5	2.0	0
8 to 14	5	8,750	12,000	5,800	64.0	32.0	3.0	1.0	0
15 to 30	6	8,800	11,500	6,700	72.0	20.0	6.5	1.5	0

[a]Values are expressed as leukocytes/mm^3
[b]Percentage of lymphocytes (Lymph), neutrophils (Neut), monocytes (Mono), eosinophils (Eos), and basophils (Baso) based on 100 leukocytes

TABLE III. Total Protein and Gamma Globulin Values in Calves and Fetuses

Animal	No. Animals	Total Protein (Gm%)[a]			Gamma Globulin (Gm%)		
		Mean	Max	Min	Mean	Max	Min
Colostrum-fed calves	23	5.55	7.00	4.00	1.13	2.00	0.68
Colostrum-deprived calves	19	4.04	4.70	3.00	0.42	0.80	0.17
Fetuses (>265 days)	8	4.04	4.90	3.40	0.45	0.65	0.14

[a]Gm/100 ml

TABLE IV. Immunoglobulin (Ig) in Serum of Fetuses and Calves as Detected by Immunoelectrophoresis and Radial Diffusion

Age (Days)	No. Animals	Immunoelectrophoresis[a]		Radial Diffusion[a]	
		IgM (+)	IgG (+)	IgM (+)	IgG (+)
Fetuses					
59 to 65	9	0	0	0	0
70 to 80	9	0	0	0	0
85 to 100	8	0	0	0	0
104 to 120	12	0	0	0	0
125 to 135	9	0	0	3	0
140 to 155	8	1	1	6	1
160 to 170	9	1	0	4	0
175 to 190	7	1	0	5	0
205 to 220	7	1	0	5	0
235 to 250	9	3	2	8	3
265 to 275	8	6	3	8	6
Calves[b]					
0 to 2	19	14	N.D.[c]	17	N.D.

[a]The number of samples that contained immunoglobulin
[b]Colostrum-deprived
[c]Not done

peared in the older fetuses and by 175 days of gestation serum patterns of the fetuses were similar to patterns normally found for adult bovine serum except for the absence of the gamma-globulins.

A protein band migrating in the region of hemoglobin appeared in disc electrophoresis but was not found with immunoelectrophoresis. The presence of this protein in hemolyzed serum samples and its absence in nonhemolyzed samples further suggested that it was hemoglobin.

Immunoglobulin M was detected as early as 130 days of gestation by the radial diffusion technique (Table IV). IgM was first identified by immunoelectrophoresis in a 145-day fetal serum sample. IgG, primarily γ_1, was also present in the 145-day sample. IgM was detected by immunoelectrophoresis in only 12 other fetuses; two were less than 205 days of age (Table IV). IgG was detected by immunoelectrophoresis in six fetuses, five were older than 220 days. The number of animals in each age group and the number of serum samples containing immunoglobulins are listed in Table IV.

DISCUSSION

Erythrocyte and leukocyte values were obtained from fetuses of clinically normal dams. Erythrocyte values increased steadily through gestation and fetuses 100 days or older had values within the ranges of those reported for normal adult cattle (12). Mature red blood cells were not observed in embryos and only a few appeared in fetuses 40 days of age. Similar results have been reported for the development of erythrocytes in hematopoetic tissue (21).

Lymphocytes were identified in the peripheral blood of a fetus at 45 days of gestation, the same age that the lymphoid thymus was recognized in histological sections (15). It has been reported that the appearance of lymphocytes in the peripheral blood of the human fetus coincided with the development of the lymphoid thymus which was first recognized at about 50 days of gestation (10). The similarity for the time of appearance of lymphocytes in the bovine fetus and human fetus may be expected since the gestational period of both is approximately 280 days. Absolute leukocyte values reached normal adult values by 190 days of gestation; however, the differential values were not in the ranges of reported adult values until 265 days of gestation (12). In the 265 to 275 day fetuses and zero to three day neonates the granulocyte values were higher than normal adult values. This increase in granulocytes near parturition has been reported by others (1, 4). However, inversion of lymphocyte and neutrophil values as reported by others was not observed (9, 12). Phagocytic cell types and IgG appeared at approximately the same time in gestation (Tables II and IV). Although this similarity may be due to coincidence, it is suggested here and by others (5) that the presence of blood monocytes (circulating macrophages) provide a cell type necessary for the immune response to be initiated, at least to certain antigens. Initiation of IgM and IgG synthesis in human fetal spleen first developed at about 140 days of gestation approximately the same time in gestation that IgM and IgG were first observed in the serum of a bovine fetus (20).

Colostrum-fed calves had total protein values and gamma-globulin values similar to values reported by others (19). The total protein values were similar in the colostrum-deprived calves and fetuses older than 265 days of gestation. Differences between total protein values of colostrum-fed and colostrum-deprived calves were only partially accounted for by gamma-globulin values. The difference in results may have resulted from colostral whey proteins which were probably transferred to the serum of colostrum-fed calves. The radial diffusion test appeared to be more sensitive than immunoelectrophoresis. The difference in the sensitivity of the two techniques was reflected in the larger number of samples that contained IgM and IgG by radial diffusion than by immunoelectrophoresis. The Gamm-ulin reagent, barium sulfate method was less specific than either of the above, and the interpretation of the results was difficult when samples contained hemoglobin. It was also reported that the zinc sulfate test was unrealiable for the detection of gamma-globulin in calf serum when hemoglobin was present (7).

The appearance of certain serum proteins at specific times suggested the *de novo* synthesis of serum proteins during gestation. The time sequence of appearance of serum proteins in the bovine was similar to the sequence reported for other species (6, 17).

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