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Association between colostrum quality, passive transfer of colostral immunoglobulin, and postnatal growth in neonatal Holstein calves

A. García-Mendoza¹, D.X. Vega-Manríquez¹, V. Cuevas-Reyes², M.J. Flores-Najera³, F.J. Almendárez-Navarro¹, E. Felix Santiago¹, J.M. Vázquez-García¹, G. Ballesteros-Rodea¹, R. Sims⁴, M. Mellado⁵, C.A. Rosales-Nieto¹,a,*

⁴Department of Agricultural Sciences, Texas State University, San Marcos 78666, U.S.A.

ABSTRACT: Calves that receive sufficient colostrum shortly after birth present improved passive immunity vital for optimum calf health and future production. Management practices and the dam's parity can influence colostrum quality and the transfer of immunoglobulins to newborn calves. Cows' parity is a modulating factor, with increased IgG in older cows, although scarce information exists regarding first- and second-parity cows. This study aimed to determine the impact of parity on colostrum immunoglobulin (IgG) concentration, transfer of passive immunity, postnatal growth, and incidence of diseases in Holstein calves. The study included 60 animals, Holstein cows and their progeny that were distributed into three groups: G1 (first-parity, n=10+10), G2 (second-parity, n=10+10), and G3 (third-parity, n=10+10). The birthweight and gender of calves were recorded. Immediately after birth, colostrum IgG was determined by colostrometer and refractometry. Blood samples from calves were collected at birth and 48 h of life to determine the transfer of passive immunity (TPI). Subsequently, the incidence of diseases, daily liveweight gain, and mortality were recorded weekly up to weaning. Birth weight tended to differ among groups (G1: 33.0±0.9; G2 38.1±2.5; G3: 38.3±1.4 kg; p=0.07). IgG concentration in colostrum at birth was greater (P < 0.05) in G3 than in G1 and G2, independently of the method used. Serum immunoglobulin concentration at birth was similar (P > 0.05) among groups, but at 48 h was higher (P < 0.05) in G3 calves compared to G1 but not to G2. Postnatal growth and weaning weight did not differ (P > 0.05) among groups. Calves of all groups presented diarrhea (P > 0.05). Two calves died, one from G1 and one from G2, with no mortality rate differences (P > 0.05) among groups. In conclusion, the older the cows, the higher the colostrum IgG concentration, increasing the passive immunity transfer in calves. Birth weight of calves tended to differ among groups, but the postnatal growth performance was similar. These results warrant more research to determine whether the reduced colostrum quality produced by first- and second-parity cows does not negatively affect the calves' post-weaning performance and age at first pregnancy.

Keywords: Maternal age; newborns; colostrometer assay; refractometry assay; postnatal development

Corresponding Author:

Cesar A. Rosales-Nieto, Department of Agricultural Sciences, Texas State University, San Marcos 78666, U.S.A.

E-mail address: nieto cesar@hotmail.com; cesar.rosales@txstate.edu

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¹Facultad de Agronomía y Veterinaria, Universidad Autónoma de San Luis Potosí, San Luis Potosí 78321, México.

²Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Valle de México, Texcoco 56250, México.

³Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental La Laguna. Blvd. José Santos Valdés No. 1200 Pte. Matamoros, Coahuila, México. C.P. 27440.

⁵Departamento de Nutrición Animal, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila, 25315, México.

^aPresent address: Department of Agricultural Sciences, Texas State University, San Marcos 78666, U.S.A.

INTRODUCTION

Profitability in the dairy herds depends on reproductive and productive efficiency. The animal's long-term productivity depends on its early immunity; therefore, maximizing survival and attaining the full growth potential of newborn calves is challenging (Van Amburgh et al., 2008; Soberon et al., 2012; Ghaffari et al., 2020). Failing to do so leads to perinatal mortality in newborns, drawing the consumer's attention from the animal welfare point of view (Cuttance and Laven, 2019; Verdon, 2021).

