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Effect of maternal cells transferred with colostrum on the health of neonate calves

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ABSTRACT

The objective of this research was to evaluate the influence of cells from colostrum on the health of neonate calves. Animals were distributed in 2 groups: COL+ ($n = 9$) which received fresh colostrum from their own damns; and COL− ($n = 10$) which received frozen colostrums from donors. Heifers were assessed before colostrum intake – D0; D2; D7; D14; D21 and D28. Heifers were monitored by clinical examination, hematological profile and serum iron. COL− had a higher diarrhea intensity score (typically 3) on D7. Moreover, a single case each of bronchopneumonia and navel inflammation were observed in COL− calves. COL− had fewer red blood cells (RBC) ($6.5 \pm 0.8 \times 10^6$/μL) and less hemoglobin (Hgb) (8.3 ± 1.4 g/dL) than COL+ (RBC = $7.2 \pm 0.8 \times 10^6$/μL; Hgb = 9.6 ± 1.3 g/dL) at D14 ($P \leq 0.05$). COL− had more anemia on D21 ($P = 0.03$) and on D28 ($P = 0.02$). Iron was lower in COL− (5.6 ± 2.7 μM/L) than COL+ (10.7 ± 6.2 μM/L) ($P = 0.03$) on D7. Lymphocytes was lower in COL− than COL+ on D7 ($3.8 \pm 1.0 \times 10^3$/μL COL+ and $5.4 \pm 2.2 \times 10^3$/μL COL−, $P = 0.02$). COL− calves had more anemia and lower serum iron concomitant with diarrhea on D7. The number of leukocytes was relatively consistent in the COL+ calves, while COL− calves showed an increasing number of of lymphocytes starting on D7.

1. Introduction

The period immediately following birth is a critical window of adaptation for the neonate. The neonate must develop functional independence from the protected maternal internal environment after birth. During this adaption process, the initial näive immune system of the calf is enhanced and activated progressively as a result of exposure to microorganisms in the maternal environment. Protection of the neonate during the preweaning period is initially dependent on the transfer of maternal immune factors from colostrum, but undergoes progressive maturation to a fully functional state (Chase et al., 2008).

Colostrum contains high concentrations of nutrients, immune components and hormones to support growth and maturation of the physiological development of the calf. Colostrum contains immunologically important cytokines, antibodies and a large number of maternal leukocytes for the immune priming and early protection of the neonate. The role of colostral antibodies in neonatal protection has been well established. However, the role of the maternal leukocytes and cytokines in colostrum have yet to be fully established.

Bovine colostrum contains about 1×10^6 cells/mL, of which 30% are viability (Godden et al., 2012). Macrophages represent the predominant leukocytes ($69 \pm 15\%$) in colostrum. Colostrum also contains T cells (16%) and B cells (11%) (Park et al., 1992).

Watson (1980) suggested that colostrum-derived leukocytes could be transferred to the neonatal calf. The passage of cells through the gastrointestinal epithelium has been reported in several species (including cattle) (Sheldrake and Husband, 1985; Tuboly et al., 1988; Williams, 1993; Reber et al., 2006). Fluorescent maternal leukocytes were detected in the blood, Peyer's patch and lymph nodes in newborn calves (Liebler-Tenorio et al., 2002; Reber et al., 2006; Aldridge et al., 1998).

These studies indicated that the transfer of cells from colostrum (COL+) to the tissues of the neonate enhanced the innate and specific immune response in neonate calves. Calves fed colostrum with viable cells had higher number of neutrophils and greater bactericidal activity than calves feed cell-free colostrum. The phenotypic profile of the calves that received cell free colostrum suggested a bias toward pro-inflammatory responses (Reber et al., 2008a, 2008b). Evidence of specific immune effects of cells from colostrum can be found in an increased of number of blood lymphocytes, enhanced proliferative response to antigen, superantigen and mitogens, and enhanced antibody production after

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stimulation with antigen in calves receiving viable maternal leukocytes (Riedel-Caspari and Schmidt, 1991a; Riedel-Caspari and Schmidt, 1991b; Donovan et al., 2007). Moreover, circulating blood mononuclear cells from these animals expressed a greater percentage of activation markers on their surface (Reber et al., 2008a, 2008b, Reber et al., 2006; Langel et al., 2015). On the other hand, feeding calves fresh colostrum suppressed B cells differentiation in the mesenteric lymph nodes relative to colostrum deprived calves (Aldridge et al., 1998).

Colostrum management represents a risk factor for disease during the first three months of life, specially diarrhea (21%) and respiratory disease (22%) (Windeyer et al., 2014). Total serum total protein and total immunoglobulin levels have been used to evaluate the efficacy of passive immune transfer However, studies characterizing the importance on health of transfer of viable maternal cells with colostrum to neonate are scarce. Langel et al. (2015) did not find a difference in the diarrhea associated fecal score between birth and 45 days of age in dairy calves fed cell-free colostrum (CFC) or whole colostrum. However, calves that did not receive maternal cells had more evidence of respiratory disease than calves that received viable maternal cells on day 38 of their study.

The hypothesis of this study is that the transfer of viable maternal leukocytes with colostrum, and the activity of their subsequent products, enhanced the neonatal protective environment and the impact of maternal antibodies in protecting the calf. Further, maternal antibody and transferred maternal immune cells and cytokine synergize in the development of the innate and adaptive immunity in the preweaning calf. This synergy provides a mechanism to reduce the incidence and severity of disease during the preweaning period. Thus, the objectives of this research were to evaluate the influence of viable maternal cells delivered with colostrum on the health and hematological development of calves specifically during the first 28 days of life.

2. Materials and methods

2.1. Farm and animals

This research was approved by University of Sao Paulo Animal Care and Use Committee number n°2934/2013. The experiment was conducted on a commercial farm localized at Sao Paulo- Brazil between July and October of 2014.

Holstein cows were moved from dry-cow pasture to the maternity barn 30 days before the expected delivery date of each cow. Natural suckling was prevented by monitoring the birth of each calf by veterinarians from research team. Healthy calves from eutocic deliveries were selected for the study following a clinical examination. The clinical examination was performed using a standard protocol.

Holstein heifer calves were distributed in two groups: COL+ ($n = 9$) which received whole colostrum containing viable immune cells from their own damns, and COL− ($n = 10$) which received frozen colostrum containing no viable maternal cells from donor cows.

