Effect of colostral volume, interval between calving and first milking, and photoperiod on colostral IgG concentrations in dairy cows

Dawn E. Morin, DVM, MS, DACVIM; Stephanie V. Nelson, DVM; Eric D. Reid, MS; Dusty W. Nagy, DVM, PhD, DACVIM; Geoffrey E. Dahl, PhD; Peter D. Constable, BVSc, PhD, DACVIM

Objective—To identify cow and management factors associated with colostral IgG concentration in dairy cows.

Design—Prospective observational study.

Animals—81 multiparous Holstein-Friesian cows from a single herd.

Procedures—Serum was obtained at the start of the nonlactating period, and cows were assigned to 1 of 4 photoperiod groups: natural day length (n = 22 cows), long days (16 h of light/d [21]) or short days (8 h of light/d [20]) for the entire nonlactating period, or natural day length followed by short days for the last 21 days of the nonlactating period (18). Serum and colostrum were collected at the first milking after calving. Regression analysis was used to investigate associations between colostral IgG concentration and the interval between calving and first milking, colostral volume, photoperiod, length of the nonlactating period, and season of calving.

Results—Colostral IgG concentration decreased by 3.7% during each subsequent hour after calving because of postparturient secretion by the mammary glands. The interval between calving and first milking and the colostral volume were significantly and negatively associated with colostral IgG concentration, with the former effect predominating. Photoperiod had no effect on colostral IgG concentration or volume. Serum protein concentration at calving correlated poorly with colostral IgG concentration.

Conclusions and Clinical Relevance—Dairy producers should harvest colostrum as soon as possible after calving to optimize transfer of passive immunity in neonatal calves. Photoperiod can be manipulated without adversely affecting colostral IgG concentration.

Neonatal calves must ingest colostrum during the first day after birth to acquire passive immunity via the active uptake of maternal IgG across the intestinal epithelium. Suboptimal transfer of passive immunity in dairy calves results in an increased risk of morbidity and death. Approximately 64% of US dairy producers manually feed a fixed volume of colostrum to calves, which makes the IgG concentration of colostrum an important determinant for the adequacy of transfer of passive immunity. Approximately 36% of US dairy producers allow a calf to acquire colostral IgG by suckling its dam; these producers rely on a calf ingesting a sufficient mass of IgG to ensure adequate transfer of passive immunity, which in turn depends on colostral IgG concentration and the volume ingested.

Numerous cow and management factors impact colostral IgG concentration. These factors include lactation number, breed, yield of colostrum at first milking, timing of first milking relative to calving, environmental temperature, and season. Although colostral IgG concentration appears to decrease as colostral volume increases and as the interval between calving and first milking increases, the relationship between colostral IgG concentration and time of first milking after calving has not been completely characterized, with studies being conducted more than 20 years ago or involving different mammary glands within cows. Some investigators have found no relationship between colostral IgG concentration and the interval from calving to first milking, provided the first milking was accomplished within 12 h of birth. From the Department of Veterinary Clinical Medicine (Morin, Nelson, Nagy, Constable), College of Veterinary Medicine, and Department of Animal Sciences (Reid, Dahl), College of Agricultural, Consumer, and Environmental Sciences, University of Illinois, Urbana, IL 61802. Dr. Nelson’s present address is Pittsburgh Veterinary Specialty and Emergency Center, 807 Camp Horne Rd, Pittsburgh, PA 15237. Dr. Reid’s present address is The Old Mill Troy Inc, 37 Wollert Ave, Menands, NY 12204. Dr. Nagy’s present address is the College of Veterinary Medicine, University of Missouri, Columbia, MO 65211. Dr. Dahl’s present address is the Department of Animal Sciences, College of Agriculture, University of Florida, Gainesville, FL 32611. Dr. Constable’s present address is the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907. Supported by the Cooperative State Research, Education, and Extension Service, USDA (grant No. NE 1009); and the Illinois Council for Food and Agricultural Research. Dr. Nelson was supported by the Merck-Merial Summer Veterinary Program administrated by the Center for Zoones Research at the University of Illinois. The authors thank John Scott, Jennifer Dauderman, and Heather Hill for technical assistance.

Address correspondence to Dr. Constable (constabl@purdue.edu).
milking was performed within 8 hours or 9 hours after calving. Additional studies to examine the relationship between colostral IgG concentration and the interval from calving to first milking are needed.

Studies also are needed to evaluate the effect of photoperiod on colostral IgG concentration. Housing cows in short-day conditions (8 h of light/d) during the nonlactating period increases milk production in the subsequent lactation and augments a number of cellular immune responses at calving, compared with results for cows housed in long-day conditions (16 h of light/d). Because photoperiod manipulation is increasingly being used as a noninvasive management tool to increase milk production, it is important to determine the effect of photoperiod on colostral volume and composition, including IgG concentration.

