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Effect of oxytocin use during colostrum harvest and association of cow characteristics with colostrum yield and IgG concentration in Holstein dairy cows

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ABSTRACT

Our objectives were to determine the effect of oxytocin use during colostrum harvest on colostrum yield (CY) and IgG concentration in Holstein dairy cows on a commercial dairy in New York and to describe associations of cow characteristics with these outcomes. Animals were enrolled between July and October 2023 using a randomized block design, with day of enrollment as the unit of randomization. A median (range) of 10 (3 to 19) cows were enrolled/d. Treatments were 1) 40 IU Oxytocin (OXY40), 2) 20 IU Oxytocin (OXY20), and 3) an untreated control group (CNTR). Oxytocin was administered intramuscularly (IM) approximately 45 s before unit attachment in a rotary parlor. Colostrum weight was measured using the colostrum bucket-embedded scale or a platform scale when the yield was less than the smallest bucket scale. The concentration of IgG ([IgG]) in colostrum was determined using radial immunodiffusion (RID) and used to calculate total IgG, and dry matter (%) was determined by oven drying. Individual cow characteristics such as parity, calf sex, weight, dystocia score, stillbirth, milk production in wk 4 of lactation, and for multiparous cows, dry period length, previous lactation dry off linear score (LS) were collected. Data were analyzed separately for primiparous and multiparous cows using backward stepwise elimination to produce final mixed effects ANOVA models Primiparous cows (n = 201) were randomized to 35.8% (n = 72) OXY40, 32.8% (n = 66) OXY20, and 31.3% (n = 63) CNTR. Multiparous groups (n = 435) were randomized to 34.7% (n = 151) OXY40, 29.7% (n = 129) OXY20, and 35.6% (n = 155) CNTR. The median (range) CY was 6.0 (0 to 20.6) kg and [IgG] was 98.5 (0.1 to 293.6) g/L in the study population. In primiparous cows, OXY40 had a higher colostrum yield (LSM [95% CI]) of 5.4 (4.9 to 5.9) kg compared with both OXY20 (4.1 [3.5 to 4.7] kg) and CNTR (3.8 [3.3 to 4.3] kg) ($P < 0.001$). In multiparous cows, OXY40, OXY20, and CTNR did not differ in CY (5.9 [5.3 to 6.5], 5.7 [6.3 to 5.1], and 5.4 [6.0 to 4.8] kg, respectively, $P = 0.43$), but colostrum yield was greater in parity 2 compared with all other parities, cows giving birth to male calves, cows with the highest milk production at wk 4 of lactation, and with a dry period of >65 d. Oxytocin use did not affect [IgG] in either primiparous or multiparous cows ($P > 0.56$), but [IgG] was highest in cows in parity ≥4 and lowest in cows dry >65 d. In summary, oxytocin use at 40 IU IM in primiparous was associated with a higher CY but not [IgG]. Oxytocin use likely addressed disturbed milk ejection and therefore increased CY in heifers milked for the first time in a rotary parlor. This study confirms cow characteristics associated with colostrum production within a single herd.
Key Words: colostrum, harvest, oxytocin, IgG

INTRODUCTION

Colostrum management continues to represent a crucial factor in dairy calf preweaning health (Lopez and Heinrichs, 2022). Transfer of passive immunity (TPI) is the typical measure by which colostrum feeding practices are evaluated, and providing sufficient amounts of high-quality colostrum containing nutrients, growth factors, immunoglobulin and other bioactive factors results in high TPI. Excellent **TPI** (≥25 g/L serum IgG, ≥ 9.4% serum Brix) has been shown to decrease preweaning disease probability and mortality, as well as increase average daily gain in heifer calves (Lombard et al., 2020; Crannell and Abuelo, 2023; Sutter et al., 2023). These data underline the importance of the new consensus recommendations for achieving high rates of excellent TPI rather than merely surpassing a minimum standard recommended previously (Godden et al., 2019). The first

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

step to achieving these goals is to have enough high-quality colostrum available to feed to newborn calves.

Individual cow-factors such as parity, sex and weight of the calf, as well as farm-management factors such as dry period management, including prepartum nutrition and dry period length that can be harnessed to improve the quantity and quality of colostrum have received attention in recent years (Fischer-Tlustos et al., 2021; Hare et al., 2023; Westhoff et al., 2024). Relatively less is known about the harvesting effects on colostrum yield and quality.

In most management systems, the first milking to harvest colostrum either follows a dry period of variable length or represents the first milking in the life of a first lactation animal. Despite the importance of obtaining a high quantity of high-quality colostrum, research is lacking regarding best practices for milking routines, milking machine settings, and the use of oxytocin to enhance milk let down. In a recent observational study including 19 herds in New York, 57.9% (n = 11) of producers reported using oxytocin regularly for colostrum harvest (Westhoff et al., 2023a). Oxytocin plays a pivotal role in obtaining the milk or colostrum stored in the alveolar component of the udder, and endogenous release of this hormone is facilitated by tactile stimulation, such as suckling of the calf, hand milking, or contact of the teat with the teat cup liner (Bruckmaier and Wellnitz, 2008). The use of exogenous oxytocin during colostrum harvest is thought to counteract inhibition of oxytocin release from the pituitary during the colostral period that can occur without obvious signs of stress in dairy cattle in parturient, mostly primiparous cows, during the first few milkings or also for a longer period (Bruckmaier et al., 1992). However, despite the widespread industry use of oxytocin at first milking, data to support the routine use of exogenous oxytocin for colostrum harvest is sparse (Sutter et al., 2019), and the anticipated positive effect on colostrum yield is not documented. Given the influence of different milking environments on milk letdown (Bruckmaier and Wellnitz, 2008), the use of oxytocin at colostrum harvest also needs to be investigated in different parlor and milking management systems. In particular, data is lacking for rotary parlors that have gained increasing prevalence (Edwards et al., 2012), including for harvest of colostrum.

The effect of breed, age of the dam, prepartum nutrition, seasonality, and dry period length have been well described (Godden et al., 2019), although we lack an understanding of the exact mechanisms of how each of these factors influences colostrogenesis. Additional work has drawn attention to additional cow-specific factors that were associated with higher colostrum yield, such as dry period length, calf sex and weight, as well as the cow's milk production (Immler et al., 2021; Rossi et al., 2023; Westhoff et al., 2023b; Westhoff et al., 2023a).