In ruminants, no exchange of immune factors occurs in utero due to the nature of the placenta (Chucri et al., 2010), resulting in agammaglobulinemia in newborns (Barrington and Parish, 2001; Gulliksen et al., 2008). Therefore, within 12 h post-partum, newborn ruminants require sufficient colostrum to reduce diseases and survive as the gut permeability to absorb immunoglobulins rapidly and progressively decreases (Moore et al., 2005; Stelwagen et al., 2009). Colostrum is responsible for protecting against environmental antigens until the proper functioning of the immune system in the newborn because it contains proteins, essential and non-essential amino acids, lipids, lactose, growth factors, immunoglobulins, and bioactive components (McGrath et al., 2016; Balan et al., 2019; Lopez and Heinrichs, 2022). Three types of immunoglobulins are found in colostrum that contribute to the transfer of passive immunity (IgG, IgA, and IgM; Larson et al., 1980; Stelwagen et al., 2009; Mc-Grath et al., 2016), with IgG accounting for more than 75% of the total (Korhonen et al., 2000). High-quality colostrum must contain >50 g/L IgG (McGuirk and Collins, 2004). Therefore, high-quality colostrum must be provided immediately after birth to improve passive immunity and the health status of calves to ensure calves' good health, growth, and pre-weaning survival (McGuirk and Collins, 2004; Godden et al., 2019). Colostrum can be evaluated before providing it to newborn calves because selecting colostrum with adequate IgG concentration is the only remaining uncontrolled variable influencing passive immunity.

Insufficient colostrum consumption or consumption of colostrum with reduced levels of immunoglobulins leads to an unsuccessful TPI, which increases morbidity and mortality in newborn calves (Windeyer et al., 2014; Godden et al., 2019; Gavin et al., 2018; Verdon, 2021). A newborn with a reduced transfer of passive immunity that does not die decreases its feed intake and postnatal growth (Faber

et al., 2005; Uetake, 2013; Roche et al., 2015) and increases its post-weaning mortality (Cuttance et al., 2017). By contrast, calves with satisfactory transfer of passive immunity have lower pre-weaning morbidity and mortality and reduced antibiotic treatments due to an improved immune system (Berge et al., 2009; Williams et al., 2014). In the long term, these calves have greater postnatal development through increased daily liveweight gain (Dewell et al., 2006; Berge et al., 2009), reduced mortality in the post-weaning period, increased feed efficiency, reduced age at first calving (Brickell et al., 2009), and improved milk yield in their first lactation (Faber et al., 2005; Van Amburgh et al., 2008).

Different factors modify colostrum quality; it varies among genotypes and individuals within a breed (Morrill et al., 2012; Puppel et al., 2019), parity (Shearer et al., 1992; Gulliksen et al., 2008), maternal age (Puppel et al., 2019), maternal health status (Puppel et al., 2019), the season of calving (Shearer et al., 1992; Gulliksen et al., 2008; Puppel et al., 2019), milk yield (Wasowska and Puppel, 2018), dry period length (Puppel et al., 2019), and nutrition (Puppel et al., 2019). However, data reporting the effect of parity on colostrum quality is confounded with the calving season and maternal age, as no difference in colostrum quality between first and second-parity cows has been observed (Quigley et al., 1994; Gulliksen et al., 2008; Moore et al., 2005; Bartier et al., 2015; Silva-del-Rio et al., 2017). Therefore, the objectives of this study were to evaluate the impact of parity on colostrum IgG concentration assessed by two methods (colostrometer and refractometry; Bartier et al., 2015), transfer of passive immunity in serum (refractometry; Elsohaby et al., 2019a), and postnatal growth rate, the incidence of diseases, and mortality up to weaning in Holstein calves.

MATERIALS AND METHODS

The study was conducted on a commercial dairy farm in Central Mexico (21°73'N, 100°96'W). The climate in this location is semi-arid and temperate. The average annual temperature is 16.7 °C, with an average annual precipitation of 220 mm. All animal procedures were consistent with international guidelines (FASS, 2010) and national policies (NAM, 2010) for the care and use of laboratory animals.

Animals and experimental procedure

A total of 60 animals, 30 pregnant Holstein cows and afterwards, their progeny (n = 30) from the in-

house flock were used to investigate the effect of parity on colostrum IgG concentration, immunoglobulin levels at birth and 48 h of life, birth weight, and postnatal development and weight at three months of age. The groups were G1 (first-parity heifers and their progeny; n=10), G2 (second-parity cows and their progeny; n=10), and G3 (third-parity cows and their progeny; n=10). All cows calved in the same week period.

Heifers management

First-parity cows were managed in 30 x 30 m² pens with 85% dirt surface and 15% concrete surface. The diet meets the nutritional requirements for both the development and maintenance of the fetus. Thirty days before parturition, pregnant heifers were moved to a smaller pen, where they were offered a diet richer in protein and energy (NRC, 2001). The diet was based on corn silage (40%), alfalfa hay (47%), and 13% concentrate (18% CP; Trouw Nutrition, Nuevo Leon, Mexico), contributing to 2.2 Mcal of metabolizable energy and 14.1% of crude protein. On average, heifers calved at 27 months of age.