Dams and donor were milked immediately after delivery using a portable milking machine at the maternity unit. Colostrum was collected after cleaning teats with soap and water, dipping each teat in a 1% iodine solution and drying with a paper towel. Each calf received colostrum of similar quality. Colostrum quality was defined by the immunoglobulin concentration using a colostometer (70–120 g/L) and assessment with a Brix refratometer (23–32°). The median somatic cell count of fresh colostrum was obtained using a direct microscopic count, and had a mean of 1.9×10^6/mL (Gomes et al., 2011).

Donor colostrum was stored in two plastic 2 L bottles for each COL− calf, and frozen at −20 °C (24 h to 3 months) before thawing for use. The first bottle was slowly warmed to 37 °C in a water bath, and fed to each COL− calf within 3 h of birth. A second 2 L feeding was given about 6 h later. After thawing, an aliquot of 20 mL was diluted 1:1 with Phosphate Buffer Saline (PBS) and centrifuged at 800 × g, 4 °C by 15 min. The fat and whey were removed after centrifugation. The cells were washed in 20 mL of PBS, and the viability was assessed using Trypan blue stain. No viable cells were observed in any frozen colostrum used in this research.

Each COL+ calf was fed no later than 6 h after birth using a bottle containing two litters of fresh colostrum from its own damns. A second 2 L of whole colostrum was held at 4 °C until it was warmed to 37 °C for a second feeding 6 h later.

COL− and COL+ calves were moved to individual pens. They were maintained in these for the 28 days of the study. Routine farm management was applied. After colostrum intake, calves received 6 l of pasteurized milk from the dairy herd per day that was divided between two feedings, plus starter feed (Rumileite 20®, Guabi) and water *ad libitum*.

2.2. Clinical evaluation protocol

Calves were given a general clinical examination that included: vital signs, hydration status, ocular mucous, capillary refill and palpation of lymph nodes (Dirksen et al., 2008). Furthermore, fecal and bronchopneumonia scores were assessed in accordance with the Calf Heath Scoring Criteria previously published by The University of Wisconsin (Madison) by McGuirk (2008).

Fecal scores were assigned as 0 - normal consistence, 1 - pasty, semiformed; 2 - pasty with largest amount of water; or 3 - liquid with fecal content adhered in the perineum and tail. Calves were assessed as having diarrhea when the scores were 2 or 3. Bronchopneumonia was scored using a combination of the following parameters: rectal temperature, cough, nasal and ocular secretion and ear position with a score of 0–3 for each based on severity of each. Calves were assessed as having bronchopneumonia when the sum of these scores was >5.0. Umbilical region was evaluated by inspection and palpation to detect inflammation.

2.3. Blood samples

Blood samples were collected in 9 mL vacutainer tubes (BD, San Jose, CA, USA) containing either *Ethylenediaminetetraacetic acid* (EDTA, 5.4 mg per tube) or without anticoagulant by external jugular puncture. Calves were assessed before colostrum intake (D0); 24–48 h (D2); 7 days (D7); 14 days (D14); 21 days (D21) and 28 days after bith (D28).

2.4. Hematology

The absolute red blood cell (RBC) number, hemoglobin (Hbg), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and absolute total leukocyte count (WBC) were obtained using an automatic counter (ABC Vet, ABX®). Leukocyte differential (lymphocyte, monocytes, basophils, eosinophils and granulocyte-neutrophils per 100 WBC) was performed by manual method according to cells morphology employing an optical microscope with 1000× magnification.

2.5. Iron concentration

The serum iron concentration was determined using a commercial kit (Ferro UIBC (CTLF), SI250, Randox®) in according to manufacturer instructions.

2.6. Statistical analysis

Statistical analyses were realized by SPSS 19.0 (IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.).

Data was tested for normality distribution by Shapiro-Wilk. Parametric data were expressed as mean and standard deviation. Tests that did not generate a parametric distribution were expressed as

median, minimum and maximum values. Differences between groups were evaluated by application of unpaired *t*-test (for continuous variables) and Mann-Whitney (for non-normal or discontinuous measures).

Parametric data were analyzed across time using a repeated measure ANOVA, and significant differences between time points in the series were assessed using the Bonferroni post-hoc. Friedman tested in association with Wilcoxon test and the Bonferroni correction ($P \leq 0.003$) were used to analyze non-parametric data.

Not all calves had clinical signs of disease. Therefore, the frequencies of positive and negative signs for each parameter were evaluated using the Chi-Square test.

Differences were considered significant when $P \leq 0.05$ (*), or to represent a tendency if $P \leq 0.10$ (†).

3. Results

3.1. Clinical evaluation

The results for vital sign measurements are shown in Fig. 1. Mean values of the vital signs demonstrated few differences between the COL+ calves and COL− calves. The mean HR in the COL− calves (179.0 ± 28.9 bpm) group was higher than COL+ calves (144.3 ± 21.1 bpm) on D0 ($P = 0.01$). COL− calves (61.8 ± 10.9mpm) had higher respiratory rate than COL+ calves (54.4 ± 11.8mpm) on D14 ($P = 0.10$). We observed a statistical tendency for a difference in mean T between the two groups on D14, with COL− calves having higher mean T ($P = 0.10$).

Heart rate, respiratory rate and temperature were evaluated over the first month of life. We observed a difference in HR between D2 (145.3 ± 17.2 bpm) and D14 (120.4 ± 14.1 bpm) for the COL+ calves ($P = 0.01$), and D0 (179.0 ± 28.9 bpm) and D21 (113.8 ± 22.0 bpm) in the COL− calves ($P = 0.00$). Differences were also observed in respiratory rate between D2 compared with D21 and D28 in both groups of calves ($P \leq 0.05$). Moreover, ANOVA analysis revealed differences between D0 and D2 ($P = 0.004$) in COL+ calves, and D14 compared with D21 ($P = 0.02$) and D28 ($P = 0.04$) in COL− calves. The range of body temperature was 38.6 ± 0.4 °C and 39.1 ± 0.5 °C for the COL+ calves, and 38.6 ± 0.5 °C and 39.2 ± 0.4 °C for COL− calves over the course of the study. T means for each group did not change during the period from birth to 28 days of age.

The incidence of dehydration was similar in each group. We observed mild dehydration of 5.0 to 7.0% (based on clinical exam) on D21 (for COL+ calves) and D28 (for COL− calves).