The quality of colostrum, particularly the concentration of IgG, is important in maximizing TPI. Brix measurements of colostrum have been used widely as an estimate of IgG concentration, and provide a simple, cost-effective, and rapid on-farm management tool for colostrum management. However, the use of this method to estimate colostral IgG concentration by a refractory index has recently been debated in the scientific community due to concerns about the accuracy of measurements (Kessler et al., 2021; Schalich et al., 2021; Lombard et al., 2022). Continued investigation of the relationship between the 2 measurements in appropriately large sample sets varying in colostral IgG concentration across a wide range is warranted.

Motivated by these knowledge gaps, we hypothesized that the use of exogenous oxytocin during the first postpartum milking would alter colostrum yield and IgG concentration in cows milked in a rotary parlor. We further hypothesized that individual cow characteristics would affect these outcomes, and that use of a Brix refractometer is a sufficiently accurate method for on-farm colostrum management. Therefore, our study had 3 objectives: (1) investigate the effect of exogenous oxytocin at 2 different doses on colostrum yield, IgG concentration, Brix% and dry matter of primiparous and multiparous Holstein dairy cows milked in a rotary parlor, and (2) investigate the associations of individual cow characteristics on the same outcomes, and (3) describe the relationship of colostrum Brix measurements with IgG concentrations measured by the reference method radial immunodiffusion (RID), and determine the ability of Brix values to correctly classify samples below 2 IgG levels (<50 and < 100 g/L).

MATERIALS AND METHODS

Study Population

This study was conducted between July and October 2023 on a commercial dairy in New York State milking approximately 5,200 cows. Approval of the study was granted by the Cornell University Institutional Animal Care and Use committee (protocol 2022–0167). The average daily milk yield was 43.1 kg per cow. The farm used DHIA services including the individual-cow SCC option. The SCC values together with the linear somatic cell score (**LS**; a logarithmic transformation of SCC) were extracted from the herd management software (DC305, VAS) for each monthly DHIA sampling. Cows in this herd were milked 3 times daily in a 100-stall rotary parlor (RP3100HD, DeLaval International AB), but the first milking colostrum (hereafter referred to as colostrum) was only harvested twice daily (0930 h and

1630h). The vacuum was set to supply a milkline vacuum of 38 kPa (11.2 in Hg). The pulsators were set to a pulsation rate of 60 cycles per min and a ratio of 65:35. Dry cows were housed in a naturally ventilated 3-row freestall close-up pen adjacent to the maternity unit with recycled manure solids as bedding, and fed a controlled-energy diet based on wheat straw, corn silage, haylage and grain formulated to supply 1,360 g/d of estimated MP and 32.6 Mcal/d estimated ME. Close-up heifers were housed in the same manner, but fed a controlled-energy TMR with the same forages and custom grain to supply 943 g/d estimated MP and 23 Mcal/d estimated ME. Cows were dried off 60 d before expected calving in parity 1, and 45 d before expected calving in parities ≥2. Close-up pens were walked by farm personnel hourly and cows were moved to a bedded straw pack (3.8 × 9.5 m) in a just-in-time manner at the first signs of stage II labor (Carrier et al., 2006). Parturition was overseen by farm personnel. Farm personnel removed newborn calves from their dams immediately after birth, recorded sex, presence of twins, time of calving, and calving score (on a scale of 1 to 3 with 1 indicating no assistance, 2 indicating light assistance, and 3 indicating a dystocia of any kind with required assistance, (Carrier et al., 2006)). Calves were weighed on a commercial platform scale (EziWeigh 5i, Tru-Test, Auckland, New Zealand) before the first feeding of colostrum from a colostrum bank. Weight for twins was recorded as the sum of the weight of both calves. When a calf was stillborn, weight information was not routinely collected.

Study Design

The study was designed as a randomized block design with blocks of 3 consecutive days with day of enrollment as the unit of randomization. We performed randomization using a random number generator (Research Randomizer, (Urbaniak, 2012)). All animals enrolled within one day received the same treatment to facilitate correct administration of treatments by farm personnel. Animals of all parities were eligible for enrollment in the study for their first milking after parturition at the morning milking (0930h) only. Cows milked for the first time in the afternoon (1730h) were not enrolled. Animals were moved to the nearby rotary parlor as soon as they were ambulatory after parturition. The milking routine for colostrum harvest was as follows: cleaning of teats with a treat brush cleaner, forestripping of 2 streams of colostrum from each teat, predipping of teats with an iodine-based teat disinfectant, and cleaning and drying of teats with an individual cloth towel. The tactile stimulation (i.e., sum of durations of brushing, forestripping, and wiping) lasted for approximately 10 s. The lag time between the first tactile stimulus and the attachment of the milking unit was approximately 60 s.

Treatments administered to individual animals were 1): 40 IU Oxytocin IM (**OXY40**), 2) 20 IU Oxytocin IM (**OXY20**), and 3) and untreated control group (**CNTR**). Oxytocin (20 IU/mL, VetOne) was administered with a 3 mL syringe and 20 G 2.5 cm needle when cows entered the rotary parlor, approx. 45 s before unit attachment. Colostrum was harvested into sanitized translucent polypropylene buckets equipped with an embedded scale indicating the content in 0.5 lb increments (De Laval). Milking clusters were removed manually based on visual assessment of the colostrum flow (i.e., when the flow of colostrum terminated) and buckets were removed by farm personnel. After milking, cows were dipped with an iodine-based teat disinfectant and moved to a freestall pen.

Colostrum Sampling

The weight of colostrum was documented immediately after milking from the embedded scale in 0.5 lb (0.23 kg) increments. When colostrum yield was less than the smallest scale (10 lb, 4.54 kg), the weight was determined using a platform scale (SF-888 Weighology Heavy Duty Digital Postal Scale, 0.1 kg increments) and subtracting the bucket weight of 8.44 lb/3.82 kg. Composite colostrum was carefully mixed in the bucket with a 2-oz sanitized ladle to avoid foaming. A thoroughly mixed sample was then taken, and a Brix measurement was performed immediately using a digital dairy refractometer (Misco DD-1, Palm Abbe). Refractometers were calibrated to zero daily before first use with distilled water and cleaned with alcohol pads between measurements. Two aliquots of composite colostrum samples were placed on ice for transport to the lab and stored at −20°C for subsequent analyses.

Laboratory Analysis

The concentration of immunoglobulin G (IgG) in colostrum was determined as previously described using radial immunodiffusion (Mann et al., 2020). Briefly, whole colostrum was thawed and warmed to room temperature, thoroughly mixed, and diluted 8-fold with sterile saline warmed to 37°C. Diluted colostrum was vortexed vigorously and pipetted onto Bovine IgG plates (Triple J Farms, Kent Laboratories) according to manufacturer specifications. A colostrum sample of known concentration was included on every plate as a quality control measure. The diameter of diffusion rings was determined in duplicate using a magnifying loupe with embedded scale with 0.1 mm division (ManiPros LED).