Multiparous cows

Pregnant cows went through a 40-d dry period before parturition. The diet provided during the dry period satisfied protein and energy maintenance requirements (60% sorghum stubble and 40% corn silage). Two weeks before parturition, the protein content and starches were increased. The diet was based on corn silage (40%), alfalfa hay (47%), and 13% concentrate (18% crude protein; Trouw Nutrition), contributing 2.2 Mcal of metabolizable energy and 14.1% of crude protein. All cows and heifers in the facility were vaccinated twice a year against bovine viral rhinotracheitis, bovine viral diarrhea BVD strains 1 and 2, bovine respiratory syncytial virus (BRSV), clostridiosis, and pneumonic pasteurellosis. Deworming was not applied. Pregnant cows calved in their pen. Cows in labor were not assisted unless dystocic parturition was detected. Once parturition had occurred, the dam and calf had contact for 10 min.

Colostrum management

At calving, cows and calves were separated, the dam was milked, and the colostrum was collected. The calves were placed in individual pens $(1.2 \times 2.5 \text{ m})$.

A colostrum subsample (250 mL) was collected

for immediate evaluation using a colostrometer and a Brix refractometer (Bartier et al., 2015). Colostrum samples were analyzed for specific gravity using a colostrometer (Biogenics, OR, USA; Fleenor and Stott, 1980; Pritchett et al., 1994). Briefly, the samples were transferred to a clean, dry 100 mL measuring cylinder, and the colostrometer was gently floated on the sample for one minute before taking the reading (Fleenor and Stott, 1980). This methodology was performed twice for each colostrum sample.

In addition, colostrum samples were analyzed with a digital Brix refractometer (Vee Gee BX-1, IL, USA). This method provides a reliable estimate of IgG in first-milking bovine colostrum (Quigley et al., 2013; Silva-del-Rio et al., 2017). Briefly, 2 to 3 drops of colostrum were placed on the measuring prism and left for 1 minute to allow temperature adjustment before taking the reading (Bielmann et al., 2010).

Calves management up to weaning

At parturition, the birth weight and sex of calves were recorded. Cows and their calves remained together for 10 min. Newborn management consisted of navel submersion in an iodine solution, and no vaccines were applied. All calves received colostrum during the first two h of life at a rate of 10% of their live weight (Godden et al., 2019). The following colostrum intakes were given at 24, 36, and 48 h after birth, at a rate of 10% of their live weight. Each calf received their dam's colostrum. Afterwards, calves received milk at a rate of 5% of their liveweight. Simultaneously, on d 15 of life, calves were offered concentrate (Sprayfo® Trouw Nutrition, Monterrey, Mexico) at a rate of 0.5 kg/day, progressively increasing to 2.0 kg. The concentrate was mixed with alfalfa hay. Calves were weaned at three months of age, and live weight was recorded to determine daily weight gain during the preweaning period.

Transfer of passive immunity

Blood samples (6 mL) from all calves were obtained in sterile vacuum tubes with 2-inch needles using the jugular vein bleeding method 10 minutes after birth and at 48 h of age. Blood was allowed to clot at room temperature for 60 m, and then serum was separated by centrifugation at 2000 x g for 20 min. Subsequently, serum samples were analyzed using a digital Brix refractometer (Vee Gee BX-1, IL, USA; Deelen et al., 2014). Approximately 250 μL of serum was used, and the Brix score was determined by transmitting light through the sample

in the prism and recording the reading on a digital scale. Before each analysis, the refractometer was thoroughly cleaned and calibrated with distilled water at room temperature. These readings gave the serum IgG content, reflecting passive immunity's transfer to calves.

Newborn diseases and mortality

Each calf was monitored for three months to determine the incidence of diseases. During the experiment, two calves died (one from G1 and G2). Their information was considered for the mortality rate and birth weight variable but not for daily weight gain and weaning weight.

Statistical analysis

Data were analyzed using the SAS statistical package version 9.3 (2010). Each cow and calf were considered an experimental unit. We acknowledge the experiment's limitation as the sample size is reduced. The sample size considered for this experiment was based on budgetary constraints (Anvari and Lakens, 2021; Lakens, 2022). Nevertheless, a post hoc power analysis (Proc POWER) indicated that the power of the analysis is 0.63 for an alpha level of 0.05. This pilot study can still provide reliable results to test the feasibility of a larger experiment.