We observed ocular red mucous in half of calves in both groups on D0. Pale mucous was observed in calves from both groups from D2 to D28. However, the frequency of pale mucous in COL− calves (60.0%) showing signs of anemia was higher than in COL+ calves (11.1%) on D21 ($P = 0.05$).

Some calves in each group presented with larger subiliac, mandibular or superficial cervical lymph nodes on initial physical exam. However there was no difference in the frequency of enlarged lymph node presentation between the two groups ($P = 0.17$).

Diarrhea was the most common disease problem observed over the entire course this study beginning on D2 (Fig. 2). Other disease problems observed during the study were navel inflammation (by palpation) and respiratory disease (scored as described in the methods). The frequency of diarrhea in both groups was high on D7 (COL+ calves 55.5%; COL− calves 70.0%), and D14 (COL+ calves 77.8%; COL− calves 60.0%). Later observations yielded a decrease in diarrhea frequency on D21 (COL+ calves 55.5%; COL− calves 50.0%), and D28 (COL+ calves 44.4%; COL− calves 50.0%). The frequency of diarrhea diagnosis (fecal score 2 and 3) was similar between groups for all points of measurement ($P \geq 0.05$).

Bronchopneumonia was defined as a total respiratory score was higher than 5.0. Respiratory disease was detected in only one COL− calf in the study on D21. In addition, three COL− calves had umbilical inflammation between D7 and D28.

3.2. Hematology values

The results of the RBC analysis are shown in Fig. 3. The absolute RBC number was similar for both sets of calves from birth (COL+ calves 7.6 ± 1.0 × 10^6/μL; COL− calves 7.6 ± 0.7 × 10^6/μL) on D0 and D7 (COL+ calves 7.1 ± 0.8 × 10^6/μL; COL− calves 7.0 ± 1.0 × 10^6/μL). COL− calves had lower absolute numbers of RBC on D14 ($P = 0.05$), D21 ($P = 0.09$) and D28 ($P = 0.10$) than COL+ calves. The absolute number of RBC was higher immediately after birth (D0) than on D2 ($P = 0.02$) in COL+ calves. RBC also was higher on D0 than on D2 ($P = 0.00$), D14 ($P = 0.02$) or D21 ($P = 0.00$) in COL− calves.

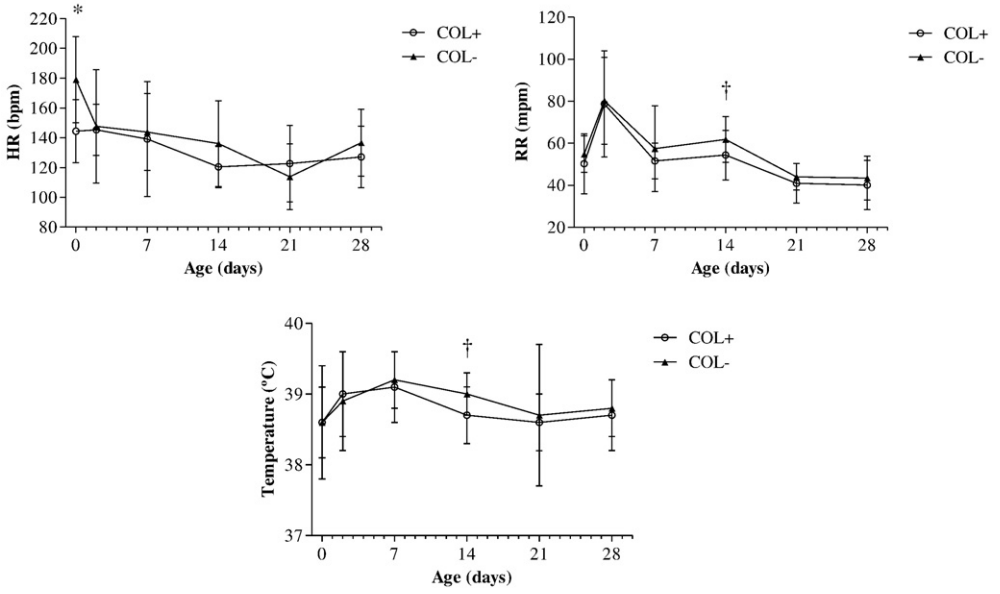

Fig. 1. Mean vital signs of the calves fed whole colostrum with viable maternal cells (COL + $n = 9$) or viable cell-free colostrum (COL − $n = 10$) at birth over the course of the trial. *Differences between groups detected by *t*-test was considered if $P \leq 0.05$ or (*) tendency to $P \leq 0.10$ (†).