Colostral IgG is derived predominantly from maternal plasma by selective transport of IgG, particularly IgG1, across mammary epithelial cells. The transport of IgG into bovine colostrum has been associated with a transient decrease in serum total protein, globulin, and IgG concentrations during the parturient period, particularly in dairy cows. The rate of IgG movement into plasma and the extent of expansion of plasma volume during pregnancy can also impact serum protein concentrations.

Therefore, the main objectives of the study reported here were to characterize the effects of colostral volume and the interval from calving to first milking on colostral IgG concentration and to determine the effects of photoperiod during the nonlactating period on colostral volume, IgG concentration, and nutrient composition. Secondary objectives of the study were to compare serum concentrations of total protein, total globulin, IgG, non-IgG globulin, and albumin on the day of calving with those at the start of the nonlactating period and to determine whether any of these factors were sufficiently correlated with colostral IgG concentration to be useful for predicting the colostral IgG concentration. Results of the study should be beneficial for helping dairy producers make informed decisions about management practices that impact the quality of colostrum fed to calves.

Materials and Methods

Animals—Multiparous Holstein-Friesian cows (n = 81) were obtained from a local commercial dairy farm. The cows were pregnant (approx 7 months of gestation); ending lactation at this stage of gestation would provide an estimated nonlactating period of 60 days before anticipated parturition. Cows were transported to the University of Illinois Dairy in 3 groups, which arrived during the months of January (n = 25 cows), May (25), and October (31). All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee.

Health-care program—Cows were given a complete physical examination at arrival and were assessed to be healthy. All 4 mammary glands of each cow were infused with cephapirin benzathine at arrival. Cows were injected with a multivalent vaccine containing chemically altered strains of infectious bovine rhinotracheitis and parainfluenza-3 viruses, modified-live bovine respiratory syncytial virus, killed cytopathic and noncytopathic strains of bovine viral diarrhea virus, and 5 serovars of Leptospira; cows also were injected with a bacterin containing the J-5 strain of Escherichia coli. The multivalent vaccine was administered again 2 weeks after the end of lactation, and the E.coli bacterin was administered again 4 weeks after the end of lactation. At 3 and 6 weeks after the end of lactation, cows were injected with a vaccine containing killed rotavirus, coronavirus, and K99 E.coli and Clostridium perfringens type C toxoid.

Experimental procedures—Cows were allocated to 1 of 4 pens (6 or 7 cows/pen) located in a light-controlled barn. Allocation was based on a randomization procedure (ie, random number generator), after blocking for milk production during the preceding lactation. The pens contained sand-bedded free stalls, concrete alleyways, automatic waterers, and a feeding system for individual provision of a total mixed ration. Photoperiod was the only variable that differed among pens. Pens were separated by floor-to-ceiling walls that eliminated incident light. Each pen had separate light controls. Metal halide lamps (6 lamps/pen) provided a minimum intensity of 250 lux at eye level of the cows (measured 1 m [39.37 inches] above the floor). Cows exposed to long-day photoperiod (n = 21) were provided 16 h of light/d for the entire 60-day nonlactating period. Cows exposed to short-day photoperiod were provided 8 h of light/d for the entire 60-day nonlactating period (n = 20) or were exposed to natural day length for the first part of the nonlactating period and to short-day photoperiod for only the last 21 days of the nonlactating period (n = 22). The remainder of the cows (n = 22) were exposed to a photoperiod that mimicked ambient lighting conditions for natural day length. Time of sunrise and sunset for Champaign County, Ill (longitude, 88.2° west; latitude, 40.1° north), were obtained from the US Naval Observatory website and used to adjust light duration at weekly intervals to match ambient conditions. Cows were moved into individual straw-bedded box stalls within the group pens when the estimated date of calving approached. Each calf remained with its dam until the cows were returned to the commercial dairy farm on the day of calving.

Physical examinations were performed every 2 weeks during the nonlactating period to confirm that cows remained healthy. Cows were monitored throughout the day and evening as the estimated date of calving approached, and parturition was assisted when necessary. The exact time of parturition was recorded for 56 cows.

A complete physical examination was performed after calving. Cows were milked with a portable milking machine at a standardized time of day, which resulted in a large range in the interval between calving and first milking. Weight of the colostrum obtained at the first milking was determined by use of a hanging scale.

Sample collection—A blood sample was collected from the coccygeal vein of each cow into a clot tube at the time of arrival, and a second sample was collected at the time of first milking after calving. Serum was
harvested and frozen at –20°C for subsequent analysis of IgG and protein concentrations. A 50-ml aliquot of first-milking colostrum was obtained for each cow and frozen at –20°C for subsequent IgG determination, and a 40-ml aliquot was obtained and tested to determine fat and protein concentrations.

