Hematological and immunological development from birth to six months of age in Holstein calves

[*Desenvolvimento hematológico e imunológico em bezerros Holandeses do nascimento aos seis meses de idade*]

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ABSTRACT

The hematological and immunological development of calves from birth to 6 months of age was performed by hemogram and cellular phenotype. Ten male Holstein calves were assessed in 13 moments: before colostrum intake (D0), every 2 days until the 10th day of life (D2 to D10), at the 15th day after birth (D15), and then monthly up to 6 months (D180). Calves presented hemoconcentration on day (D) 0 and showed a gradual decrease in hematimetric rates until D180. The inversion of the neutrophilic for lymphocytic profile was observed on D4. The percentage of $CD3^+$ cells on D10 was higher than D30 up to D180. The number of $CD4^+$ and $CD8^+$ cells did not change between time points. The number of $CD21^+$ lymphocytes was significantly higher at early time points of D0 up to D15, compared on D30 until D150. In conclusion, the neonatal period was marked by stress leukogram in the first 4 days, and low number of B lymphocytes. These might be risk factors for bacterial infections responsible for navel inflammation and diarrhea. The increase in the number of B cells from 30 days of age demonstrated that the calves were functional and able to generate an immune response.

Keywords: bovine, immune system, leukogram, immunophenotyping, flow cytometry

RESUMO

O desenvolvimento hematológico e o imunológico de bezerros do nascimento aos seis meses de idade foram avaliados por meio hemograma e imunofenotipagem dos linfócitos. Para tanto, 10 bezerros machos Holandeses foram avaliados em 13 momentos: antes da ingestão do colostro (D0), a cada dois dias até o 10º dia (D2 a D10), 15º dia (D15) e, em seguida, mensalmente até seis meses de idade (D180). Os bezerros apresentaram hemoconcentração em D0 e decréscimo gradual dos índices hematimétricos até D180. A inversão do perfil neutrofílico para linfocítico foi observada em D4. Os valores de CD3+ (%) em D10 foram maiores que aqueles observados em D30 até D180. CD4+ e CD8+ não apresentaram alterações entre os momentos. Os linfócitos B CD21+ aumentaram significativamente a partir dos primeiros momentos D0 até D15, em comparação com os momentos subsequentes D30 até D150. Em conclusão, o período neonatal foi caracterizado pelo leucograma de estresse nos primeiros quatro dias e o baixo número de linfócitos B. Esses fatos podem ser considerados como fatores de risco para infecções bacterianas responsáveis pelas inflamações umbilicais e pela diarreia. Por outro lado, o aumento de células B após os 30 dias de vida demonstrou que os bezerros foram capazes de gerar uma resposta imune.

Palavras-chave: bovino, sistema imune, leucograma, imunofenotipagem, citometria de fluxo

INTRODUCTION

Newborn calves are considered immune-naïve and agammaglobulinemic at birth, because of the synepitheliochorial placenta from dams, which prevents the passage of pathogens to the fetus during pregnancy. Even though this barrier maintains the sterility of the placental environment, it is also associated with two negative consequences for the neonate: agammaglobulinemia and absence of an immunological memory at birth (Barrington and Parrish, 2001).

Progesterone, prostaglandins, and the cytokines interleukin (IL)-4 and IL-10, produced by the placenta during pregnancy, are responsible for the suppression of the Th1 maternal response, which releases toxic cytokines for the fetal-placental environment. The predominance of the Th2 immune response is responsible for the susceptibility of the newborn to intracellular pathogens (Wegmann*et al.*, 1993). Furthermore, maternal and fetal cortisol released at the end of pregnancy and during labor is responsible for stress leukogram and the decrease of the functional activity of phagocytes (Chase *et al.*,2008).