The dry matter (DM) of colostrum was determined using procedures described previously (Mann et al., 2016). In brief, colostrum samples were thawed in a water bath at 37°C, mixed thoroughly by inversion, and 5 g of sample was placed in a dry aluminum dish, dried for 20 h at 100°C in a gravity convection oven (model 20GC, Quincy Labs), and DM (%) determined as the (net weight of the dry sample g/net fresh weight g) x 100.

Statistical Approach

Sample size was estimated based on the primary objective before the study using JMP PRO (v.16.0, SAS Institute). We wanted to be able to identify a difference of 1 kg in colostrum yield between cows that receive exogeneous oxytocin at any dose compared with control cows. Using a power of 0.9, an α level of 0.05, and a standard deviation of 3.0 kg, a sample size of 172 per group was determined. The calculated sample size was then inflated by a factor of 0.05 because of an assumed attrition rate of 5%, resulting in a pre-exclusion sample size of 531 animals.

For statistical analysis, information regarding treatment assignment, colostrum weight, Brix %, IgG concentration, colostrum DM content, parity, calf information, milk production at wk 4 of lactation (wk4 milk), date and time of calving, and date and time of enrollment was gathered into an Excel file (Microsoft 365 Office) for all enrolled animals from physical data sheets and on-farm recordkeeping software (DC305, Valley Ag Software). Statistical analysis was performed using JMP PRO (v.16.0, SAS Institute) and graphs produced in Graphpad Prism (v. 9.4.0). Analysis was stratified to analyze animals in parity 1 separately, and multiparous cows were grouped into 3 parity groups of parity 2, 3, and ≥4 as a covariate for the multiparous models. Cows with gestation length shorter or longer than 2 standard deviations from the median (<263, > 288 d) were excluded from analysis.

Days dry was divided into quantiles <49 (n = 100), 49–53 (n = 93), 54–58 (n = 79), 59–65 (n = 77), and >65 d (n = 84). Gestation length was divided into 3 categories in primiparous (265 to 271 d [n = 31], 272 to 278 d [n = 122], 279 to 287 d [n = 50) and multiparous cows (263 to 275 d [n = 97], 276 to 281 d [n = 281], 282 to 288 d [n = 92]). Dystocia was categorized into none (score 1, n = 577)) or any degree of dystocia (scores 2 and 3, n = 68). Calf sex was divided into singleton female (n = 402), male (n = 208), and twins (n = 35). Twins were excluded from analysis as weight information was not obtained for both calves routinely. Calf birth weight associations were only investigated for singleton calves and categorized as 24 to 35 (= 37), 35.1 to 40 (n = 134), 40.1 to 45 (n = 214), 45.1 to 50 (n = 138), and 50.1 to 60 (n = 50) kg. Milk production at wk 4 was divided into the following 4 categories in primiparous cows (≤30, 30.1 to 35, 35.1 to 40, and 40.1 to 45 kg) and multiparous cows (≤40, 40.1 to 50, 50.1 to 60, and 60.1 to 70 kg).

Population characteristics were described by treatment, and where applicable, by parity group. Differences in enrollment across treatments were analyzed with Fisher's exact test or one-way ANOVA, respectively.

For the analysis of the primary objective, the effect of oxytocin treatment on colostrum yield, IgG concentration, Brix%, DM, and total IgG, a mixed effects ANOVA were used with the fixed effects of treatment, parity group and interaction of parity group with treatment (in case of multiparous animals), and lag time (min) between calving and colostrum harvest, as well as enrollment block as a random effect.

For the analysis of the secondary objective, the association of cow characteristics with colostrum yield, IgG yield and concentration, the effect of calf sex, calf weight, dystocia, stillbirth, wk4 milk, as well as days dry, days carried calf, days in the closeup group, and previous lactation LS at dry-off for multiparous cows, were first considered in a separate model. Each model was controlling for the effect of treatment, lag time (min) between calving and colostrum harvest, as well as enrollment block as a random effect. Data for primiparous and multiparous cows were again considered separately, and parity group was forced into all models for multiparous animals regardless of *P*-value. All variables of interest with association with yield/IgG concentration at a level of $P < 0.10$ were then examined in a single model with backward stepwise elimination until all remaining variables had $P < 0.05$.

Pairwise comparisons of least squares means (LSM) were corrected with the Tukey's procedure to account for multiple comparisons, and different superscript letters ^{a,b,c,d} indicate pairwise comparison $P < 0.05$. Results are presented as LSM and 95% CI unless otherwise noted. Model assumptions of homoscedasticity and normality of residuals were visually assessed.

For the relationship between Brix% and IgG concentration, a simple linear regression analysis was performed. In addition, colostrum IgG concentration was dichotomized into <50g/L and ≥50 g/L, as well as <100g/L and ≥100 g/L and a logistic regression analysis with ROC was performed to identify the best cut point for classification, as well as the sensitivity and specificity at the determined cut point. Sensitivity and specificity with 95% CI at the determined sample prevalence was determined using MedCalc Statistical Software (v. 19.2.6).

RESULTS

Study Population, Exclusion of Records, Missing Data

The study was conducted for a total of 65 enrollment days between July and October 2023 with a median (range) number of 10 (3 to 19) cows enrolled/d, and a total of 657 animals enrolled. The median (SD) gestation length of the sample population was 275 (6) d, subsequently 5 animals with gestation length under 263 d and 5 animals with gestation length over 288 d were excluded from analysis, correcting the median (range) gestation length to 279 (263 to 288) days. For 11 enrolled animals, colostrum samples were missed, and these animals were removed from the data set. For 2 additional animals, colostrum was weighed but no sample was saved for analysis and for 39 calves BBW was not recorded; these cows remained in the data set, resulting in a total of 636 animals for final analysis. The sample population characteristics are summarized in Table 1.

Colostrum Production

The median (range) colostrum yield for all animals in the study was 6.0 (0 to 20.6) kg, a complete lack of colostrum (0 kg) was only recorded for 2 animals (parity 1, CNTR). The median (range) concentration of IgG was 98.5 (0.1 to 293.6) g/L, total IgG in colostrum was 451 (0.1 to 4,039) g, and the overall Brix % in the study was 23.6 (4.1 to 38.7). The DM of colostrum in the study was 23.5 (11.4 to 43) %.