**Sample analysis**—Serum samples obtained at the end of lactation and at calving were analyzed to determine concentrations of total protein (biuret method) and albumin (bromocresol green method) by use of an autoanalyzer. Serum total globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration.

A commercial radial immunodiffusion kit was used to measure total IgG concentration in serum and colostrum. Serum was thawed at 19° to 22°C, and 5 µL of serum then was transferred into wells of an immunodiffusion plate. Colostrum was thawed at 19° to 22°C and diluted 1:6 with sterile saline (0.9% NaCl) solution, and 5 µL of diluted colostrum then was transferred into wells of the immunodiffusion plate. Three reference samples were included on each plate to enable us to generate a standard curve. Plates were allowed to incubate for 24 hours at 19° to 22°C before results were interpreted. The diameter of each ring was measured, and IgG concentrations were calculated by extrapolation from the standard curve.

Serum concentration of non-IgG globulin was estimated by subtracting the serum IgG concentration from the calculated serum concentration of total globulin. Colostral IgG mass was calculated by multiplying the colostral IgG concentration by the volume of colostrum obtained at first milking. The number of cows with a colostral IgG mass < 153 g was determined; this cutoff point was selected because a minimum of 153 g of colostral IgG is required for optimum colostral transfer of immunoglobulins when calves are fed 3 L of colostrum at 2 hours after birth. Volume of colostrum was calculated by dividing colostral weight (as measured in pounds) by 2.27. Colostral protein concentration was determined via infrared spectrophotometry.

**Statistical analysis**—Data were summarized as mean ± SD. Pearson correlation coefficients were used to determine linear relationships between continuous variables. Differences in serum variables between the end of lactation and calving were determined by use of paired t tests. The effects of photoperiod during the nonlactating period (4 levels) and cow group (3 levels; used as a proxy for season of calving) on serum and colostral variables were evaluated by use of an ANOVA. The Tukey Studentized range test was used for between-group comparisons whenever there was a significant F test for photoperiod.

Univariable regression analysis and forward and backward stepwise multivariable linear regression analyses were used to identify the most important factors associated with colostral IgG concentration. A value of $P < 0.20$ was required for entry into forward stepwise regression analysis, and a value of $P < 0.05$ was required to remain in forward and backward regression analyses. For all other analyses, significance was defined at values of $P < 0.05$. A statistical software program was used for all analyses.

### Results

Cows remained healthy throughout the nonlactating period; the nonlactating period ranged from 40 to 106 days (mean ± SD, 62 ± 9 days). Serum total protein, albumin, total globulin, and non-IgG globulin concentrations were lower at calving than at the end of lactation, but serum IgG concentration was similar at both times (Table 1).

The interval between calving and first milking for the 56 cows for which time of parturition was recorded ranged from 0.3 to 23.8 hours (mean ± SD, 10.9 ± 6.7 hours). Colostral values for all cows were summarized (Table 2).

The effects of colostral volume and the interval from calving to first milking on colostral IgG concentrations were evaluated by use of regression analysis. Analysis of residual plots during regression analysis indicated that

<table>
<thead>
<tr>
<th>Variable</th>
<th>End of lactation</th>
<th>Calving</th>
<th>Difference</th>
<th>$P$ value*</th>
</tr>
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<tbody>
<tr>
<td>Total protein (g/dL)</td>
<td>7.91 ± 0.38</td>
<td>6.71 ± 0.66</td>
<td>−1.20 ± 0.54</td>
<td>&lt; 0.001</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>3.89 ± 0.27</td>
<td>3.60 ± 0.28</td>
<td>−0.29 ± 0.26</td>
<td>&lt; 0.001</td>
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<tr>
<td>Total globulin (g/dL)</td>
<td>4.01 ± 0.45</td>
<td>3.11 ± 0.53</td>
<td>−0.91 ± 0.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Albumin-to-globulin ratio</td>
<td>0.99 ± 0.15</td>
<td>1.19 ± 0.21</td>
<td>0.20 ± 0.17</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IgG (g/dL)</td>
<td>1.52 ± 0.40</td>
<td>1.43 ± 0.37</td>
<td>−0.09 ± 0.42</td>
<td>0.066</td>
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<tr>
<td>Non-IgG globulin (g/dL)</td>
<td>2.50 ± 0.42</td>
<td>1.68 ± 0.34</td>
<td>−0.82 ± 0.47</td>
<td>&lt; 0.001</td>
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</table>