The immature immune system of newborn calves at birth emphasizes the critical role of passive immunity transfer from the dam. The bovine colostrum is rich in immunoglobulin G1 (IgG1), cells, and cytokines. Mononuclear leukocytes are the predominant cells in the mammary secretion, especially $CD14^+$ macrophages (39.6%), B lymphocytes (24.5%), $CD4^+$ T lymphocytes (13.8%), and $CD8^+$ T lymphocytes (19%) (Park *et al.*, 1992). These cells are absorbed by the newborn intestinal mucosa and have been identified in the blood stream and lymph nodes after fresh colostrum intake (Liebler-Tenorio*et al.*, 2002;Reber*et al.*, 2006).

The duration of immune factor presence in colostrum, especially IgG1, and the precocity of immune system development are the main factors in the epidemiological distribution of infectious diseases in calves. For determining the vulnerability periods, it is necessary to establish the hematological and immunological profiles of calves during early life.

Several studies have been performed to establish the normal values for hematological parameters. However, few published papers included repeated measurements of hemogram components in calves during the first 6 months of life. In addition, previous studies were performed using older technology (manual) (Brun-Hansen *et al.*, 2006). Data on the profile of lymphocyte subsets in calves are scarce (Kampen*et al.*, 2006). At this time, we are aware of only two international papers published on this topic, and the results may not be representative for calves under Brazilian conditions (Wilson *et al.*, 1996; Kampen*et al.*, 2006).

The hypothesis of this study is that variations in calves' hematological and immunological components occur during development, and these variations could indicate the window of susceptibility to antigens that are common in calf rearing. Therefore, a longitudinal study was designed to follow the cellular profile of Holstein calves from birth up to 6 months of age. This profile was evaluated by hemogram and phenotyping of the lymphocyte subsets.

MATERIALS AND METHODS

This research was approved by the Ethics Committee on Animal Use of the Faculty of Veterinary Medicine and Animal Science, University of São Paulo (Protocol # 2305/2011).

Ten Holstein cows and their calves were sourced from a dairy farm located in Nova Odessa, São Paulo, Brazil. These cows produced 25–30L of milk per day at the peak of lactation.

The dams were vaccinated against foot and mouth disease and *Brucella abortus* according to the Brazilian official program. These animals were also vaccinated in the pre-partum period by subcutaneous injection of 5mL of commercial multivalent vaccine containing inactivated strains of Bovine viral Diarrhea Virus (BVDV) type 1 (5960) isolated in Iowa (USA) and BVDV type 2 (53637) isolated in Ontario (USA); live/thermosensitiveBovine Herpesvirus 1 (Cooper) and Parainfluenza-3 Virus (RLB 103); live-attenuatedBovine Respiratory Syncytial Virus (strain 375) diluted in the adjuvant with "Quil A, cholesterol and amphigen." The vaccinations for bovine respiratory disease were administered twice, 8 and 4 weeks before the expected delivery dates.

Immediately after delivery, the dam was moved into a parlor where colostrum was collected aseptically from each quarter. Calves were fed with fresh colostrum from their respective dams. The calves were fed within 6h of birth with 2L of colostrum and with additional 2Lat12h after birth. An esophageal tube feeder was used only when the calves did not suckle from the bottle. The calves were transported from a dairy farm to the Ruminant Hospital at University of São Paulo,140km, in the second week after birth.

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They were then fed with 6L of milk replacer (Sprayfo® Violeta, Stolen, Deventer, Holanda), commercial feed (Ração para bezerros®, Agromix, Sao Paulo, SP, Brazil), hay, mineral salt (Fosbovi 20®, Tortuga, Mairinque, SP, Brazil),and water *ad libitum* until weaning. The quantity of the feed was increased at the beginning of the transition period and the maximum amount was established to be 3kg/day.

The calves were assessed before colostrum administration (D0), every 2 days until the 10th day of life (D2 to D10), at the 15th day after birth (D15), and then monthly up to 6 months of age (D180).