Effects of oxytocin on colostrum yield, Brix, IgG concentration, total IgG, and Dry Matter

In primiparous animals, OXY40 had a higher colostrum yield (LSM [95% CI]) of 5.4 (4.9 to 5.9) kg compared with both OXY20 (4.1 [3.5 to 4.7] kg) and CNTR (3.8 [3.3 to 4.3] kg) ($P < 0.001$, **Figure 1**). Treatment did not have an effect on colostrum yield in multiparous cows ($P = 0.43$, **Figure 1**).

Table 1. Characteristics of the sample population (n = 236) enrolled in the study and randomized to receiving 40 IU oxytocin (OXY40), 20 IU oxytocin (OXY20), or no oxytocin (CNTR) intramuscularly immediately before colostrum harvest

Sample characteristic (n; median [range])	OXY40 (n = 223)	OXY20 (n = 195)	CNTR (n = 218)	OVERALL (n = 636)	P[1]
Parity					0.72
1	72	66	63	201	
2	49	45	54	148	
3	35	33	45	113	
≥4	67	51	56	174	
Days dry					0.61
2	63 (48 to 146)	65 (50 to 136)	64 (455 to 152)	64 (48 to 152)	
3	50 (43 to 84)	50 (40 to 58)	50 (38 to 119)	50 (38 to 119)	
≥4	51 (38 to 149)	51 (40 to 144)	49 (43 to 120)	50 (38 to 149)	
Dry off linear					0.46
2	0.7 (0.1 to 8.4)	0.8 (0 to 7.9)	0.9 (0 to 6.1)	0.8 (0 to 8.4)	
3	2 (0.1 to 5.6)	1.8 (0.1 to 4.1)	1.7 (0.1 to 5.4)	1.8 (0.1 to 5.6)	
≥4	2.6 (0 to 8.7)	2.3 (0.1 to 7.4)	3.2 (0 to 8)	2.5 (0 to 8.7)	
Parity-specific gestation					0.62
1	276 (265 to 287)	276 (265 to 287)	274 (267 to 285)	275 (265 to 287)	
2	278 (263 to 288)	279 (265 to 287)	278 (269 to 285)	278 (263 to 288)	
3	279 (271 to 287)	279 (271 to 288)	278 (267 to 288)	278 (267 to 288)	
≥4	280 (267 to 287)	279 (263 to 287)	279 (271 to 285)	279 (263 to 287)	
Calf sex					0.35
Female	139	121	135	395	
Male	66	65	73	204	
Twin	18	8	9	35	
Missing record	—	1	1	2	
Birth body weight (kg)					0.56
Female	40.8 (30.4 to 54.4)	41.7 (24.5 to 54.4)	41.3 (29.9 to 54.4)	40.8 (24.5 to 54.4)	
Male	45.8 (34.5 to 59.0)	45.4 (27.2 to 54.4)	45.5 (32.7 to 53.5)	45.4 (27.2 to 59.0)	
Missing record (n)	12	8	19	39	
Stillbirth					0.58
Singleton	3	3	4	10	
Twins	1	4	1	6	
Calving Score					0.06
1	192	182	193	567	
≥1	31	1	23	67	
Missing record	—	—	2	2	

[1]P-value describes testing for differences in sample characteristics across treatment groups.

Treatment did not influence colostrum IgG concentration or Brix % in primiparous ($P > 0.56$) or multiparous cows ($P > 0.56$) as shown in **Figure 2**. Treatment also did not have an effect on total IgG in colostrum in primiparous ($P = 0.33$), or multiparous cows ($P = 0.73$). Likewise, DM did not differ by treatment in primiparous ($P = 0.96$) or multiparous cows ($P = 0.21$) (**Figure 2**).

Screening of Variables associated with Colostrum Yield, IgG Concentration, and total IgG

Parity Effect and Days Dry. Colostrum yield (kg) was higher in parity 2 (7.1 [6.6 to 7.7][a]) compared with all other parities: parity 1 (4.4 [4.0 to 4.9][b]), parity 3 (4.8 [4.2 to 5.5][b]) and parity ≥4 (5.1 [4.6 to 5.6][b] ($P < 0.001$). Colostrum IgG concentration (g/L) in parity 1 (84.3 [78.8 to 89.8][a]) and parity 2 (84.2 [77.8 to 85.5][a]) were both lower than in parity 3 (117.9 [110.6 to 125.2][b]) and ≥4 had the greatest concentration among all groups (139.1 [133.2 to 137.4][c]) ($P < 0.001$). Total IgG in colostrum (g) was highest in parity ≥ 4 (661 [596 to 725][a]) compared with parity 3 (508 [431 to 586][b]), and both were not different from parity 2 (580 [511 to 649][ab]), but parity 1 had the lowest yield overall (377 [322 to 432][c]) ($P < 0.001$).

The category of dry period length showed a relationship with colostrum yield ($P < 0.001$), such that cows dry for less than 49 d had the lowest yield (kg) (4.1 [3.4 to 4.8][a]), followed by dry period lengths of 49–53 d (5.7 [3.9 to 5.4][ab]), 54–58 d (5.9 [5.2 to 6.7][bc]), 59 to 65 d (6.9 [5.2 to 6.7][cd]), and ≥65 d (7.5 [6.7 to 8.2][d]).

The dry period categories showed an inverse relationship with IgG concentration (g/L) ($P = 0.04$) compared with yield such that cows dry for less than 49 d had the highest IgG concentration (123.7 [114.1 to 133.3][a]), and the lowest concentration dry for over 65 d (103.7 [93.6 to 113.9][b]), whereas cows dry in all other categories were not different from other groups (49 to 53 d: 120.0 [110.1 to 129.9]; 54 to 58 d: 109.1 [99.5 to 118.8]; 59 to 65 d: 110.3 [97.5 to 122.6][ab]) did not differ from the other categories.

Total IgG (g) differed between dry period categories ($P < 0.001$) such that cows dry for less than 49 d had the lowest total IgG (446 [358 to 534][a]), followed by cows 49–53 d (491 [400 to 583][ab]), 54–58 d (583 [492 to 673] [bcd]), 59 to 65 d (703 586 to 820][cd]), and over 65 d (758 [664 to 853][d]).

Days in the close-up group was not associated with colostrum yield, IgG yield, or IgG concentration ($P \geq 0.35$).

Gestation Length, Calf Sex and Weight. Gestation length, calf sex and BBW were not associated with colostrum yield ($P > 0.10$), IgG concentration ($P > 0.10$), or total IgG yield ($P > 0.11$) in primiparous animals.