*Values were considered significant at $P ≤ 0.05$ (paired t test).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cows</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (kg [lb])</td>
<td>76</td>
<td>7.0 ± 4.9 (15.4 ± 10.8)</td>
<td>0.5–30.4 (1.1–67.0)</td>
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<tr>
<td>IgG concentration (g/L)</td>
<td>78</td>
<td>41 ± 25</td>
<td>8–121</td>
</tr>
<tr>
<td>IgG mass (g)</td>
<td>75</td>
<td>248 ± 185</td>
<td>11–702</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>55*</td>
<td>9.8 ± 3.0</td>
<td>4.3–15.5</td>
</tr>
</tbody>
</table>

*Physical properties of the colostrum prevented accurate analysis in some samples.
the best fit was obtained with the logarithm (base 10) of the colostral IgG concentration. Univariable regression analysis revealed a significant association between the log_{10} colostral IgG concentration and the interval between calving and first milking \((P = 0.001; n = 56\) cows; Figure 1) and between the log_{10} colostral IgG concentration and colostral volume \((P = 0.004; \text{Table 3}); Figure 2\). Variables evaluated by use of forward and backward stepwise linear regression included interval between calving and first milking, colostral volume, serum IgG concentration at calving, serum IgG concentration at end of lactation, and duration of the nonlactating period. Colostral volume and the interval between calving and first milking were the only variables that remained in the final regression model \(R^2 = 0.27; n = 56\) cows; Table 3). An interaction term between colostral volume and interval between calving and first milking did not enter the model, which indicated that the colostral volume and the interval between calving and first milking each exerted independent and additive effects on the log_{10} colostral IgG concentration. This was consistent with the finding that IgG mass in colostrum was independent of the interval between calving and first milking (Figure 3). However, there was marked variability in the total mass of IgG in colostrum, with total IgG content in first-milking colostrum ranging from 11 to 681 g. Seventeen of 56 (30\%) cows had a total IgG mass < 153 g. Considered together, analysis of these results indicated that IgG concentration in colostrum decreased by 3.7\% during each subsequent hour after calving as a result of postparturient secretion by the mammary glands of a fluid with a much lower IgG concentration than that of colostrum.

Colostral IgG concentration was weakly but significantly correlated \((r = 0.35)\) with serum total protein, total globulin, and non-IgG globulin concentrations at the end of lactation; however, colostral IgG concentration was not correlated with serum protein concentrations at calving (Table 4). Neither colostral volume (data not shown) nor

![Figure 1](image1.png)  
**Figure 1**—Scatterplot of the relationship between colostral IgG concentration and the interval between calving and first milking for 56 multiparous Holstein-Friesian cows. The fitted regression line (solid line) reveals a significant correlation \(R^2 = 0.18; P = 0.001\). The equation for the regression line is as follows: \(\text{log}_{10}\text{IgG concentration} = 1.751 - 0.0162 \times \text{time}\), which is equivalent to IgG concentration \(= 10^{(1.751 - 0.0162 \times \text{time})}\), with time in hours. The logarithmic relationship indicates that the colostral IgG concentration decreased by 3.7\% for each additional hour after calving.

![Figure 2](image2.png)  
**Figure 2**—Scatterplot of the relationship between colostral IgG concentration and colostral volume at first milking for 75 multiparous Holstein-Friesian cows. The fitted regression line (solid line) reveals a significant correlation \(R^2 = 0.11; P = 0.004\). The equation for the regression line is as follows: \(\text{log}_{10}\text{IgG concentration} = 1.080 - 0.0080 \times \text{volume}\), with volume measured in liters. The logarithmic relationship indicates that the colostral IgG concentration decreased by 3.7\% for each additional liter of mammary gland secretions.

![Figure 3](image3.png)  
**Figure 3**—Scatterplot of the relationship between total IgG mass in colostrum and the interval between calving and first milking for 56 multiparous Holstein-Friesian cows. A linear relationship was not identified between 

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate</th>
<th>SE</th>
<th>P value*</th>
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<tr>
<td>y-intercept (g/L)</td>
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<tr>
<td>Interval between calving</td>
<td>-0.0139</td>
<td>0.0046</td>
<td>0.006</td>
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<tr>
<td>and first milking (h)</td>
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<tr>
<td>Colostral volume (L)</td>
<td>-0.0137</td>
<td>0.0062</td>
<td>0.031</td>
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*Values were considered significant at \(P \leq 0.05\). NA = Not applicable.

The recommended IgG mass (153 g) that should be fed to a Holstein-Friesian calf during the first 2 hours after birth to optimize the transfer of passive immunity is indicated (horizontal dashed line). Notice that 17 of 56 (30\%) cows had a total IgG mass < 153 g.