The calves were given a general clinical examination that included vital signs, hydration, ocular mucous, capillary refill, and palpation of the lymph nodes. The organ systems were evaluated when animals showed any changes in the general clinical examination and data excluded at moment presented (Feitosa, 2014).

Blood samples (4mL) were collected by vein puncture of the external jugular vein, using a vacuum system, into tubes containing ethylenediamine tetraacetic acid (EDTA, 1.5mg/mL). All samples were transported under refrigeration in an isothermal box.

Hematological parameters were obtained using an automatic system (ABC Vet, Horiba ABX®, Montpellier, FRA). Differential counting of leukocytes was performed by observing the cell morphological characteristics at 1000xmagnification (Novo *et al*., 2015).

The phenotyping of blood lymphocytes was performed according to the recommendations of Della Libera *et al*. (2012). The proportions of blood lymphocyte subsets were determined by flow cytometry (FACSCalibur™, Becton Dickinson Biosciences, San Jose, CA, USA). One hundred microliters of whole blood with EDTA was added to the flow cytometry tubes. Red blood cells (RBCs) were lysed by adding FACS Lyse Solution (BD Biosciences). Monoclonal antibodies (VMRD, Pullman, WA, USA) against various bovine leukocyte surface molecules (Table 1) were added to a final concentration of 1μg/mL.The samples were stained for 30min at 4°C and washed thrice with phosphate buffered saline (PBS). Subsequently, the primary antibodies were labeled with 1μg/mL fluorescein isothiocyanate - FITC, 1μg/mL phycoerythrin- rPE (Invitrogen, Carlsbad, CA, USA), and 10μg/mL peridinin-chlorophyll proteins - PercP (Becton Dickinson Biosciences), and incubated for 30min in the dark at 4°C. The IgG1 specific antibody was conjugated with PerCP, the IgG2a specific antibody was conjugated with rPE, and the IgM specific antibody was conjugated with FITC. Finally, cells were washed twice and suspended in 300μL of cold, sterile PBS.

Samples were analyzed by flow cytometry using FacsCalibur equipment (BD Biosciences, San Jose, CA, USA). Gate windows in forward angle scatter and 90° light scatter were established to allow the inclusion of the lymphocyte population.

Table 1. Primary and secondaryantibodies used to identify blood mononuclear cells

Primary	Clones	Secondary	Isotypes	Specificity
CD3	MM1A	PerCP®, rat anti-mouse	IgG1	T lymphocytes
CD4	IL-A11	rPE®, goat anti-mouse	IgG2a	T Helper
CD8	BAQ111A	FITIC®, goat anti-mouse	IgM	T cytotoxic
CD21	BAQ15A		IgM	B Lymphocytes

Flowjo analysis software (Treestar Inc, San Carlos, USA) was used to analyze the events (50.000 cells/samples) acquired by flow cytometry. All samples were gated on forward angle and side scatter to establish the lymphocyte

population. The lymphocyte subsets were analyzed based on the expression of specific fluorescence for CD3, CD4, CD8 and CD21.

Results are reported as the percentage of gated cells that were positive for each cell surface marker. The absolute number of each lymphocyte subset was calculated by multiplication of the percentage and the total number of lymphocytes per milliliter of blood obtained from the hemogram.

Statistical analysis was performed using SPSS for Windows (International Business Machines [IBM] Corporation, Armonk, New York, USA). All variables had parametric distribution according to the Shapiro-Wilk test, except rod neutrophils and eosinophils. Theage effect was examined using repeated measures analysis of variance(ANOVA). Differences among time points were considered significant when $P< 0.05$.

Rod neutrophils and eosinophils were not detected at some of the evaluation time points of this research. Therefore, these parameters were determined from D0 up to D6 for rod neutrophils, and from D60 up to D180 for eosinophils. These variables had non-parametric distribution according to the Skewness-Kurtosis test. Theage effect was examined using Friedman test, but none of the tested parameters showed statistically significant difference ($P< 0.05$) among time points.