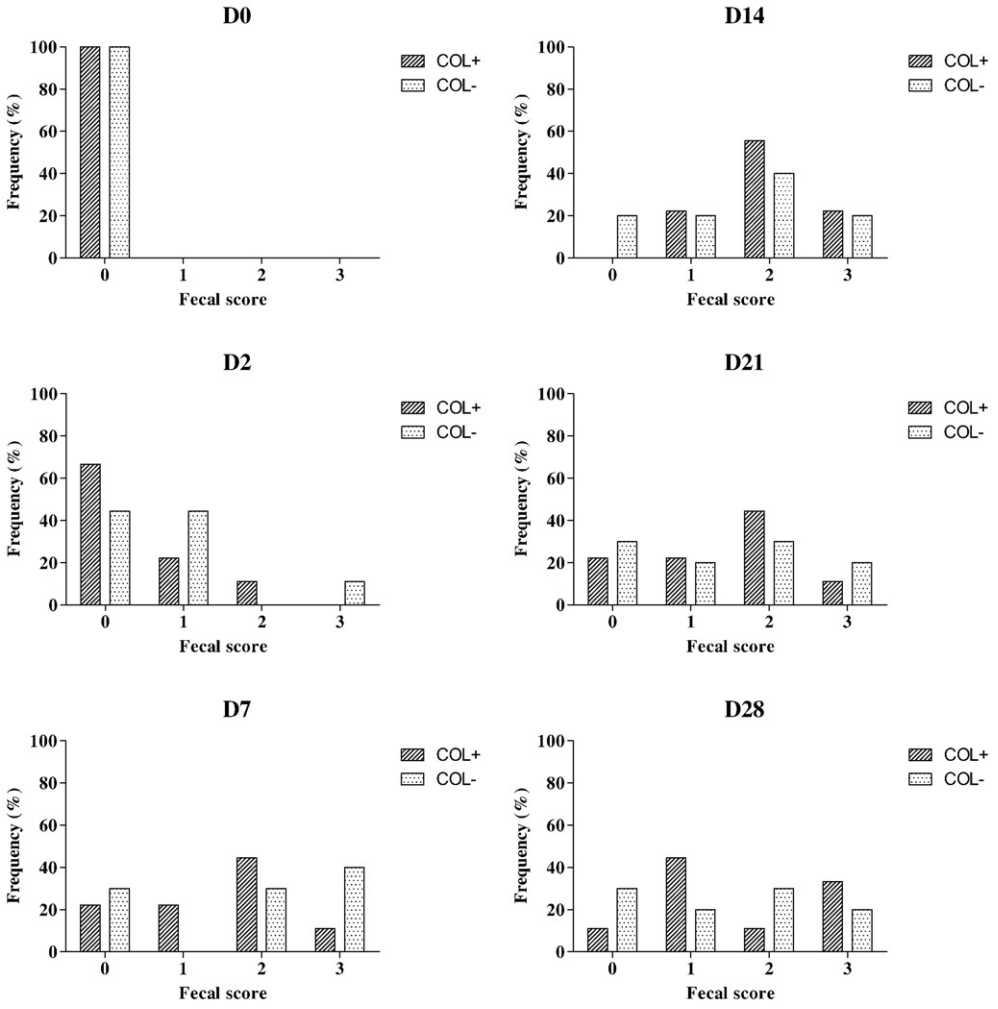

Fig. 2. Comparative fecal (diarrhea) scores of calves fed whole colostrum with viable maternal cells (COL+) or viable cell-free colostrum (COL−) over the course of this study. Legend: Fecal score 0- normal consistence, 1 - pasty, semi-formed; 2 - pasty with largest amount of water; or 3 - liquid with fecal content adhered in the perineum and tail.

Hemoglobin (Hgb) concentration peaked on D0 (COL+ calves 11.3 ± 1.7 g/dL; COL – calves = 11.1 ± 1.6 g/dL), after the values had gradual decrease until D28 (COL+ = 8.8 ± 1.6 g/dL; COL− = 7.2 ± 1.3 g/dL). Differential assessment of the two treatment groups for Hgb yielded a significant difference on D14 (COL+ calves 9.6 ± 1.3 g/dL; COL− calves 8.3 ± 1.4 g/dL, $P = 0.04$) and a tendency toward a difference on D21 (COL+ calves 8.8 ± 1.0 g/dL; COL− calves 7.7 ± 1.3 g/dL, $P = 0.10$) and D28 (COL + calves 8.8 ± 1.6 g/dL; COL− calves 7.2 ± 1.3 g/dL, $P = 0.07$).

ANOVA analysis of Hgb indicated a difference between D0 compared with D21 ($P = 0.00$) and D28 ($P = 0.00$) in COL + calves. For COL− calves, a difference was observed between D0 and D2 ($P = 0.00$), D0 and D14 ($P = 0.00$), D0 and D21 ($P = 0.00$), and D0 and D28 ($P = 0.00$). Moreover, the values observed on D2 and D7 also were also higher than on D21 ($P = 0.00$; 0.01), and D28 ($P = 0.00$; 0.01) in COL− calves.

HCT peaked on D0 (COL + = calves 36.9 ± 5.0%; COL− calves 36.1 ± 5.4%). This value declined steadily throughout the study (COL + calves 26.1 ± 5.1%; COL− calves 20.6 ± 5.4%). The mean HCT was higher in the COL + calves than COL− calves on D14 ($P = 0.06$) and D28 ($P = 0.07$). ANOVA analysis indicated higher values for HCT on D0 than all days D2 to D28 ($P \leq 0.05$) in both treatment groups groups. HCT was higher on D7 than D28 ($P = 0.04$) in COL+ calves, and when comparing D2 and D7 with D21 and D28 ($P \leq 0.05$) in COL− calves.

There were no differences between groups in the pattern of MCV, MCH and MCHC. However, ANOVA analysis indicated several difference between sampling times. MCV decreased over the study period. Significant reduction was observed relative to D0 on D2 to D28 at every sampling ($P \leq 0.05$), between D2 and D7 to D28 at every sampling ($P \leq 0.05$) and between D7 and D21 and D28 ($P \leq 0.05$) in both treatment groups. COL+ calves also showed a significant difference between D7 and D14 ($P = 0.03$).

MCH also decreased over the course of the trial. Using ANOVA analysis, we found significant differences comparing D0, D2, D7 and D14 with D21 and D28 ($P \leq 0.05$) for COL+ calves, and between D0 compared with D14 and D21 ($P \leq 0.05$), and D2 and D7 with D21 ($P \leq 0.05$) in COL− calves. MCHC increased over the course of the study. Using ANOVA analysis we observed significant differences when D0 was compared with D2, D7, D14 and D21 ($P \leq 0.05$) in each treatment group. MCHC was lower on D0 than D28, D2 than D7, and D2 than D28 in COL+ calves ($P \leq 0.05$).

The interpretation of individual RBC parameters from individual calves indicated anemia in calves over the period from D2 to D28 based on reference values established by Brun-Hansen et al. (2006). The frequency of anemia observed among the calves was 22.2% and 30.0% on D2, 11.1% and 10.0% on D7, 22.2% and 50.0% on D14, 0.0% and 40.0% on D21; and 0.0% and 50.0% on D28 for COL+ calves and COL− calves, respectively. COL− calves had a significantly higher frequency of anemia on D21 ($P = 0.03$) and on D28 ($P = 0.02$) than