Table 3—Final multivariable regression model for factors associated with log_{10} colostral IgG concentration in samples obtained at the first milking after calving from 56 multiparous Holstein-Friesian cows.
colostral IgG mass was correlated with serum protein concentrations at the end of lactation or at calving.

Serum total protein, albumin, total globulin, IgG, and non-IgG globulin concentrations at the end of lactation were only moderately correlated (r = 0.41 to 0.77) with their respective serum concentrations at calving (Table 4). Serum total protein concentration at the end of lactation was most highly correlated with serum total globulin (r = 0.81) and non-IgG globulin (r = 0.58) concentrations and only weakly correlated with serum IgG concentration (r = 0.29; Table 2). In contrast, serum total protein concentration at calving was correlated (r = 0.57) only with serum IgG concentration. Serum IgG concentration was more highly correlated with total globulin concentration at calving (r = 0.77) than at the end of lactation (r = 0.51). Serum IgG concentration was weakly correlated (r = 0.39) with serum non-IgG concentration at the end of lactation but not at calving. Serum IgG concentration was negatively correlated with serum albumin concentration at both the end of lactation (r = −0.44) and at calving (r = −0.23).

Effect of photoperiod—Eight cows were removed from the analyses involving photoperiod because they calved sooner or later than anticipated and therefore were not exposed to the photoperiod treatments for the appropriate amount of time. The number of cows exposed to the long-day photoperiod for the entire 60-day nonlactating period, short-day photoperiod for the entire 60-day nonlactating period, short-day photoperiod for the last 21 days of the nonlactating period, and natural day length for mean ± SD colostral IgG concentration (34 ± 20 g/L, 45 ± 31 g/L, 45 ± 23 g/L, and 41 ± 24 g/L, respectively [P = 0.71]) or for mean ± SD colostral weight (8.1 ± 4.2 kg [18.7 ± 9.2 lb], 7.2 ± 4.8 kg [19.8 ± 10.6 lb], 5.9 ± 3.1 kg [12.1 ± 6.8 lb], and 7.1 ± 7.1 kg [15.6 ± 15.6 lb], respectively [P = 0.50]). Time of year did not significantly affect any of the measured variables, except for mean ± SD serum albumin concentration at calving (P = 0.045), which was slightly lower in cows arriving in May and calving in July (3.5 ± 0.33 g/dL) than in cows arriving in October and calving in December (3.7 ± 0.22 g/dL).

Discussion

Major findings of the study reported here included that colostrum should be harvested as soon as possible after calving to ensure that colostral IgG Concentration is as high as possible and that there is marked variability in the IgG mass in colostrum. Furthermore, photoperiod can be manipulated without adversely affecting colostral IgG concentrations.

The amount of colostrum harvested at the first milking after calving and the IgG concentration of colostrum varied widely among cows in the study reported here. Cows yielded between 0.5 and 30.0 kg (1.1 and 66.0 lb) of colostrum, with IgG concentration ranging from < 10 to 120 g/L; the large range in IgG concentration was similar to that reported in dairy cows in Norway and beef cows in the United States. Both volume and IgG concentration influence the total mass of IgG in the colostrum. The IgG concentration of the colostrum has the greatest effect on the mass of IgG fed and therefore the effectiveness of transfer of passive immunity because most calves on US dairy farms are fed a fixed amount of colostrum by bottle, bucket, or esophageal feeder. The most important factors associated with colostral IgG concentration in the study reported here were the interval between calving and first milking and the colostral volume, with the interval between calving and first milking having a more important role. Analysis of our results indicated that colostral IgG concentration decreased by 3.7% during each subsequent hour after calving, which implies that the interval between calving and first milk-

<table>
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<tr>
<th>Variable</th>
<th>Colostal IgG (g/dL)</th>
<th>Colostal IgG mass (g)</th>
<th>Serum total protein at EL (g/dL)</th>
<th>Serum albumin at EL (g/dL)</th>
<th>Serum IgG at EL (g/dL)</th>
<th>Serum non-IgG globulin at EL (g/dL)</th>
<th>Serum IgG at calving (g/dL)</th>
<th>Serum albumin at calving (g/dL)</th>
<th>Serum total protein at calving (g/dL)</th>
<th>Serum non-IgG globulin at calving (g/dL)</th>
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<td>Serum albumin at calving (g/dL)</td>
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<td>Serum non-IgG globulin at calving (g/dL)</td>
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Value reported is r. NS = Not significantly (P ≥ 0.05) correlated.