RESULTS

The means and standard deviations of the erythrogram components and multiple comparisons between time points are shown in Table 2.

Sample time on RBC had decreased from D2 to D6 with an increasing trend throughout the study. Significant differences were seen between D0 (before colostrum intake)to D4 and D6 ($P< 0.05$).

The maximum values for hemoglobin (HGB) and hematocrit (HCT) were observed on D0. These HCT values then decreased between D2 to D10, with a slight increase thereafter (D15 to D180). HCT values on D0 were significantly different from those on D4, D6, D8 and D10 ($P< 0.05$).

The values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) decreased gradually during the first 6 months of life (D0 to D180). MCV values on D0 were

significantly different compared with those from D6 up to D150 ($P< 0.05$), with the exception of D10. Values on D2 were different from those on D8, D30, D60, D120, D150 and D180 ($P< 0.05$). The mean MCV values on D4, 6, 8 and 10 differed from those on days 60, 120 and 150 ($P< 0.05$). MCH values on D2 and D8were different compared to those on D60 ($P< 0.05$).

The means and standard deviations of the leukogram components and multiple comparisons between time points are shown in Table 3.

Total leukocytes decreased from birth up to D6, after which time, the values increased slightly and remained constant from D8 to D180. The difference between the values on D0 and those on D6 was statistically significant ($P< 0.05$).

Relative and absolute values for segmented neutrophils showed some variations from D0 to D180. Relative values (%) on D0were higher than those on D6, 30, 60, 90 and 150 ($P< 0.05$). Absolute values ($x10^3/\mu L$) on D0 were significantly different from those on D6, D60, D150 and D180 ($P< 0.05$).

The relative and absolute values for monocytes varied slightly from D0 to D180. There were no statistically significant differences between the time points, despite the slight variations observed during this study. Relative and absolute lymphocyte values increased from D0 up to D180, and displayed some variations on D6, D30-D90 and D150-180. The number (%) of lymphocytes on D0 was lower than that on D6, D15, D30, D60, D90, D150 and D180 ($P< 0.05$).

Rod neutrophils and eosinophils were not detected at some of the time points included in this study. The minimum and maximum values observed for rod neutrophils were 1% ($0.1 \times 10^3/\mu L$) on D6 and 3.8% ($0.6 \times 10^3/\mu L$) on D2, respectively. Eosinophil numbers varied between 1% ($0.1 \times 10^3/\mu L$) on D90 and 3% ($0.2 \times 10^3/\mu L$) on D150. There were no statistically significant differences between time points for these parameters.

The means and standard deviations for lymphocyte subsets and multiple comparisons between time points are shown in Figure 1. Variations were detected in the proportions of $CD3^+$ and $CD21^+$ cells.

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Table 2. Means and standard deviations of erythrogram components of Holstein calves from birth up to six months of age

Moments	RBC (x 10^6/µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (%)
D0	8.5±1.3[a]	12.5±2.3[a]	39±6[a]	46 ± 1[af]	14.5±1.3[ab]	32±2[a]
D2	7.2±1.3[ab]	10.5±1.9[a]	31±5[ab]	43 ± 1[ac]	14.5±0.7[a]	33±2[a]
D4	6.8±1.4[b]	10.0±1.8[a]	30±4[b]	42 ± 2[acd]	13.8±1.7[ab]	33±4[a]
D6	7.1±1.4[b]	9.3±2.5[a]	30±4[b]	42 ± 2[cde]	13.2±3.0[ab]	31±8[a]
D8	7.5±1.2[ab]	10.2±1.3[a]	31±4[b]	41 ± 1[de]	13.5±0.9[a]	33±4[a]
D10	7.7±1.1[ab]	9.7±1.6[a]	31±4[b]	41 ± 2[ae]	13.3±1.1[ab]	33±3[a]
D15	8.5±1.2[ab]	10.0±1.6[a]	33±3[ab]	40 ± 3[bcde]	12.1±1.8[ab]	31±5[a]
D30	10±1.4[ab]	11.1±1.6[a]	36±6[ab]	37 ± 3[bde]	11.7±1.2[ab]	31±3[a]
D60	9.8±1.7[ab]	10.0±2.0[a]	34±6[ab]	37 ± 2[b]	10.6±1.1[b]	29±3[a]
D90	9.2±1.8[ab]	10.8±1.1[a]	33±4[ab]	38 ± 4[bcde]	12.3±1.6[ab]	33±2[a]
D120	9.6±0.5[ab]	10.1±0.6[a]	33±5[ab]	36 ± 3[b]	11.2±1.2[ab]	31±2[a]
D150	9.7±1.7[ab]	10.1±1.2[a]	34±6[ab]	36 ± 2[bg]	10.7±2.1[ab]	29±2[a]
D180	9.2±2.1[ab]	9.7±1.5[a]	33±7[ab]	33 ± 9[bdef]	10.3±2.0[ab]	29±5[a]