In multiparous cows, gestation length was associated with colostrum yield (kg) (263 to 275 d: 5.1 [4.4 to 5.8][a]; 276 to 281 d: 5.9 [5.1 to 6.1][ab]; 282 to 288 d: 6.4 [5.7 to 7.1][b], $P = 0.02$) as well as colostrum IgG concentration (g/L) (263 to 275 d: 120 [111 to 129][a]; 276 to 281 d: 116 [109 to 122][ab]; 282 to 288 d: 106 [96 to 115][b], $P = 0.04$). Gestation length was not associated with total IgG yield ($P = 0.67$).

Calf sex was associated with colostrum yield such that cows giving birth to a singleton female calf had lower colostrum yield (5.1 [4.6 to 5.6] kg) than those giving

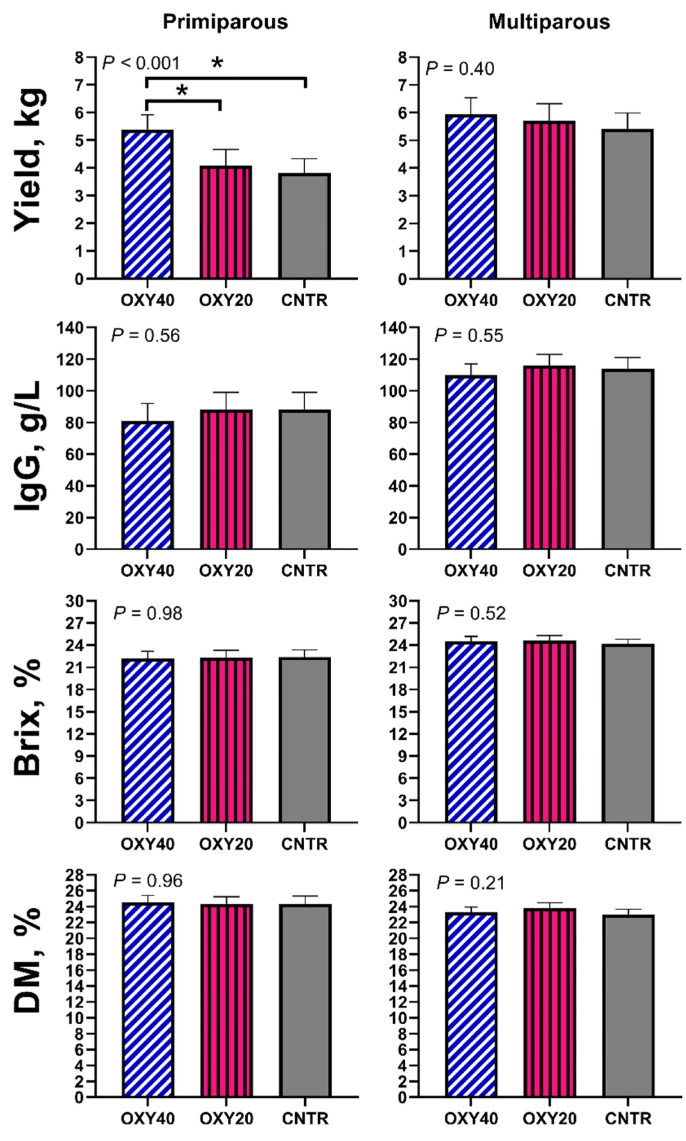

Figure 1. Yield, IgG concentration, Brix and dry matter (DM) in colostrum harvested from primiparous (n = 201) and multiparous cows (n = 435) treated intramuscularly with 40 IU oxytocin (OXY40), 20 IU oxytocin (OXY20), or no treatment at the time of colostrum harvest. P-values shown for treatment effect from mixed effects ANOVA with the fixed effects of treatment, parity group (multiparous model only), lag time from calving to milking and the random effect of enrollment block. * denotes pairwise Tukey's posthoc test corrected comparison with $P < 0.05$.

birth to a male calf (6.2 [5.6 to 6.8] kg, $P = 0.002$). Calf sex did not have an association with IgG concentration ($P = 0.48$). Cows giving birth to a male calf had higher total IgG (640 [534 to 813]) than cows giving birth to female calves (525 [466 to 585], $P = 0.02$).

Calf BBW was associated with colostrum yield (kg) of multiparous cows ($P < 0.0001$), such that with increasing weight categories of the calf, colostrum yield increased (24 to 34.9 kg: 3.8 [2.1 to 5.5][ab], 35 to 39.9 kg: 4.3 [3.5 to 5.2][a], 40 to 44.9 kg: 5.4 [4.8 to 6.0][ab], 45 to 49.9 kg: 6.0 [5.4 to 6.7][bc], 50 to 60 kg: 7.3 [6.4 to 8.2][c] kg). Calf weight was not associated with IgG concentration of multiparous cows ($P = 0.95$), but calf weight was associated with total IgG yield (g) ($P < 0.001$) (24 to 34 kg: 396 [204 to 589][ab]; 35.1 to 40 kg: 458 [363 to 553][a]; 40.1 to 45 kg: 541 [476 to 607][ab]; 45.1 to 50 kg: 631 [560 to 702][bc]; 50.1 to 60: 732 [628 to 836][c]).

Effect of Dystocia and Stillbirth. Dystocia and stillbirth were not associated with colostrum yield, IgG concentration, or total IgG in either primiparous or multiparous animals ($P \geq 0.13$).

Previous Lactation Linear Score at Dry Off. The last LS of the previous lactation in multiparous cows did not show to have any effect on either outcome ($P \geq 0.15$).

Milk production in wk 4

Milk production at wk 4 did not have an association with colostrum yield in primiparous cows ($P = 0.18$). Milk production at wk 4 was associated with multiparous colostrum yield ($P < 0.001$) such that cows with the highest production at wk 4 had the greatest colostrum yield (kg) (60.1 to 70 kg: 8.0 [7.0 to 9.0][a]), followed by cows in the second highest group (50.1 to 60: 6.0 [5.6 to 6.5][b]), compared with cows producing 40.1 to 50 kg (4.6 [4.0 to 5.1][c]), and for cows producing ≤40.0 kg (5.0 [3.7 to 6.2][bc]).

Milk production in primiparous animals at wk 4 did not have an association with colostrum IgG concentration ($P = 0.55$) or total IgG yield ($P = 0.15$). Milk production at wk4 tended to be associated with IgG concentration in multiparous cows ($P = 0.09$), but total IgG of multiparous cows was associated ($P < 0.001$) with wk4 milk production, and followed a similar pattern of differences as colostrum yield with the highest yield (g) in the highest producing group (60.1 to 70 kg: 830 [707 to 953][a]), the second highest IgG yield in the group producing 50.1 to 60 kg (611 [552 to 670][b]), and the lowest yield in the group 40.1 to 50 kg (462 [394 to 530][c]), with no difference in the lowest milk yield group (≤40 kg: 614 [461 to 766][abc]).