Table 4—Correlations between colostral IgG concentration, colostral IgG mass, and serum protein concentrations in samples obtained from 81 multiparous Holstein-Friesen cows at the end of lactation (EL) and at calving.
ing should be as short as possible. The interval between calving and first milking was negatively correlated with IgG concentration for dairy calves in Denmark ($r = -0.35^6$) and in Germany ($r = -0.19^2$); a similar negative correlation ($r = -0.42$) between the interval between calving and first milking was detected for the $\log_{10}$ IgG concentration in the study reported here.

The substantial decreases in colostral IgG concentration associated with a delay in the interval from calving until first milking and with increased colostral volume are clinically relevant because Holstein-Friesian calves should be fed at least 153 g of total IgG within the first 2 hours after birth.$^{35}$ According to a national survey,$^7$ US dairy producers who manually feed colostrum to calves do not administer the first feeding until a mean $\pm$ SE of $3.3 \pm 0.1$ hours after birth. The findings of the study reported here will not enable dairy producers to predict the absolute IgG concentration in a given colostrum sample, but they will enable them to appreciate the magnitude of impact that management decisions have on colostral IgG concentration and optimization of transfer of passive immunity. The interval between calving and first milking and the colostral volume accounted for only 27% of the variability in colostral IgG concentration among cows, which indicated that other unidentified factors are potentially more important than these 2 factors. One known factor, lactation number, could not be assessed in the study reported here because records were not available from the producer. The negative association between colostral IgG concentration and colostral volume, independent of the effect of the interval between calving and first milking, provides strong support to the view that dilution by accumulation of mammary gland secretions provides an important contribution to low colostral IgG concentrations in Holstein-Friesian cows.$^{36}$ Additional studies should be conducted to determine whether our findings can be applied in general to first-lactation cows.

The marked variability in IgG mass in colostrum (Figure 3), with 30% of cows producing an IgG mass $< 153$ g, probably plays an important role in the incidence of failure of transfer of passive immunity in dairy calves. One potential cause for the marked variability in IgG mass in colostrum from dairy cows is the $\beta_M$ genotype 2.2, which is expressed by 9% of US beef cattle$^{10}$ and an unknown percentage of US dairy cattle. The $\beta_M$ genotype 2.2 is associated with an 11-fold increase in the incidence of failure of transfer of passive immunity in beef calves, although it has not been determined whether impaired transfer of IgG, into colostrum in dams or decreased intestinal absorption of suckled IgG, by calves.$^{37}$ We believe that the results of the study reported here emphasize the marked cow-to-cow variability in colostral IgG mass in dairy cattle, with a calculated CV for IgG mass of 67% for Holstein-Friesian cows (n = 56 cows). The large variability in IgG mass was similar to that in cows of the Black and White Danish breed (CV = 69% [n = 71 cows])$^6$ and the Red Danish breed (CV = 53% [60])$^6$ and in unaffected mammary glands of Holstein-Friesian cows (CV = 57% [66]).$^{39}$

The findings of our study support the application of an inexpensive and accurate on-farm test for measure-

ment of colostral IgG concentrations to decrease the prevalence of inadequate transfer of passive immunity, which was 17% in US dairy calves in 2007.$^{41}$ Currently available on-farm methods for estimating colostral IgG concentration (such as a hydrometer, refractometer, or immunoassay) are too expensive (ie, immunoassay) or have inadequate sensitivity (eg, hydrometer sensitivity $= 0.37$ or 0.47)$^{42}$ for identifying colostrum with a low IgG concentration ($< 50$ g/L). However, specificity of the hydrometer is sufficiently high (specificity $= 0.93$ or 0.94)$^{43}$ for use as an on-farm method to identify colostrum with a low IgG concentration. In other words, a hydrometer should be used to identify poor-quality colostrum that should be discarded. The net result of a test-discard strategy based on a hydrometer value is that some good-quality colostrum will be discarded. The total volume discarded will not impact the overall success of a colostral testing program because a dairy calf should be fed 3 L of colostrum within 2 hours after birth, and the mean weight of colostrum obtained 2 hours after birth is 8.8 kg (19.4 lb)$^{35}$; use of hydrometer testing in this manner in 1 study$^{42}$ resulted in the availability of 6.2 L (hydrometer 1) or 6.8 L (hydrometer 2) of good-quality colostrum that could be fed to each calf, assuming each dam gave birth to a single live calf. The major limitations with hydrometer or refractometer testing of colostrum are that colostral specific gravity more closely reflects colostral protein concentration than it does IgG concentration and that it is markedly influenced by the month of calving.$^{45}$