(RBC) red blood cells; (MCV) mean corpuscular volume; (MCH) mean corpuscular hemoglobin; (MCHC) mean corpuscular hemoglobin concentration. Lower case letters in the same column show difference between moments (P< 0.05) using ANOVA for repeated measure.

Table 3. Means and standard deviations of leukogram components of Holstein calves from birth up to six months of age

Moments	Leukocytes (x 10^3/µL)	Segmented Neutrophils (%)	(x 10^3/µL)	Monocytes (%)	(x 10^3/µL)	Lymphocytes (%)	(x 10^3/µL)
D0	15 ± 4[a]	62 ± 12[a]	9.04 ± 3[a]	11 ± 1[a]	1.45 ± 0.48[a]	25 ± 10[a]	3.52 ± 1.99[a]
D2	13 ± 4[ab]	54 ± 19[ab]	7.49 ± 4[ab]	9 ± 2[a]	1.22 ± 1.13[a]	33 ± 14[ab]	4.12 ± 1.64[a]
D4	10 ± 2[ab]	34 ± 19[ab]	3.58 ± 2[ab]	15 ± 1[a]	1.57 ± 0.53[a]	44 ± 14[ab]	4.27 ± 1.17[a]
D6	9 ± 4[b]	31±6[b]	2.99±1[b]	14±2[a]	1.37±0.98[a]	54±9[b]	4.39±1.29[a]
D8	11 ± 4[ab]	37 ± 15[ab]	4.52 ± 3[ab]	13 ± 2[a]	1.47 ± 0.84[a]	49 ± 15[ab]	4.87 ± 1.13[a]
D10	12 ± 4[ab]	46 ± 11[ab]	5.88 ± 3[ab]	10 ± 2[a]	1.33 ± 0.61[a]	43 ± 13[ab]	4.55 ± 1.15[a]
D15	10 ± 4[ab]	35 ± 14[ab]	3.85 ± 3[ab]	15 ± 2[a]	1.39 ± 0.47[a]	49 ± 10[b]	4.94 ± 1.93[a]
D30	11 ± 2[ab]	25±13[b]	2.88 ± 2[ab]	14 ± 1[a]	1.62 ± 0.60[a]	56 ± 13[b]	6.37 ± 1.22[a]
D60	11 ± 3[ab]	38 ± 9[b]	4.39 ± 2[b]	15 ± 1[a]	1.64 ± 0.46[a]	43 ± 5[b]	5.06 ± 1.48[a]
D90	12 ± 4[ab]	37 ± 16[b]	4.74 ± 3[ab]	14 ± 1[a]	1.59 ± 0.35[a]	45 ± 13[b]	5.12 ± 1.35[a]
D120	10 ± 3[ab]	38 ± 14[ab]	4.10 ± 2[ab]	13 ± 2[a]	1.49 ± 1.19[a]	44 ± 9[ab]	4.3 ± 0.99[a]
D150	9 ± 4[ab]	33 ± 13[b]	2.96 ± 2[b]	11 ± 1[a]	1.12 ± 0.69[a]	50 ± 10[b]	4.51 ± 2.62[a]
D180	10 ± 4[ab]	31 ± 11[ab]	2.99 ± 2[b]	10 ± 1[a]	1,11 ± 0,69[a]	54 ± 11[b]	5.15 ± 2.18[a]