Multivariable Models for the Outcomes Colostrum Yield, IgG Concentration, and Total IgG. For primiparous cows and the outcome of colostrum yield, treatment was the only variable of interest (**Table 2**). A multivariable model was not built for IgG concentration and total IgG yield as none of the variables of interest entered the model.

For multiparous cows, an initial multivariable model with the variables parity group, days dry category, milk production at wk 4, gestation length, calf sex, and calf weight category was created for the outcome of colostrum yield, and all variables of interest except gestation length remained in the model which was controlled for the effect of treatment, lag time from calving to harvest, as well as enrollment block as a random effect (**Table 2**).

For multiparous cows, an initial multivariable model was built for IgG concentration. Parity and days dry remained as the only variables of interest controlled for the effect of treatment, lag time from calving to harvest, as well as enrollment block as a random effect (**Table 3**). For the outcome total IgG yield, an initial multivariable model with the variables parity group, days dry category, milk production at wk 4, calf sex, and calf weight category was created and all variables of interest except for BBW remained in the model which was controlled for the effect of treatment, lag time from calving to harvest, as well as enrollment block as a random effect (**Table 4**).

Association of Brix with IgG. The relationship between Brix% and IgG concentration showed an R^2 of 0.63 (**Figure 2**). Out of the 633 samples, 69 (10.9%) and 319 (50.4%) had an IgG concentration <50 g/L and <100 g/L, respectively. The AUC of the ROC for colostrum classified as <50 and ≥50 g/L had a value of 0.957, and the identified cut-point to maximize correct classification was 20.6%. At this cut-point, sensitivity (95% CI)

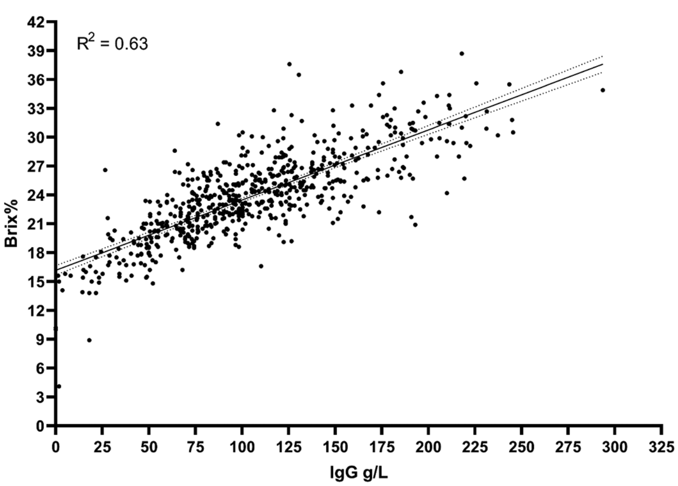

Figure 2. Relationship between colostrum IgG concentration determined by radial immunodiffusion (IgG g/L), and Brix % determined by digital refractometry in 633 colostrum samples. R^2 value was determined by linear regression analysis. Solid line shows the fit of the simple linear regression, dotted lines represent 95% confidence intervals.

Table 2. Mixed effects multivariable model for variables associated with colostrum yield (kg) in primiparous (n = 201) and multiparous (n = 371) Holstein cows

	Primiparous[1]		Multiparous[2]	
	LSM (95% CI)	*P*	LSM (95% CI)	*P*
Treatment		0.002		0.52
OXY40	5.4 (4.5 to 6.1)[a]		6.0 (5.3 to 6.9)	
OXY20	4.0 (3.3 to 4.8)[b]		5.9 (5.2 to 6.6)	
CNTR	3.8 (3.0 to 4.5)[b]		5.6 (4.9 to 6.2)	
Parity				0.04
2			6.7 (5.9 to 7.5)[a]	
3			5.3 (4.5 to 6.2)[b]	
≥4			5.4 (4.8 to 6.1)[b]	
Calf Sex				0.001
Female			5.4 (4.8 to 6.0)[a]	
Male			6.3 (5.6 to 6.9)[b]	
Dry period length (d)				<0.001
<49			4.1 (3.2 to 4.9)[a]	
49 to 53			4.8 (4.0 to 5.6)[ab]	
54 to 58			5.9 (5.1 to 6.7)[bc]	
59 to 65			6.8 (5.8 to 7.8)[cd]	
>65			7.6 (6.7 to 8.4)[d]	
Milk production wk 4 (kg)				<0.001
≤40			4.4 (2.9 to 5.9)[ab]	
40.1 to 50			4.7 (4.1 to 5.3)[a]	
50.1 to 60			6.1 (5.6 to 6.6)[b]	
60.1 to 70			8.1 (7.1 to 9.1)[c]	

[1]Lag time from calving to harvest and enrollment block were included as a covariate, R^2 adjusted = 0.01.
[2]Lag time from calving to harvest and enrollment block were included as covariates, R^2 adjusted = 0.32.

(i.e., the proportion of correctly identified samples < 50 g/L) was 92.8 (83.9 to 97.6) % (64/69) and specificity (the proportion of correctly identified samples ≥50 g/L) was 87.2 (84.2 to 89.9) % (491/563).

For the classification of <100 and ≥100 g/L the AUC of the ROC was 0.878 and the Brix cut point was 23.9%. At this cut-point, sensitivity was 82.8 (78.2 to 86.7) % (264/319) and specificity was 77.4 (72.4 to 82.0) % (243/314).

DISCUSSION

Use of oxytocin for colostrum harvest

Our first objective was to investigate the effect of exogenous oxytocin on colostrum yield, IgG concentration, Brix, and DM measurements. Endogenous oxytocin release following stimulation of the teat leads to plasma concentrations in the 20–50 pg/mL range in mid-lactation cows (Lollivier et al., 2002). Exogenous oxytocin administered intramuscularly at doses of 20 to 40 IU must be considered supraphysiological as a similar dose of 50 IU increased plasma oxytocin above the physiological range within approx. One min after injection, and led to peak concentrations approx. 3–4 times the upper physiological value (Mačuhová et al., 2004). In our study, use of the higher dose of oxytocin was associated with a higher colostrum yield in primiparous animals only, whereas we did not see differences in IgG concentration, or DM in primiparous or multiparous animals. These findings contrast with results reported by Sutter et al. (2019) where IgG concentration of colostrum (measured by ELISA) from cows receiving 20 IU oxytocin intramuscularly 3 min before udder stimulation was approx. Six g/L higher than in the control group. To explain the difference in IgG concentration, the authors hypothesized that oxytocin use could have altered tight junctions and IgG transfer during milking, a process by which the blood-milk barrier (BMB) integrity could be reduced transiently to

Table 3. Mixed effects multivariable model for variables associated with colostrum IgG concentration (g/L) in multiparous (n = 371) Holstein cows

	Multiparous[1]	
	LSM (95% CI)	*P*
Parity		<0.001
2	89 (80 to 99)[a]	
3	114 (105 to 124)[b]	
≥4	136 (129 to 144)[c]	
Dry period length (d)		0.03
<49	124 (114 to 133)[a]	
49 to 53	120 (110 to 130)[ab]	
54 to 58	109 (99 to 119)[ab]	
59 to 65	110 (98 to 123)[ab]	
>65	104 (94 to 114)[b]	

[1]Lag time from calving to harvest, treatment ($P = 0.40$), and enrollment block were included as a covariate, R^2 adjusted = 0.37.