It is important to mention that calves in the study reported here were allowed to stay with their dams until they were returned to the farm of origin on the day of calving. Therefore, it is possible that some calves sucked 1 or more mammary glands before colostrum was harvested. However, sucking was rarely observed by study personnel who were in the barn most of the time. Suckling could have contributed to the effect of interval between calving and first milking on colostral IgG concentration because sucking presumably would have been more likely to occur the longer a calf was with its dam. However, a study$^{19}$ in which individual mammary glands within cows were milked 2, 6, 10, and 14 hours after calving revealed that colostral IgG concentrations decrease significantly over time, even without sucking. In that study,$^{19}$ colostral IgG concentrations in samples collected 6, 10, and 14 hours after calving were 17%, 27%, and 33% lower, respectively, than were concentrations in samples collected 2 hours after calving; those measured values were similar to predicted decreases of 14%, 26%, and 36% determined by use of a nonlinear regression equation developed in the study reported here (IgG concentration = $10^{(1.751 - 0.0162 \times \text{time})}$), with time measured in hours. Our results may be most applicable to the 44% of US dairy herds that do not remove calves from their dams immediately after birth$^2$; colostrum is manually fed in some of those herds, similar to the study reported here, whereas producers in other herds rely totally on a calf sucking its dam to obtain IgG.

Serum total protein concentration was lower at calving than at the end of lactation in cows in the study reported here, which is consistent with findings reported elsewhere.$^{13,30}$ For example, in 4 cows monitored over the course of a year, serum total protein concentration
was highest approximately 1 month before calving and lowest at calving, with a 10% to 30% decrease during the late nonlactating period.30 The decrease in serum total protein concentration that is evident during the last 3 to 4 weeks before calving is generally attributed to a reduction in serum IgG concentration that results from movement of IgG from the serum into the colostrum. For example, mean serum IgG concentration decreased from 0.84 g/dL at approximately 1 month before calving to 0.67 g/dL at calving in 15 beef cows.13 A more marked decrease was detected in 13 Holstein cows, with the mean serum IgG concentration decreasing from 0.57 to 0.14 g/dL; the larger decrease in serum IgG concentration in dairy cows than in beef cows was attributed to movement of a larger total mass of IgG into colostrum. It should be mentioned that mean total IgG concentration (ie, the sum of IgG1 and IgG2 concentrations) at calving in the multiparous cows in the study reported here was 1.68 g/dL.

The decrease in serum total protein concentration between the end of lactation and calving in the study reported here cannot be attributed to a decrease in serum IgG concentration. In contrast, serum IgG concentrations were similar at the end of lactation and at calving, whereas serum concentrations of albumin and non-IgG globulin were lower at calving than at the end of lactation. The decrease in serum total protein, albumin, total globulin, and non-IgG globulin concentrations between the end of lactation and calving may have been attributable, in part, to expansion of the plasma volume during late gestation.31 However, expansion of the plasma volume should dilute all serum proteins to the same extent; this was not the case in our study because the change in serum albumin concentration was less than the change in total globulin and non-IgG globulin concentrations. This suggests that globulins other than IgG were selectively transported into colostrum or that the periparturient increase in fat percentage of the liver impacted hepatic production of proteins. In another study,31 a decrease in β1-globulin concentrations in maternal serum was detected beginning approximately 5 weeks before calving and continuing until calving, with no change in serum concentrations of albumin or α-, β2-, or γ1-globulins; however, the decrease in β2-globulin concentrations was much less substantial than the decrease in γ1-globulin concentrations (ie, IgG1 concentrations because electrophoresis did not allow quantification). In another study,28 the mean serum concentration of γ-globulins was 20% higher 1 month before calving than at calving, with the mean concentration of β- and γ-globulins combined being 46% higher and no difference in serum albumin concentration; the change in β-globulin concentration alone was not reported. Mean serum γ-globulin concentration in 8 multiparous Holstein cows was 3.23 g/dL at the end of lactation, compared with 1.87 g/dL at calving, which was a decrease of approximately 40%, whereas serum albumin concentration was not different at the 2 time points.27 All isotypes of immunoglobulin are selectively accumulated in colostrum when indexed against albumin.23 We did not measure transport of non-IgG globulin into colostrum in the study reported here, but the minimal change in serum albumin concentration between the end of lactation and calving, compared with the change in globulin concentration, appears to be consistent with findings reported elsewhere.

One possible reason that we did not detect a decrease in serum IgG concentration between the end of lactation and calving in the study reported here is that the multiparous cows were injected with 11 antigens during the nonlactating period. Vaccination, especially booster vaccination, stimulates production and circulation of antigen-specific IgG. The vaccination schedule used in the study reported here is not unusual for contemporary dairy farms, so our findings may be more relevant than the results of earlier studies. To our knowledge, studies conducted to evaluate serum protein or immunoglobulin changes during the nonlactating period typically have not included information on the vaccination history.