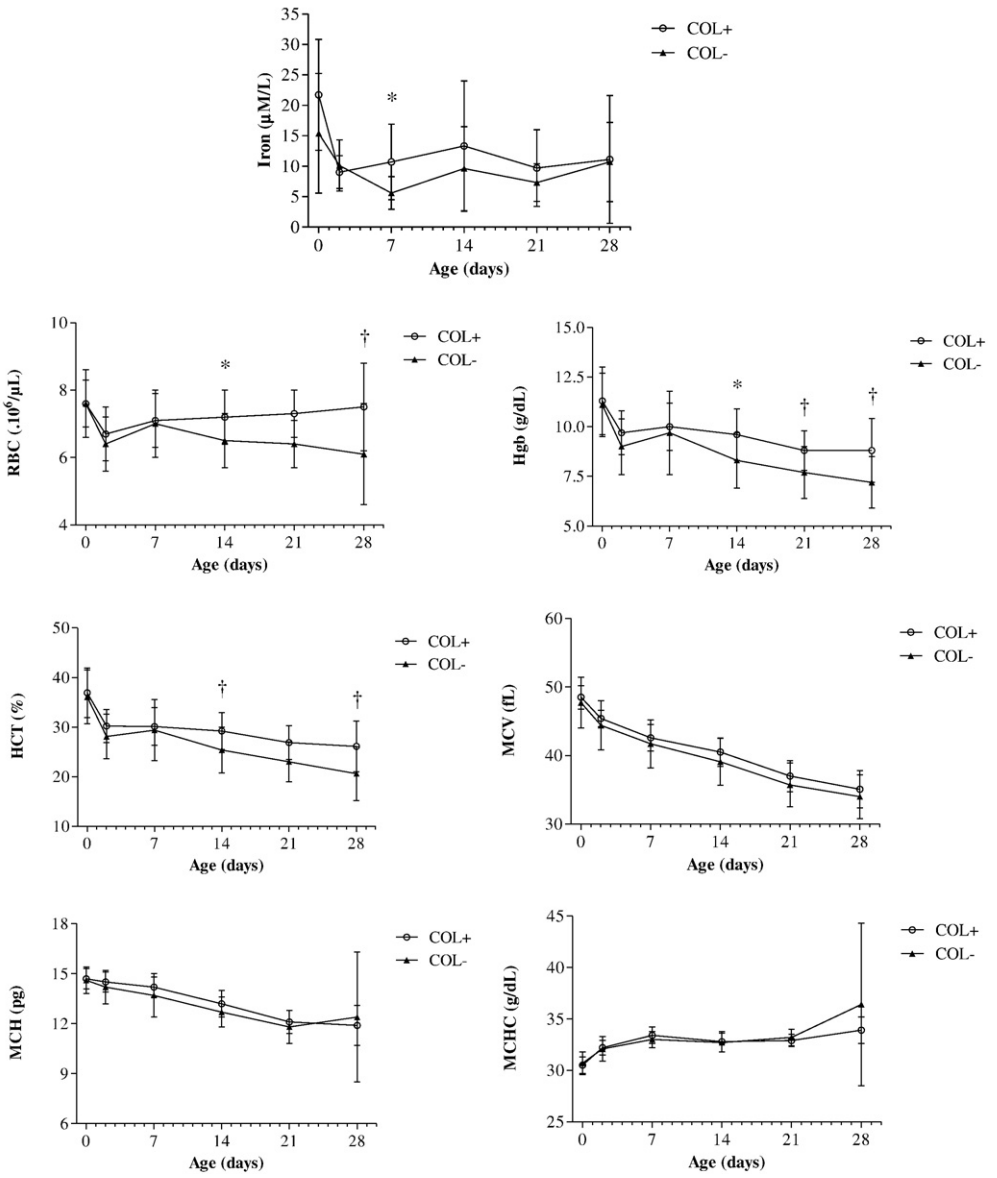

Fig. 3. Mean of the hematological parameters measured for calves fed whole colostrum with viable maternal cells (COL+) or viable cell-free colostrum (COL−) over the course of the study. Differences between COL+ and COL− groups for each parameter was considered if $P < 0.05$ (*) or tendency if $P \leq 0.10$ (†).

COL+ calves. Normocytic hypochromic anemia was the predominant finding based on the red cells morphology.

COL + (21.7 ± 9.1 μM/L) and COL− (15.4 ± 9.8 μM/L) calves had comparable iron concentration at birth. However, the iron concentration decreased in each groups on D2 (COL + = calves 9.0 ± 2.7; COL− calves 10.1 ± 4.2 μM/L). Later in the trial, iron concentration was stable for COL + calves (9.7 ± 6.3–13.3 ± 10.7 μM/L). In contrast COL− calves showed two sharp decreases in serum iron concentration on D7 (5.6 ± 2.7 μM/L) and the second on D21 (7.3 ± 3.1 μM/L). There was a statistically significant difference in iron concentration between the treatment groups on D7 ($P = 0.03$).

The number of WBC and distribution of lymphocytes and neutrophils is shown in Fig. 4. WBC varied only slightly over the course of the study, from D0 up to D28 in COL+ calves (7.7 ± 3.6–12.1 ± 5.5 × 10^3/μL), and in COL− calves (6.9 ± 1.7–11.8 ± 5.8 × 10^3/μL) ($P \geq 0.05$). WBC mean was higher on D0 than D28 ($P = 0.03$) in COL− calves.

In the COL + calves, the absolute number of neutrophils was stable measured on D0, D2 and D7. The number declined at later samplings. In contrast, COL− calves demonstrated a consistent decrease in the number and percentage of neutrophils over the course of the study. COL+ calves had a tendency toward a higher percent of circulating neutrophils on D7 (COL+ calves 62.2 ± 14.5%; COL− calves 49.6 ± 12.6%, $P = 0.09$). ANOVA analysis indicated a higher percent of neutrophils in circulation on D0 compared to D14 ($P = 0.02$) and D0, D2 and D7 compared to D21 and D28 ($P \leq 0.05$) in COL+ calves; and between D0 and D2 compared to D21 and D28 ($P \leq 0.05$) for COL− calves. The Absolute number of neutrophils (as × 10^3/μL) was higher on D0 than D21 and D28 in COL+ calves, and D0 than D28 in COL− calves ($P \leq 0.05$).

The percent of circulating lymphocytes tended to be higher in COL− calves than COL+ calves on D7 (COL+ = 36.5 ± 14.5%; COL− = 49.7 ± 12.5%; $P = 0.09$). ANOVA analysis indicated significant differences between D0 compared with D7, and D21 compared with D28, and D2 compared with D21 and D28 for both treatment groups ($P \leq 0.05$). Further, COL+ calves demonstrated a significant difference when D7 was compared with D14, D21 and D28 ($P \leq 0.05$).