Lower case letters in the same column show difference between moments (P< 0.05) using ANOVA for repeated measure.
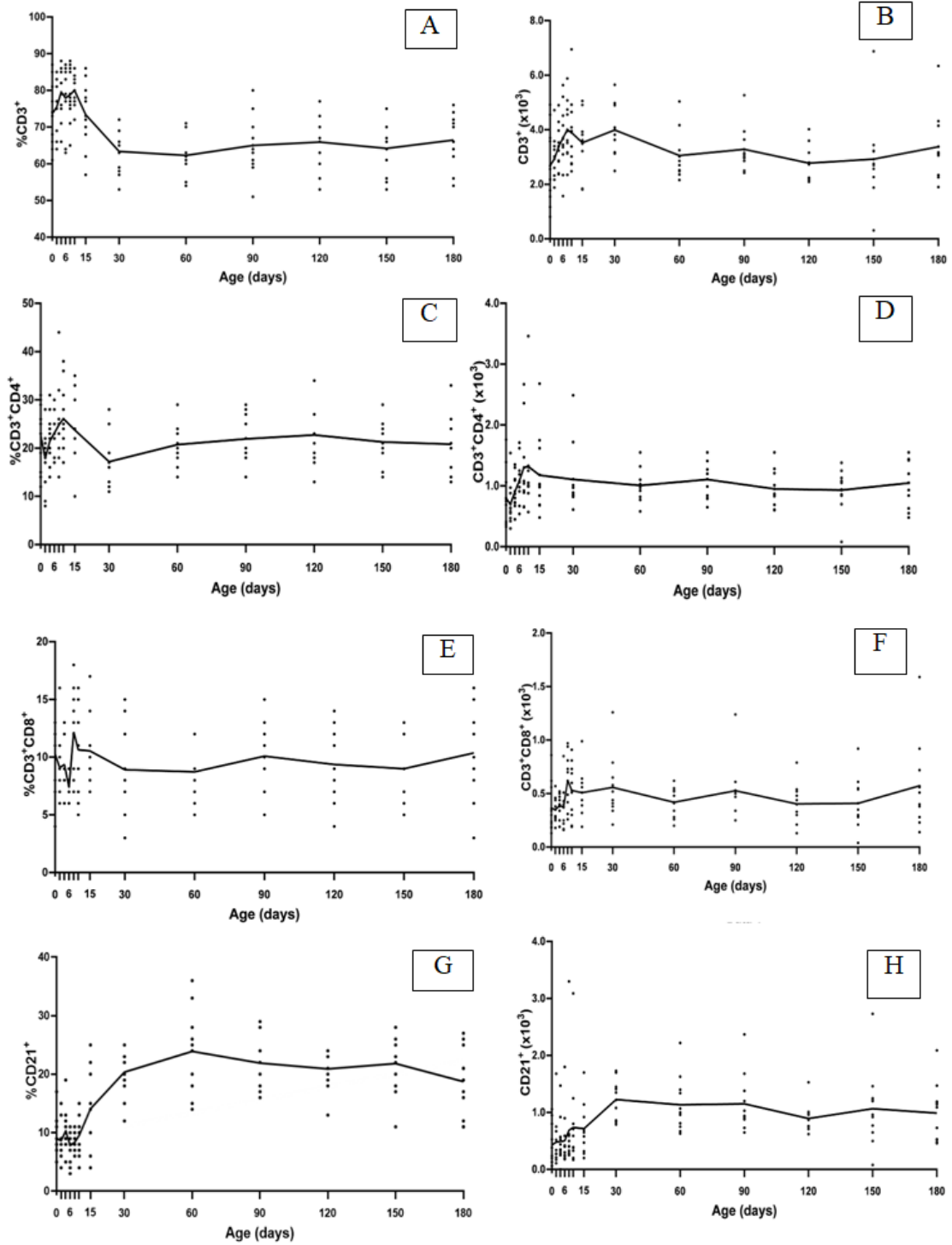
Figure 1. Mean and individual values of T and B lymphocytes population in blood of Holstein calves from birth up to six months of age