Another possible reason that we did not detect a decrease in IgG concentration between the end of lactation and calving is that we measured total IgG concentrations rather than IgG1 concentrations. Although mammary epithelial cells express receptors for both IgG1 and IgG2 in the periparturient period, IgG2 receptors are fewer and of low affinity, compared with the high-affinity receptors for IgG1; therefore, the extent of selective transport is less for IgG2.29 Maximum entry rates into the mammary glands at 3 days and 1 day before calving were 125 and 60 g/500 kg/d for IgG1 and IgG2 in 8 cows injected with radiolabeled immunoglobulin.28 In that study,28 the ratio of IgG1 to IgG2 in colostrum was 7:1, compared with a ratio of 1:1 in plasma. Because we measured total IgG concentrations in the study reported here, the expected reduction in serum IgG concentrations might have been partially masked by relatively stable concentrations of IgG2. The half-life of IgG1 and IgG2 in serum and the rate of IgG entry into serum, neither of which were measured in the present study, would also have been expected to impact our measurements.

Photoperiod conditions during the nonlactating period had no effect on colostral yield or colostral concentrations of IgG, fat, or protein in multiparous Holstein cows in the study reported here. Cows exposed to short-day conditions during the nonlactating period had been found to yield more milk in the subsequent lactation than those exposed to long-day conditions.23,24 A similar effect on colostral yield was not detected in the study reported here; colostrum yield at the first milking after calving did not differ among cows in the 4 photoperiod groups. Short-day conditions during the nonlactating period also are associated with enhanced cellular immune responses at calving.25 To our knowledge, effects of photoperiod conditions during the nonlactating period on humoral immune responses have not been investigated. In the study reported here, photoperiod conditions during the nonlactating period had no effect on colostral IgG concentrations or serum concentrations of IgG or non-IgG globulin. Therefore, it appears that the beneficial effects of short-day photoperiods on milk yield and cellular immune responses can be achieved without adversely impacting colostral characteristics.

In the study reported here, colostral IgG concentrations decreased as the interval between calving and first...
milking increased and as colostral volume increased, with the former effect predominating. Serum total protein, globulin, and IgG concentrations in cows at calving did not correlate well with colostral IgG concentrations. Photoperiod conditions during the nonlactating period did not impact the yield or IgG concentration of colostrum harvested at the first milking after calving. Finally, we did not observe the expected decrease in serum IgG concentration between the end of lactation and calving, possibly because cows were injected with numerous antigens during the nonlactating period. To reduce the risk of failure of transfer of passive immunity in dairy calves, producers should harvest colostrum as soon as possible after a cow calves and preferably within 2 hours after calving if the cow is strong enough to permit milking. This will enable producers to feed the dam’s colostrum to its calf. Transfer of passive immunity is optimized when colostrum is fed to calves within the first 2 hours after birth.1-10

References
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From this month’s AJVR

Use of an in vitro biotinylation technique for determination of posttransfusion survival of fresh and stored autologous red blood cells in Thoroughbreds

Sean D. Owens et al

Objective—To evaluate N-hydroxysuccinimide (NHS)-biotin labeling of equine RBCs and determine posttransfusion survival of autologous equine RBCs stored in citrate phosphate dextrose adenine-1 (CPDA-1) for 0, 1, 14, and 28 days.

Animals—13 healthy adult Thoroughbreds.

Procedures—Serial dilutions of biotin and streptavidin-phycoerythrin (PE) were evaluated in vitro in blood collected from 3 horses. One horse was used to determine RBC distribution and recovery. Twelve horses were allocated to 4 groups for in vivo experiments in which blood was collected into CPDA-1. Blood was labeled with biotin and reinfused or stored at 4°C for 1, 14, or 28 days prior to labeling with NHS-biotin and reinfusion. Posttransfusion blood samples were collected 15 minutes and 1, 2, 3, 5, 7, 14, 21, 28, and 35 days after reinfusion. Biotin-labeled RBCs were detected via flow cytometry by use of streptavidin-PE. Posttransfusion lifespan of RBCs and RBC half-life were determined.

Results—Optimal biotin concentration was 0.04 pg of biotin/RBC, and the optimal streptavidin-PE ratio was 1.2 µg of streptavidin-PE/1 X 10^7 RBCs. Posttransfusion lifespan of autologous RBCs was 99, 98, 66, and 59 days after storage for 0, 1, 14, and 28 days, respectively. Storage did not result in significant alterations in RBC lifespan. Mean posttransfusion RBC half-life was 50, 45, 33, and 29 days for 0, 1, 14, and 28 days of storage, respectively.

Conclusions and Clinical Relevance—Biotin can be used to label equine RBCs for RBC survival studies. Posttransfusion survival of equine autologous RBCs was greater than previously reported. (Am J Vet Res 2010;71:960–966)