Birth weight, daily liveweight gain, weaning weight, colostrum quality, and blood BRIX readings were analyzed using linear mixed model procedures (PROC MIXED). The fixed effects in the model were parity of cows and calf's gender. Birth weight, daily lightweight gain, and weaning data were included as covariates in the model. Daily liveweight gain was fitted in a linear regression model of liveweight on time postpartum, and estimates of the regression coefficients were obtained as a measure of weight change by a unit of time.

Disease incidence and mortality rate were analyzed using the generalized linear mixed model procedures with a binomial distribution and logit link function (PROC GLIMMIX). The fixed effect was parity of cows. Birth weight, daily lightweight gain,

and weaning data were included independently as covariates.

The correlations among birth weight, daily weight gain, weaning weight, and IgG evaluated by a colostrometer and a Brix refractometer were predicted using PROC GLM with the MANOVA option to allow the removal of significant fixed effects. It was considered strong if the correlation value was > 0.5 and near 1.0. It was considered low if the correlation value was < 0.5 and weak when the value approached 0.0. Group was considered fixed effect.

All 2-way interactions among the fixed effects were included in each model, and non-significant (P > 0.05) interactions were removed from the model. Significant differences among means for parity classes within variables were analyzed using the CONTRAST statement of SAS (2010).

RESULTS

Brix readings in colostrum

The colostrum IgG concentration (mg/mL \pm SEM) was 73.5 \pm 4.0 for G1 cows, 82.8 \pm 2.9 for G2 cows, and 87.9 \pm 3.3 for G3 cows assessed by the colostrometer. The colostrum IgG concentration estimated by the Brix was 23.0 \pm 0.8 for G1 cows, 25.1 \pm 0.6 for G2 cows, and 26.2 \pm 0.7 for G3 cows. Colostrum IgG concentration differed (P < 0.05) among groups by both methods, the colostrometer (hydrometer) and the Brix refractometer (Table 1). With both methods, an orthogonal contrast for the colostrum IgG concentration showed that G1 and G3 groups differed (P < 0.01); no statistical difference was observed between G1 and G2 or between G2 and G3 (P > 0.05). With both methods, colostrum IgG concentration did not differ between calf's gender (P > 0.05).

Transfer of Passive Immunity

Parity did not influence serum immunoglobulin concentration at birth in newborns calves (G1: 0.22 g/L; G2: 0.22 g/L; G3: 0.21 g/L; P > 0.05; Table 2) but affected the serum immunoglobulin concentration at 48 h of life (G1:14.4 g/L; G2:15.0 g/L; G3:17.8 g/L;

Table 1. The effect of the dam's parity on colostrum IgG concentration (means ± SEM) assessed by colostrometer and Brix refractometer.

Method	Parity			Significance	Contrast		
	G1	G2	G3		G1 vs. G2	G1 vs. G3	G2 vs. G3
n	10	10	10				
Colostrometer (mg/mL)	73.5 ± 4.0	82.8 ± 2.9	87.9 ± 3.3	0.03	0.10	0.01	0.31
Brix (%)	23.0 ± 0.8	25.1 ± 0.6	26.2 ± 0.7	0.03	0.08	0.01	0.30

P < 0.05; Table 2). At birth, an orthogonal Contrast showed no differences between the G1 and G2 groups and G1 and G3 groups (P > 0.05; Table 2). At 48 h, an orthogonal contrast showed no differences between the G1 and G2 or between G2 and G3 but between G1 and G3 groups (P < 0.05; Table 2). Neither birth weight nor calf's sex influences immunoglobulin concentration at birth nor 48 h (P > 0.05).

Newborn birth weight and postnatal development

In G1, nine females and one male were born, and seven females and three males were born in G2 and G3. Parity tended to influence birth weight (G1:33.0 kg; G2:38.1 kg; G3:38.3 kg; P = 0.07; Table 3); but not daily liveweight gain G1:551 g/d; G2:515 g/d; G3:497 g/d; P > 0.05; Table 3) or weaning weight (G1:82.4 kg; G2:84.9 kg; G3:82.9 kg; P > 0.05; Table 3). Birth weight between G1 and G2 and between G1 and G3 differed (P < 0.05; Table 3), but not between G2 and G3 (P > 0.05). Weaning weight was positively related to birth weight (P < 0.05). Weaning weight increased by 5.2 kg when birth weight increased by 10 kg. Birth weight, daily liveweight gain, and weaning

weight were not influenced by sex (P > 0.05).