The mean relative values of CD3^{+} T lymphocytes did not change substantially in the first 10 days of life and then decreased (D15). The proportion of CD3^{+} (%) at D10 was higher than that observed on D30, D60, D90, D150 and D180 (P< 0.05). There were no statistically significant differences in the absolute values of CD3^{+} cells between time points.

The mean of absolute and relative values of the subsets CD4^{+} and CD8^{+} showed slight variations during the course of the study. There were no statistically significant differences between time points for these parameters.

The proportions (%) and absolute values of CD21^{+} lymphocytes increased significantly from D0 up to D30–D180. Differences in both parameters were detected between the early time points of D0, D2, D4, D8, D15 and the later time points of D30, D60, D90, D120 and D150.

By clinical parameters, all calves were healthy immediately after birth (D0). Navel inflammation and diarrhea were the most frequently observed disease conditions during the first fifteen days after birth. The frequencies of navel inflammation were 20% (2/10) from D2 up to D10 and increased to 50% by D15 (5/10,). Four cases of diarrhea were detected only on D15.

DISCUSSION

This study followed the hematological and immunological profiles of Holstein calves from birth up to 180 days of life.

Hemoconcentrationwas observed at birth, higher values of RBC, HGB and HCT in the first days of life are related of a physiological hemoconcentration due to fetal growth in a placental environment with low concentrations of O_2. The components of the erythrogram are consistent with established reference ranges, according to Novo *et al.*, (2015), Benesi*et al.* (2012a); Brun-Hansen *et al.,* (2006). The subsequent decrease values is associated with the higher oncotic pressure and plasma dilution after colostrum administration. Moreover, low serum iron levels in neonates can compromise erythropoiesis (Mohri*et al.*, 2004; Mohri*et al.*, 2007).

The MCV values of newborn calves were highest at birth (46 fL) and then decreased subsequently. Erythrocyte size also decreased with advancing age, which probably occurs through the replacement of RBCs containing fetal HGB by smaller RBCs containing adult HGB (Knowles *et al.*, 2000; Lee *et al.*, 1971). The decrease in hematimetric rates was concomitant with the increase of RBCs from the 10th day of life. These data are in agreement with variations reported in previous studies (Brun-Hansen *et al.*, 2006).

The leukocytosis by neutrophilia observed around birth might be caused by the increase of maternal and fetal cortisol at the end of pregnancy and during labor. No evidence of eosinopenia profile was reported (Fagliari*et al.,* 1998; Benesi*et al.,* 2012b), since eosinophils cell concentration of calves are in small numbers in the blood of both young calves and adult animals. Transient increase in circulating neutrophils occurs because of the release of bone marrow cells and the marginal compartment for blood circulation. Furthermore, lower expression of L-selectin adhesion molecules on the surface of these cells in the presence of high cortisol levels has been reported (Nagahata*et al.*, 2000). Despite the reported neutrophilia, it is worth noting that the phagocytosis and bactericidal activity are decreased in neonate calves (Hauser *et al.*, 1986).

Leukogram analysis and phenotyping of lymphocyte subsets allowed us to conclude that the total number of lymphocytes increased because of the increase in the proportion of CD21^{+} B cells after 30 days of life. The relative level of B lymphocytes detected in the first week after birth was low (9%), a value slightly higher than the mean value of 4% reported by Kampen*et al.* (2006).