The absolute values of lymphocytes showed a gradual increase in each groups over the course of the study that was steady and progressive. The absolute value for lymphocytes was higher in COL− calves than COL+ calves on D0 (COL+ calves 2.7 ± 1.0 × 10^3/μL; COL− calves

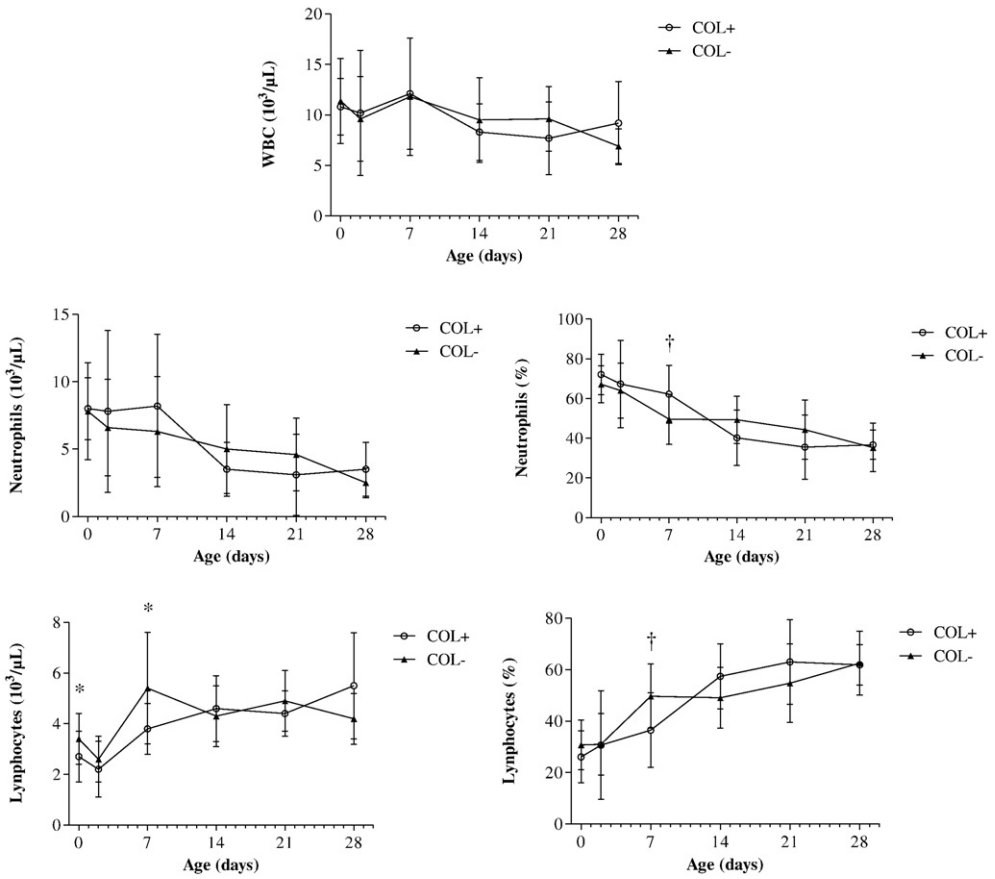

Fig. 4. Mean leukocyte assessments for calves fed whole colostrum with viable maternal cells (COL+) or viable cell-free colostrum (COL−) over the course of the study. Differences between COL+ and COL− groups for each parameter was considered if $P < 0.05$ (*) or tendency if $P \leq 0.10$ (†).

$3.4 \pm 1.0 \times 10^3$/μL, $P = 0.01$) and D7 (COL+ calves $3.8 \pm 1.0 \times 10^3$/μL; COL− calves $5.4 \pm 2.2 \times 10^3$/μL, $P = 0.02$). ANOVA analysis indicated lower mean absolute values of lymphocytes on D0 than D14 and D21 ($P \leq 0.05$) in COL+ calves, and D0 and D2 compared with D21, and D2 compared with D28 ($P \leq 0.05$) in COL− calves.

Monocytes, eosinophils and basophils were not normally distributed in this study. The minimum and maximum values observed in this study were 0.00 to 0.86×10^3 monocytes/mL, 0.00 to 0.47×10^3 basophils/mL and 0.00 to 0.26×10^3 eosinophils/mL. This is reported for all calves in the study and no significant differences between treatment groups were observed at any sampling point in the study.

4. Discussion

This research evaluated the influence of viable maternal cells transferred in colostrum on the health of calves during the first 28 days of life.

The colostrum utilized in this study (fresh or frozen) was selected based on both the immunoglobulin concentration (≥50.0 g/L) and the Brix index (21.0°) using a standard reported by Quigley and coworkers (Quigley et al., 2013). The median of somatic cell count in the colostrum of the damns and donor was 1.9×10^6 cell/mL. The heifers fed whole colostrum received about 8.0×10^9 total maternal cells in four litters of colostrum within six hours after birth. Thirty percent (2.3×10^6/mL) of these cells were probably viable (as reported by Godden et al., 2012).

In a previous study, we demonstrated that no viable maternal cells survived the freezing and thawing of pooled colostrum at −20.0 °C for a minimum period of 24 h. On the other hand, the refrigeration of colostrum at 4.0 °C for six hours did not reduce the cellular viability >30.0% (to about 20% viable) relative to freshly collected colostrum (Novo et al., 2014). Langel et al. (2015) reported using a model with similar treatments. However, they used liquid nitrogen to freeze colostrum quickly, and then fed calves with frozen colostrum from their own dams. In our study, COL− calves received frozen colostrum from a pool of donor cows collected and frozen from the same farm prior to the start of the calf study.

Clinical evaluation of calves at birth that continued through 28 days of age was carried out using a clinical examination as defined in the methods section of this paper. This included the assessment of fecal scores and respiratory scores, and was combined with an assessment of RBC and WBC parameters after what has been previously published (Dirksen et al., 2008; Mcguirk, 2008; Poulsen and McGuirk, 2009).

Calves from the COL+ and COL− treatment groups had differences in the parameters of general physical exam on D0 and D7. COL− calves had higher heart rate than COL+ calves on D0. This difference might be explained by the presence of two heifers in COL− group with tachycardia immediately after birth based on reference values (100.0–150.0 bpm) as previously reported by Mee (2008). Subsequent HR measurement of these calves normalized during the rest of the study. Therefore, the difference in HR in the COL− calves and COL+ calves was not an effect of failure to receive viable maternal cells.