Correlations between body weight measurements and immunoglobulins present in colostrum

The correlations among birth weight, daily weight gain, weaning weight, and the quantity of immunoglobulins present in colostrum (Brix and colostrometer) are shown in Table 4. The correlation between Brix and colostrometer readings for colostrum Ig content was strong and significant (Table 4). The correlation between birth weight and weaning weight was moderate and tended to be significant (Table 4). The correlation between daily liveweight gain and weaning weight was high and significant (Table 4). The rest of the correlations were not significant (Table 4).

Prevalence of diseases and mortality rate

All calves presented diarrhea; therefore, the incidence of diseases did not differ among groups (P > 0.05). Two deaths were recorded, one from G1 and one from G2. The mortality rate did not differ (P > 0.05) between groups.

Table 2. Effect of dam's parity on immunoglobulin serum concentration (g/L) in calves at birth and 48 h of life (means \pm SEM).

Variable	Parity		Significance	Contrast			
	G1	G2	G3		G1 vs. G2	G1 vs. G3	G2 vs. G3
n	10	10	10				
Birth (g/L)	0.22 ± 0.002	0.22 ± 0.02	0.21±0.02	0.16	0.72	0.17	0.08
48 h (g/L)	14.4±0.7	15.0±1.0	17.8±1.2	0.05	0.72	0.05	0.29

Table 3. The effect of the dam's parity on the birth weight, daily liveweight gain, and weaning weight of Holstein calves (means ± SEM). Sex is combined for the effect of parity.

Variable	Parity		Significance	Contrast			
	G1	G2	G3		G1 vs. G2	G1 vs. G3	G2 vs. G3
n	10	10	10				
Females	9	7	7				
Males	1	3	3				
Birth weight (kg)	33.0 ± 0.9	38.1 ± 2.6	$38.3 {\pm} 1.4$	0.07	0.05	0.04	0.95
Weaning weight (kg)	82.4 ± 2.7	84.9 ± 3.7	82.9 ± 2.7	0.71	0.93	0.82	0.73
Daily liveweight gain (g/d)	551±25	515±46	497±24	0.72	0.57	0.91	0.64

Table 4. Correlations (r) among birth weight (BWT), daily weight gain (DWG), weaning weight (DWG), and the quantity of immunoglobulins present in colostrum (Brix and Colostrometer). The data combined groups.

Variable	BWT	DWG	WWT	Brix	Colostrometer
BWT	NA	NS (-0.32)	0.06 (0.35)	NS (0.19)	NS (0.17)
DWG		NA	*** (0.76)	NS (-0.06)	NS (-0.07)
WWT			NA	NS (0.05)	NS (0.04)
Brix				NA	*** (0.99)
Colostrometer					NA

NA: Not applicable; NS: P > 0.05; ***: P < 0.001

DISCUSSION

There have not been many studies examining how a cow's parity affects the colostrum quality using Brix refractometry, nor have there been many on how this quality impacts IgG absorption by calves. This study addresses that gap by assessing the relationship between cow's parity and colostrum quality, measured by Brix refractometry. We acknowledge that justifying the sample size that will be collected is an important step when designing an empirical study. We also acknowledge the experiment's limitation, as the sample size is low. Nevertheless, in animal research, the sample size can be reduced by different factors, including but not limited to budget constraints (Anvari and Lakens, 2021; Lakens, 2022). This pilot study can still provide reliable results to test the feasibility of a larger experiment. We hypothesized that parity in Holstein cows influences colostrum IgG concentration, passive immunity transfer, postnatal calves' development up to weaning, incidence of diseases, and mortality rate. Our results demonstrated that parity influenced colostrum quality, indicating that colostrum IgG concentration in third-parity cows was higher than in first-parity cows; however, no statistical difference was detected between first and second-parity cows or between second-parity and third-parity cows. Furthermore, the transfer of passive immunity was greater in calves from third-parity cows than in calves from first-parity cows. There was no statistical difference in the transfer of passive immunity among calves from first and second-parity cows. Birth weight tended to differ among calves from cows with different parity, but not the daily liveweight gain and weaning weight. This study has limitations due to the resource constraints that reduce the sample size and the colostrum chemical composition that was not assessed. Still, considering the relevance of the results on this topic for the dairy industry, the present findings provide a basis for management guidelines for colostrum, which could improve the survival of young Holstein calves.