The cellular profile in the calves during the first weeks of life can be influenced by variations in the levels of progesterone, prostaglandin, and cytokines IL-4 and IL-10 transferred from dams to newborn calves through pregnancy (Chase *et al.*, 2008). Despite the Th2 cytokine profile at birth, the increase in the number of B lymphocytes presenting CD21^{+} receptor, which is expressed only in activated B cells, was observed only on the 30th day of life.

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The late activation of B cells in neonates may be related to the low number of dendritic cells and low expression of major histocompatibility complex (MHC) class II by monocytes during the neonatal period (Reber*et al.*, 2008a; Reber*et al.*, 2008b). Once a pathogen is recognized, it is internalized and processed by antigen-presenting cells (APCs). The peptides generated by APCs are expressed on the membranes of MHC II cells, which present the peptide to helper T lymphocytes (CD4$^+$). The binding between MHC II and T lymphocyte receptor triggers events that result in the clonal expansion of T helper cells and enhanced cytokine production, which stimulate the proliferation of B lymphocytes and their differentiation to memory cells and plasma cells, which in turn produce antigen-specific immunoglobulins (McHeyzer-Williams, 2003).

Activated B cells could also act as APCs (McHeyzer-Williams, 2003). However, the reduced density of the CD21$^+$ population in the first month of life can negatively impact B cell self-stimulation.

B cells migrate from the bone marrow to the secondary lymphoid tissues, where they go through the maturation process. Initially, naïve B cells are located in the primary lymphoid follicles, where they bind antigens and become activated. Then, the cells migrate to the germinal center portion for the selection and clonal expansion of antigen-specific B cells, where B lymphocytes are transformed to antibody-secreting plasma cells and memory B cells. The germinal center grows in size as the immune response is triggered by pathogens. It regresses and finally disappears when the infection is resolved. Germinal centers are present in the lymph nodes for about 3–4 weeks after initial antigen exposure, and subsequently leave via the efferent lymphatic vessels (Rudinand Thompson, 1998). The increase in activated B lymphocytes observed in this study could have been triggered by antigenic exposure. This phenomenon indicates that the maturation of specific immune system progresses in small steps from birth until puberty. Kampen*et al.* (2006) and Chattha*et al.* (2009) also reported a similar increase in lymphocytes after 30 days.

The low number of B lymphocytes may be responsible for colonization and infection by extracellular microorganisms that cause diarrhea and navel inflammation. These diseases were the main cause of data exclusion in this experiment on D8, D10 e D15.

The proportion of CD3$^+$ T lymphocytes decreased from the 10th day of life. Variations in the proportions of CD3$^+$ T lymphocytes could not be compared with those observed in the other studies owing to the scarcity of longitudinal studies evaluating the expression of this receptor from birth up to 6 months of age.

The proportions of CD4$^+$ and CD8$^+$ T lymphocytes were stable throughout the study period, with values of 23% and 10% at birth, and 21% and 10%, respectively, at 6 months of age. A similar proportion has been reported in earlier studies (Allen *et al.*, 1996; Ayoub and Yang, 1996; Kampen*et al.*, 2006; Kulberg*et al.*, 2004; Wilson *et al.*, 1996).

Hematologic and immune components of calves, as determined by hemoconcentration, stress leukogram, and the predominance of CD3$^+$ T cells,varied with age, especially in the first month of life, when compared to CD21$^+$ Bcells until 30 days of age. Subsequently, the lymphocyte populations increased due to the increase in CD21$^+$ B cells.

Variation in the cell populations indicates that the neonatal period is a phase of increased susceptibility to infectious diseases. Therefore, extra care is neededto maintain the health and survival of the calves during this phase (Murray and Leslie, 2013).

CONCLUSION

This research highlights the neonatal period as a critical stage in calf rearing. The study results also indicate that hematological profiles and subsets of lymphocytes of calves were functional and able to generate immune response in the calves after 30 days of life.

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