The higher respiratory rate and body temperature on D14 of the COL− calves than COL+ calves and the finding of pale mucous on D21 in the same calves indicates a less robust physiological development of the heifers fed with frozen colostrum free of viable maternal cells.

Diarrhea was disease condition most frequently observed during this study. The frequency of diarrhea observed in this study was higher for both treatment groups in this study (COL+ calves at 44.4–77.8%) and COL− calves at 50.0–70.0%) than the prevalence (19.1%) and incidence (21.2%) reported by previously (Bartels et al., 2010; Windeyer et al., 2014). The farm where this research was conducted has given vaccines during the dry period with *Escherichia coli,* Rotavirus and

Coronavirus antigens as the primary targets. If the vaccines had good efficacy, then the expectation would be that a reduced occurrence of diarrhea should be observed. In other studies, Meganck et al. (2015) demonstrated that similar vaccination of dams reduced the frequency of diarrhea from 39.9% to 14.3% in neonate calves. This was not observed in our study, suggesting that the vaccinations were not of the same value under our farm management program.

The frequency of diarrhea was similar between groups, although COL− calves had high intensity of this disease (average fecal score of 3) on D7 than COL+ calves. Riedel-Caspari (1993) reported similar data to our study after experimental infection with enteropathogenic *Escherichia coli* in calves fed with cell-depleted colostrum (COL−) relative to colostrum containing live maternal cells (COL+). These calves from both treatment groups developed mild diarrhea on D7, but the authors reported that COL+ calves were less affected than those fed with pooled colostrum without viable maternal cells. COL− calves had a slight higher rectal temperature during most of the investigation, especially between D9 and D16. This finding may be explained by a greater *Escherichia coli* burden based on the differential recovery of fecal *E. coli* colony-forming units from COL− calves. The reduction in *E. coli* from the COL+ calves may have resulted from direct antimicrobial effects of viable leukocytes from colostrum, or from the effect of cytokine or other factors contained in maternal colostrum associated with transferred cells (live or dead) that stimulated the calf's endogenous resistance to infection.

In this research, only one COL− calf was diagnosed with bronchopneumonia on D28. Langel et al. (2015) evaluated the health of calves that received viable cell free-cell and viable maternal cell containing whole colostrum using fecal and respiratory scores. They did not detected differences in the incidence of diarrhea or bronchopneumonia between the treatment groups during the first month of life.

Navel inflammation was observed in three COL− calves (30.0%) but none in the COL+ calves. The diagnosis of diarrhea, bronchopneumonia or naval inflammatory problems could be associated with poorer physiological and innate immune development in the calves fed with viable cell free frozen colostrum.

The values of RBC, Hgb and HCT were higher for COL+ calves than COL− calves from D14 until the end of the trial. Normocytic hypochromic anemia was the predominant finding given an assessment of red cell morphology. Low serum iron in the COL− calves (a significant finding relative to COL+ calves) may have compromised erythropoiesis (Mohri et al., 2007, Mohri et al., 2004).

There are some unusual findings in RBC related parameters in the blood of all neonate calves. We observed higher than typical numbers of RBC in circulation at birth. This was likely due to a low concentration of placental oxygen. The subsequent decrease in the number of circulating RBC, Hgb and HCT values might be associated with the higher oncotic pressure and plasma dilution of oxygen after colostrum delivery. Moreover, neonate calves generally have low serum iron levels at birth (Mohri et al., 2007, Mohri et al., 2004).

In general, the number of neutrophils and lymphocytes changed inversely during the course of this study (Fig. 4). Neutrophils were found at the highest numbers near the time of birth, but lymphocytes increased in absolute number over the course of the study. This was true for calves in both treatment groups. The high number of neutrophils at D7 might be explained by the release of maternal and fetal cortisol in late pregnancy and delivery, or the maturation and release of bone marrow stores of neutrophils following signals from tissues interacting with microbes. This pattern with age has previously been published by Mohri et al. (2007), Brun-Hansen et al. (2006) and Novo et al. (2015).

An interpretation of dynamics of neutrophils in this study indicates a rapid increase of neutrophils in circulation in COL+ calves peaking on D7 followed by migration of these cells to the mucosal tissues of the gut and respiratory tract. In COL− calves a slow and gradual decrease in circulating neutrophils associated with the onset of diarrhea was observed. The absolute number of circulating lymphocytes peaked in COL− calves on D7. The absolute number of circulating lymphocytes in COL+ calves featured a steady, gradual increase over the course of this investigation. The distribution of leukocytes was more in line with the typical profile seen in developing calves in the COL+ calves.

The lower overall incidence of disease in the COL+ calves may be explained by the influence of viable maternal cells from colostrum, and the impact of products that are produced by transferred maternal cells during the development of the innate and adaptive immunity in the calf. Maternal leukocytes can cross the gastrointestinal epithelium by diapedesis to enter mucosal tissue spaces and enter the circulatory system during the first 24 h after birth in calves (Liebler-Tenorio et al., 2002; Reber et al., 2006). Previous studies demonstrated the participation of colostrum-derived leukocytes in the development of innate and adaptive immune phenotype markers and responses in bovine neonate (Riedel-Caspari and Schmidt, 1991a, 1991b; Donovan et al., 2007; Reber et al., 2008a, 2008b, Reber et al., 2006; Langel et al., 2015).

5. Conclusion

We documented that calves fed with viable maternal cell free frozen colostrum had a less robust physiological development profile, pale mucous membranes, and evidence of anemia during natural episodes of diarrhea during this study. Moreover, COL− calves had lower absolute number of circulating erythrocytes, lower hemoglobin and RBC volume that appeared to be associated with lower serum iron concentrations at the time that the diarrhea started (D7). The distribution of leukocytes was closer to previously reported typical values in the COL+ calves than COL− calves, which had a significantly greater number of circulating lymphocytes on D7.