Our results indicated that IgG colostrum concentration was higher in G3 cows than in cows from G1 or G2. We did not observe differences in the IgG concentration in colostrum between G1 and G2 cows or between G2 and G3 cows. Data reporting the effect of parity on the content of IgG in colostrum could be confounding with the calving season and maternal age, as no difference in colostrum quality between cows from first to second parity has been reported (Quigley et al., 1994; Gulliksen et al., 2008; Moore

et al., 2005; Bartier et al., 2015; Silva-del-Rio et al., 2017). Also, Zarei et al. (2017) found no significant increase in IgG and IgM concentration with increasing parity. In cows, the maternal immune system improves with age as older cows have been exposed to antigens for a longer time than younger cows; thus, older cows produce colostrum with higher Ig levels (Shearer et al., 1992; Conneely et al., 2013; Vlasova and Saif, 2021). Therefore, a positive relationship has been reported between Ig concentration in colostrum and parity in cows (Ahmann et al., 2021). The immune system between first-parity cows and second-parity cows is similar, and the Ig concentration in colostrum was similar between groups. Nevertheless, these observations do not explain the lack of statistical difference between G1 and G2 cows. Our results align with several authors (Bartier et al., 2015; Chuck et al., 2017; Ahmann et al., 2021) who observed that firstcalf heifers had a considerably lower colostral IgG content than cows in their third parity or later.

Our results indicate that parity did not influence serum immunoglobulin concentration of calves at birth; however, after colostrum ingestion, serum immunoglobulin concentration was higher in calves from G3 cows. A similar serum IgG concentration among groups at birth was expected as newborn calves are agammaglobulinemia (Barrington and Parish, 2001; Gulliksen et al., 2008) because no exchange of immunoglobulins occurs in utero due to the nature of the placenta (Chucri et al., 2010). Neonatal calves can respond to environmental conditions loaded with microorganisms, yet the response is delayed due to the immaturity of protective mechanisms (Barrington and Parish, 2001). After colostrum ingestion, we observed a sustained increase in serum IgG, which agrees with previous reports (Topal et al., 2008; Hare et al., 2020). According to our results, serum IgG concentration of calves at 48 h of life indicated that the passive immunoglobulins transference was higher in calves from G3 cows. Yet, the transfer of passive immunity was similar between calves from G1 and G2 cows. Indeed, the transfer of passive immunity is highly associated with colostrum quality (Lichtmannsperger et al., 2023), and G3 cows had greater colostrum IgG concentration than G2 cows. Increasing colostrum quality results in enhanced intestinal development, improving nutrient absorption and transfer of passive immunity in newborn calves (Yang et al., 2015). There was no evidence for differences between G1 and G2 cows. Nevertheless, the dairy industry needs to develop guidelines that indicate the use of colostrum

from third-parity cows to improve the transfer of passive immunity and, thus, the health of calves.

It has been well documented that birth weight is reduced, and postnatal growth performance is delayed in progeny from first-calf heifers compared with multiparous cows (Holland and Odde, 1992; Kertz et al., 1997). Indeed, a positive genetic relationship between birth weight and adult weight has been reported (Lamb and Barker, 1975; Holland and Odde, 1992). Notably, postnatal growth performance highly correlates with newborn health because of the highquality colostrum ingested (Furman-Fratczak et al., 2011; Yang et al., 2015; Elsohaby et al., 2019b; Lopez et al., 2020). Furthermore, the relationship between birth weight and progeny growth rate was characterized by correlation analysis. This point is important, as a positive relationship between birth weight and postnatal growth and development has been reported previously (Furman-Fratczak et al., 2011). Thus, heavier progeny at birth grows faster than lighter progeny and can have greater fertility (Lamb and Barker, 1975). Our results align with previous observations showing that birth weight from first-calf heifers was lower than that of calves from two- or third-parity cows. However, we observed that daily liveweight gain and weaning weight were similar regardless of parity class. This lack of difference in the postnatal growth performance among groups was reflected in the similar prevalence of diseases and mortality among calves from cows with different

parities. To better understand if colostrum from first- and second-parity cows has any adverse effects on newborns' post-weaning performance and first pregnancy, additional research is needed.

CONCLUSIONS

It was concluded that parity influenced the colostrum IgG content and TPI and tended to influence the birth weight of calves. However, postnatal growth up to weaning among calves from cows with different parity was similar. Further research, including larger sample sizes and comprehensive colostrum composition analyses, is warranted to determine the relationship between colostrum composition and IgG concentrations on the TPI and the impact on the post-weaning growth performance and reproductive efficiency at their first breeding. These results are very relevant to the dairy industry in developing neonatal management guidelines to improve the transfer of passive immunity and, thus, the health of newborn calves.

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CONFLICT OF INTEREST

None declared

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