Table 4. Mixed effects multivariable model for variables associated with total IgG yield (g) in multiparous (n = 371) Holstein cows

	Multiparous[1]	
	LSM (95% CI)	*P*
Parity		<0.01
2	546 (443 to 649)[a]	
3	599 (497 to 701)[ab]	
≥4	716 (635 to 796)[b]	
Calf Sex		0.03
Female	573 (502 to 645)[a]	
Male	667 (589 to 745)[b]	
Milk production wk 4 (kg)		<0.001
≤40	572 (384 to 760)[abc]	
40.1 to 50	485 (417 to 553)[a]	
50.1 to 60	614 (558 to 670)[b]	
60.1 to 70	810 (384 to 760)[c]	
Dry period length (d)		<0.001
<49	465 (360 to 571)[a]	
49 to 53	514 (413 to 615)[a]	
54 to 58	593 (492 to 694)[ab]	
59 to 65	732 (604 to 859)[bc]	
>65	796 (360 to 571)[c]	

[1]Lag time from calving to harvest, treatment ($P = 0.59$), enrollment block were included as a covariate, R^2adjusted = 0.13.

allow additional transfer of IgG from the blood into the colostrum. However, the dose of exogenous oxytocin to induce loss of BMB integrity has not been conclusively determined for cattle, but is thought to exceed 20 IU (Wellnitz and Bruckmaier, 2021). Our study did not identify any differences at the same oxytocin dose as used in (Sutter et al., 2019) and doubling of the oxytocin dose led to numerically lower, rather than higher IgG concentrations, as well as no difference in total IgG yield. The lack of difference in Brix and DM measurements also indicates that the composition of colostrum was not greatly altered. We interpret these findings to mean that 40 IU of oxytocin administered intramuscularly immediately before milking was not a high enough dose to alter the BMB in our study. Although the BMB is leaky during colostrogenesis and still during the first days of lactation (Wall et al., 2015), this does not appear to play a role for the transcellular transfer of IgG from blood into milk. In addition, very high dosages of exogenous oxytocin (100 IU) have been shown to only slightly alter IgG concentrations in milk through reduced BMB integrity established lactation (Wall et al., 2016). The existing knowledge on the physiology of the BMB together with our findings suggest that BMB integrity did not play a role in the observed results.

However, differences in study design might explain the discrepancies. In the study by Sutter et al. (2019), cows were individually milked in a chute immediately after calving rather than in a rotary parlor at the next scheduled milking time.

Reasons for the higher colostrum yield in primiparous animals and a lack of differences in multiparous animals can be explained by the well documented ability of exogenous oxytocin to overwrite the inhibition of endogenous oxytocin release by stress (Bruckmaier and Blum, 1998). First lactation animals are known to experience greater difficulties adapting to the novel stimuli when milked compared with multiparous cows (Kness et al., 2023). However, this effect was only seen at the higher oxytocin dose, possibly due to the short lag time between injection and the beginning of the milking process, particularly since oxytocin was given intramuscularly, and not intravenously.

Association with cow factors

Our second objective was to examine the associations of cow characteristics with colostrum and IgG yield as well as IgG concentration.

We showed here that calf sex was associated with colostrum yield in multiparous cows. In an observational study including 5,790 primiparous and 12,553 multiparous cows on 18 New York dairy farms, Westhoff et al. (2023a) found that both heifers and cows giving birth to male calves produced a greater colostrum quantity than those giving birth to females and that calf birth weight was also associated with increased yield. Similarly, Sutter et al. (2019) and Conneely et al. (2013) showed that increasing calf birth weight was associated with higher colostrum yield. As reviewed in Westhoff et al. (2024), the relationship of calf characteristics with colostrum yield of the dam could be mediated by pregnancy-related endocrine differences related to calf sex and overall conceptus weight, although we are not aware of work that has tested this specific hypothesis. Stillbirth was not associated with colostrum outcomes in this study as opposed to previous reports reviewed by Westhoff et al. (2024) showing a negative association with colostrum yield and IgG concentration. Given the smaller sample size of this current study, as well as the low percentage of stillborn calves, we were likely lacking the power to identify this difference if it existed in the study herd.

Multiparous cows in 3rd as well as ≥4th parity had higher colostrum IgG concentration and total IgG yield, whereas cows in parity 2 a substantially greater yield compared with all other groups, and an equally low IgG concentration as parity 1 animals. The association of increasing parity and higher IgG concentration has been reported previously with the same significant increase in parity 3 and greater (Conneely et al., 2013; Sutter et al., 2019). Reasons underlying this difference may be the increased circulating IgG concentrations in older cows (Herr et al., 2011), but age may also play a role in IgG transfer capacity. The association of parity with colostrum yield in this herd was influenced by the difference in dry period management of animals in parity 2, but this group produced the greatest yield of colostrum even after controlling for dry period length. This finding is consistent with results of other studies differing in management systems (Conneely et al., 2013; Westhoff et al., 2023a) but differs from others where this group had the lowest yield (Sutter et al., 2019). Given the effect of dry period management and nutrition on colostrum yield (Westhoff et al., 2024), these differences likely reflect farm-specific differences.

One of the best documented management factors with consistent relationship with colostrum production is dry period length. With increasing dry period length, colostrum and IgG yield increased whereas IgG concentration decreased. The increase in colostral yield with increasing dry period length is consistently reported in experimental and observational studies as reviewed by Westhoff et al. (2024). The difference between the shortest and longest dry period category of about 20 g/L is possibly due to a partial dilution effect. However, total IgG yield in the longest dry period category was 1.7-fold that of the shortest dry period category, suggesting a greater total IgG transfer into the mammary gland in animals with longer dry period. This finding is of interest since the mass transport of IgG occurs in the last days to weeks before calving (Bigler et al., 2023) and suggests that some of the IgG transport into the mammary gland occurs any time in late pregnancy as long as the cow is not milked, supporting the assumption that accumulation of IgG in the mammary gland likely occurs much earlier than the month before parturition (Baumrucker and Bruckmaier, 2014). However, the timeframe and hormonal signals that determine the IgG transfer are still not sufficiently understood (Bigler et al., 2023).