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References

Aldridge, B.M., Mcguirk, S.M., Lunn, D.P., 1998. Effect of colostral ingestion on immunoglobulin-positive cells in calves. Vet. Immunol. Immunopathol. 62, 51–64.
Bartels, C.J.M., Holzhauer, M., Jorritsma, R., Swart, W.A.J.M., Lam, T.J.G.M., 2010. Revalence, prediction and risk factors of enteropathogens innormal and non-normal faeces of young Dutch dairy calves. Prevent. Vet. Med. 93, 162–169.
Brun-Hansen, H.C., Kampen, A.H., Lund, A., 2006. Hematologic values in calves during the first 6 months of life. Vet. Clin. Pathol. 35, 182–187.
Chase, C.C.L., Hurley, D.J., Reber, A.J., 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. North Am. Food Anim. Pract. 24, 87–104.
Dirksen, G., Gründer, H.D., Stöber, M.R., 2008. Exame clínico dos bovinos. third ed. Guanabara Koogan, Rio de Janeiro.
Donovan, D.C., Reber, A.J., Gabbard, J.D., Aceves-Avila, M., Galland, K.L., Holbert, K.A., Ely, L.O., Hurley, D.J., 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. Am. J. Vet. Res. 68, 778–782.
Godden, S.M., Smolenski, D.J., Donahue, M., Oakes, J.M., Bey, R., Wells, S., Sreevatsan, S., Stabel, J., Fetrow, J., 2012. Heat-treated colostrum and reduced morbidity in preweaned dairy calves: results of a randomized trial and examination of mechanisms of effectiveness. J. Dairy Sci. 95, 4029–4040.
Langel, S.N., Wark, W.A., Garst, S.N., James, R.E., Mcgilliard, M.L., Petersson-Wolfe, C.S., Kanevsky-Mullarky, I., 2015. Effect of feeding whole compared with cell-free colostrum on calf immune status: the neonatal period. J. Dairy Sci. 98, 3729–3740.
Liebler-Tenorio, E.M., Riedel-Gaspari, G., Pohlenz, J.F., 2002. Uptake of colostral leukocytes in the intestinal tract of newborn calves. Vet. Immunol. Immunopathol. 85, 33–40.
Gomes, V., Madureira, K.M., Della Libera, A.M.M.P., Blagitz, M.G., Alves, M., Baptistella, F., Benesi, F.J., 2011. Dinâmica da celularidade do colostro de vacas holandesas no pós-parto imediato. Arq. Bras. Med. Vet. Zootec. 63, 1047–1053.
Mcguirk, S.M., 2008. Disease management of dairy calves and heifers. Vet. Clin. North Am. Food Anim. Pract. 24, 139–156.
Mee, J.F., 2008. Managing the calf at calving time. Proc. Am. Ass. Bov. Pract. 41, 46–53.
Meganck, V., Hoflack, G., Piepers, S., Opsomer, G., 2015. Evaluation of a protocol to reduce the incidence of neonatal calf diarrhoea on dairy herds. Prev. Vet. Med. 118, 64–70.
Mohri, M., Sharifi, K., Eidi, S., 2007. Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults. Res.Vet. Sci. 83, 30–39.

Mohri, M., Sarrafzadeh, F., Seifi, H.A., Farzaneh, N., 2004. Effects of oral iron supplementation on some haematological parameters and iron biochemistry in neonatal dairy calves. Comp. Clin. Path. 13, 39–42.

Novo, S.M.F., Reis, J.F., Sobreira, N.M., Gomes, V., 2014. Effect of Temperature and Conservation Time on the Feasibility of Bovine Colostrum Cells, in: World Buiatrics Congress. Cairns, Austrália, p. 17.

Novo, S.M.F., Freitas, R.L., Silva, C.P.C., Baldacim, V.A.P., Baccili, C.C., Reis, J.F., Hagiwara, M.K., Gomes, V., 2015. Hematological adaptation in Holstein calves during the neonatal period. Braz. J. Vet. Res. Anim. Sci. 52, 212–216.

Park, Y.H., Fox, L.K., Hamilton, M.J., Davis, W.C., 1992. Bovine mononuclear leukocyte subpopulation in peripheral blood and mammary gland secretion during lactation. J. Dairy Sci. 75, 998–1006.

Poulsen, K.P., McGuirk, S.M., 2009. Respiratory disease of the bovine neonate. Vet. Clin. North Am. Food Anim. Pract. 25, 121–137.

Quigley, J.D., Lago, A., Chapman, C., Erickson, P., Polo, J., 2013. Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. J. Dairy Sci. 96, 1148–1155.

Reber, A.J., Lockwood, A., Hippen, A.R., Hurley, D.J., 2006. Colostrum induced phenotypic and trafficking changes in maternal mononuclear cells in a peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. Vet. Immunol. Immunopathol. 109, 139–150.

Reber, A.J., Donovan, D.C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K.A., Marshall, L., Hurley, D.J., 2008a. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part I. Effects on monocyte lineage cells. Vet. Immunol. Immunopathol. 123, 186–196.

Reber, A.J., Donovan, D.C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K.A., Marshall, L., Hurley, D.J., 2008b. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. Vet. Immunol. Immunopathol 123, 305–313.

Riedel-Caspari, G., 1993. The influence of colostral leukocytes on the course of an experimental *Escherichia coli* infection and serum antibodies in neonatal calves. Vet. Immunol. Immunopathol. 35, 275–288.

Riedel-Caspari, G., Schmidt, F.W., 1991a. The influence of colostral leukocytes on the immune system of the neonatal calf I Effects on lymphocyte responses. Dtsch. Tierarztl. Wochenschr. 98, 77–116.

Riedel-Caspari, G., Schmidt, F.W., 1991b. The influence of colostral leukocytes on the immune system of the neonatal calf II Effects on passive and active immunization. Dtsch. Tierarztl. Wochenschr. 98, 165–204.

Sheldrake, R.F., Husband, A.J., 1985. Intestinal uptake of intact maternal lymphocytes by neonatal rats and lambs. Res. Vet. Sci. 39, 10–15.

Tuboly, S., Bernáth, S., Glávits, R., 1988. Medveczky I. Intestinal absorption of colostral lymphoid cells in newborn piglets. Vet. Immunol. Immunopathol. 20, 75–85.

Watson, D.L., 1980. Immunological functions of the mammary gland and its secretion-comparative review. Aust. J. Biol. Sci. 33, 403–422.

Williams, P.P., 1993. Immunomodulating effects of intestinal absorbed maternal colostral leukocytes by neonatal pigs. Can. J. Vet. Res. 57, 1–8.

Windeyer, M.C., Leslie, K.E., Godden, S.M., Hodgins, D.C., Lissemore, K.D., LeBlanc, S.J., 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Prev. Vet. Med. 113, 231–240.