Milk production at wk 4 was positively associated with colostrum yield as well as total IgG yield in this study. The association of colostrum yield with previous lactation or current lactation milk yield indicators has either shown a positive association or no relationship as recently reviewed (Westhoff et al., 2024)

Few reports are available on the association of udder health and colostrum outcomes (Westhoff et al., 2024). Our results suggest that colostrum production and milk production potential are linked to each other. In this study, we used dry off LS as a proxy for udder health status and found no association with colostrum yield and IgG concentration. Investigations into the potential effect of udder health on colostrogenesis are sparse (Maunsell et al., 1998; Enger et al., 2021) but generally do not suggest an effect of udder health on colostrum outcomes. However, the effect of mastitis on colostrogenesis may differ in relation to an individual herd's mastitis risk and merits further investigation in appropriately large study populations.

Only 2 primiparous animals had a complete absence of colostrum at harvest, a mere 0.3% of the entire study population, or 1% of the primiparous group, whereas no multiparous cows (0%) had 0-kg colostrum. This number is much lower than the 3.1 and 5.5% of primiparous and multiparous animals reported recently for 19 Holstein farms in New York State (Westhoff et al., 2023a), and the 0.3 and 6.0% primiparous and multiparous Jerseys reported by Gavin et al. (Gavin et al., 2018). A potential difference is that study personnel assessed colostrum yield in this study directly, and any production of colostrum yield was recorded as such, whereas a small amount might be recorded as 0-kg by farm personnel. Additionally, the study was conducted from July to October, and therefore sample collection excluded some of the months of the year with the highest known odds for 0-kg production (Westhoff et al., 2023a).

Relationship of Colostrum Brix and IgG determined by RID

Our third objective was to describe the relationship between Brix and IgG measured by RID in colostrum in a sample set of high variability in IgG concentrations. In our data set, IgG ranged from 0.1 to almost 300 g/L, the prevalence of samples <50 g/L was 10%, and the prevalence of samples <100 g/L was 50%. Results demonstrated the high variability of colostrum IgG concentration similar to Conneely et al. (2013), making this a valuable data set to explore the relationship of Brix and IgG.

The magnitude of the simple linear relationship of R^2 = 0.63 was in the previously reported range of 0.43 to 0.81 (0.81 estimated by likelihood ratio test of a mixed linear model (Quigley et al., 2013), as well as 0.43 (Bartier et al., 2015), 0.53 (Bielmann et al., 2010; Morrill et al., 2012), 0.56 (Quigley et al., 2013), 0.69 (Kessler et al., 2021), and 0.79 (Molla, 1980; Röder et al., 2023)) determined by correlation or by simple linear regression), demonstrating that Brix measurements are a good approximation of IgG concentrations.

Commonly reported cut-off values for determining adequate colostrum quality (≥50 g/L IgG) in different sample sets range from 18 to 23% (Godden et al., 2019), consistent with the 20.6% reported for our sample. Cut-off values determined in sample sets for high-quality colostrum (≥100g/L IgG) are rare, but establishing a specific cut-off for this IgG concentration could be useful for producers to alter the amount of colostrum fed while still achieving IgG intake. Similar to the 23.9% identified in this sample of dairy cows, Breuer et al. (2023) found 23.8% as the cut-off at a level of 100 g/L colostral IgG in beef cows. As discussed by Buczinski and Vandeweerd

(2016a) and Buczinski et al. (2021), the choice of the Brix cut-off for on-farm management should ideally be based on meta-analysis to reduce the influence of small sample sizes of individual studies on cut-off determination (Bhandari et al., 2021). The choice of cut-off within reported ranges should also consider producer preferences. If the producer prioritizes the likelihood of selection of good quality colostrum at the expense of discarding some good colostrum then a higher Brix cut-off can be chosen. If the producer wants to be more accurate in discarding only poor-quality colostrum at the expense of feeding some poor-quality colostrum, then a lower Brix cut-off can be chosen. As discussed by Buczinski and Vendeweerd (Buczinski and Vandeweerd, 2016b) 22% could be used to achieve the former, and 18% the latter. We encourage producers and veterinarians to define the most appropriate Brix cut-off individually for each herd, colostrum feeding strategies, and management priorities.

Study Limitations and Future Research

Interpretation of the presented findings should consider certain study limitations. First, the study was conducted on one commercial dairy farm in central New York. Our results likely reflect what would happen on dairy operations with similar management and colostrum harvesting approach in a rotary parlor in this same region, but external validity is limited until findings are replicated in other systems and geographic regions. Second, the study was conducted during the summer and fall months, whereas no data were obtained during the spring and winter months. As reported by Westhoff et al. (2023a), colostrum yield is seasonal. Therefore, authors that investigate the effect of oxytocin administration on colostrum quantity and quality in the future could consider enrolling dairies with different management and milking systems in different regions over the course of a year to study the possible interaction of oxytocin use with season on colostrum yield and quality. Third, in this study, we used 2 different doses of oxytocin. One is the oxytocin label dose for milk letdown (20 IU), and the other dose (40 IU) was based on the study farm's current protocol for colostrum harvest. Similarly, the route of administration and timing of injection were chosen in accordance with the existing milking routine, feasibility, and considerations for safety of research and farm personnel. Future research investigating different doses, routes of administration, and timing of injection is needed to complement the other available studies on this topic.

CONCLUSIONS

Our study showed that primiparous animals milked for the first time in a rotary parlor had greater colostrum yield when given 40 IU of oxytocin IM immediately before milking. We speculate that this difference was due to the presence of inhibition of oxytocin release and hence disturbed milk letdown in this group of animals which is overcome by use of exogenous oxytocin. Oxytocin did not affect colostrum IgG, Brix, or dry matter in primiparous or multiparous animals. Lower parity, male sex of the calf, higher milk production at wk 4 of lactation, and longer length of dry period were associated with colostrum yield in multiparous cows, and greater parity and shorter dry period length were associated with increased IgG concentration. Although dilution might explain the opposite effects seen in these cow factors, higher parity, male calf sex, higher milk production at wk 4, and longer dry period length showed the highest total IgG yield. Our study confirmed that the relationship of Brix and [IgG] is sufficiently accurate, and 20.6 and 23.9% were identified as cut-offs to best classify colostrum at a level of < 50 g/L or 100 g/L in this herd.

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