Brix refractometer scoring of immunoglobulin G in postpartum mares' colostrum

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Abstract

Newborn foals are dependent on ingestion of high-quality colostrum for their immune protection. Colostrum quality (immunuoglobulin G [IgG]) can be determined by the single radial immunodiffusion (SRID) test or estimated by Brix refractometer. Goal was to compare SRID test findings (IgG concentrations) and Brix refractometer readings (IgG percentage) in colostrum samples collected from mares during the first 12 - 18 hours postpartum. A total of 56 colostrum samples were collected from 10 postpartum mares. Postfoaling, colostrum IgG concentrations were 21,785 ± 5,592 mg/dl and IgG percentage was 27.9 ± 3.7%. At 12 hours postfoaling, IgG concentrations decreased to 1,713.9 ± 1,380.4 mg/dl and its percentage to 11.4 ± 1.6%. There was a high correlation (r = 0.9661) between SRID test results and Brix refractometry scores. In summary, Brix refractometry can be used to monitor colostrum quality.

Keywords: Mare, colostrum, immunoglobulin G, single radial immunodiffusion test, refractometer

Introduction

Passive transfer of immunoglobulins through ingestion of colostrum is critical to the survival of equine neonates.[1,2] The epitheliochorial placenta of the equine does not allow for any substantial transplacental transfer of immunoglobulins during pregnancy.[3,4] Intestinal absorption of antibodies from colostrum is highest within the first 12 hours of life and very little absorption occurs after 24 hours.[5-7]

Evaluation of colostrum quality immediately after foaling can be beneficial in predicting the efficacy of passive transfer of immunoglobulins.[8,9] Foals born to mares with good quantity and quality colostrum are more likely to have adequate passive antibody transfer. Clinical situations associated with a limited amount or poor quality colostrum include premature leakage of colostrum, inadequate mammary development in maiden mares and fescue toxicity.[10-12] Early intervention of at-risk foals by oral treatment of supplemental colostrum from a colostrum bank is an easier and less expensive alternative than intravenous plasma treatment.[12,13]

The gold standard for measurement of immunoglobulin G (IgG) concentrations in plasma or colostrum is the single radial immunodiffusion (SRID) test.[14] Unfortunately, SRID test requires ~ 18 - 24 hours to perform,[9] making it unsuitable when rapid clinical decisions have to be made. The colostrometer, a second method of colostrum evaluation, measures specific gravity or density of colostrum.[15] Although specific gravity measurements of colostrum are correlated with IgG concentrations, the colostrometer is cumbersome, the procedure is time consuming, and prone to errors due to the requirement to measure an exact volume of colostrum.[8]

Brix or sugar refractometer has been described as a rapid, inexpensive and repeatable technique to estimate the quality of equine colostrum.[8,16,17] Quality of equine colostrum as estimated by a Brix refractometer is highly correlated with the IgG content of colostrum as determined by immunoradiodiffusion,[8] or immunoturbidimetric assay.[17] In addition, the Brix refractometer score of mare colostrum collected immediately after foaling has been reported to be correlated with IgG in foal plasma collected 12 - 48 hours after birth.[17]

Other reasons for evaluation of mare colostrum besides prevention of failure of passive transfer include informed management decisions on harvesting good quality colostrum from individual mares for a frozen colostrum bank and monitoring depletion of antibodies in colostrum of mares seropositive for anti-red blood cell (RBC) antigens for the prevention of neonatal isoerythrolysis in at-risk foals.[17] It is common practice to muzzle foals born from mares that are seropositive for antiRBC antibodies for 24 hours after birth, provide an alternative source of colostrum and repeatedly strip out and discard the potentially harmful colostrum from the dam to prevent clinical disease.[18]

Objectives were to monitor the decreases in IgG content of colostrum during the first 12 - 18 hours in normal postpartum mares using SRID and Brix refractometry tests and to determine the correlation between these 2 techniques. The clinical rational was to assess whether Brix refractometry could be used in the preventive management of neonatal isoerythrolysis and in the selection of colostrum to be harvested and stored in a colostrum bank.

Materials and methods

All procedures were approved by Institutional Animal Care and Use Committee. A total of 10 late-term pregnant mares were used. All mares foaled under veterinary supervision and without assistance. An assessment of the health status of each foal was made in the early postpartum period. Foals were subsequently monitored for the time interval from birth to when the foal first stood and the time to when the foal first nursed. Continued observations were made throughout the first day to confirm that all foals were active and had nursed appropriately.

A small volume (< 1 ml) of colostrum was collected from each mare immediately after foaling and subsequently at ~ 2 hour intervals for a period of 12 - 18 hours. One drop from each fluid sample was applied to a Brix refractometer (Animal Reproduction Systems, Chino, CA) and the Brix score was recorded as a percentage.[8,16,19] The remaining colostrum samples were subsequently submitted to the Colorado Veterinary Diagnostic Laboratory for measurement of equine IgG concentrations (mg/dl) using a single radial immunodiffusion (SRID) test (Equine IgG Test Kit, Cat. # 828411, Triple J Farms, Bellingham, WA).

Data analyses

Statistical analysis was performed using R 3.6.1 statistical software. Nonlinear regression was used to fit a 4-parameter logistic curve to the data. The correlation coefficient (r value) between SRID values and Brix score was also determined. All data are presented as the mean ± standard deviation.

Results

Average intervals from birth to first standing and birth to first nursing for 10 foals were 47.9 ± 45.8 and 155.6 ± 68.2 minutes, respectively. A total of 56 colostrum samples were collected from 10 postpartum mares. Average SRID IgG concentrations of colostrum immediately after foaling was 21,785 ± 5,592 mg/dl. A steady decline in IgG was noted over time as foals started to nurse and continued nursing (Figures 1 and 2). Individually, the IgG concentrations in the colostrum of all 10 mares decreased to < 5,000 mg/dl by 9.1 ± 2.8 hours postfoaling (Figure 1). Overall, the IgG concentration decreased to 1,713.9 ± 1,380.4 mg/dl at 12 hours after foaling (Figure 2).

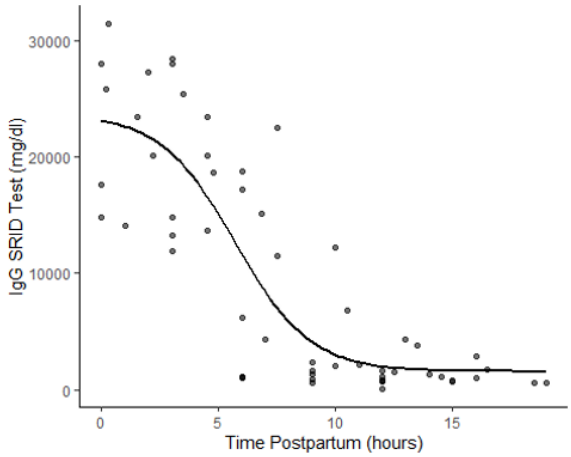

Figure 1 Concentrations of IgG (mg/dl) in equine colostrum as determined by single radial immunodiffusion test from immediately after birth to 12 - 18 hours postpartum in 10 mares; note: a nonlinear logistic regression curve was fit to data.

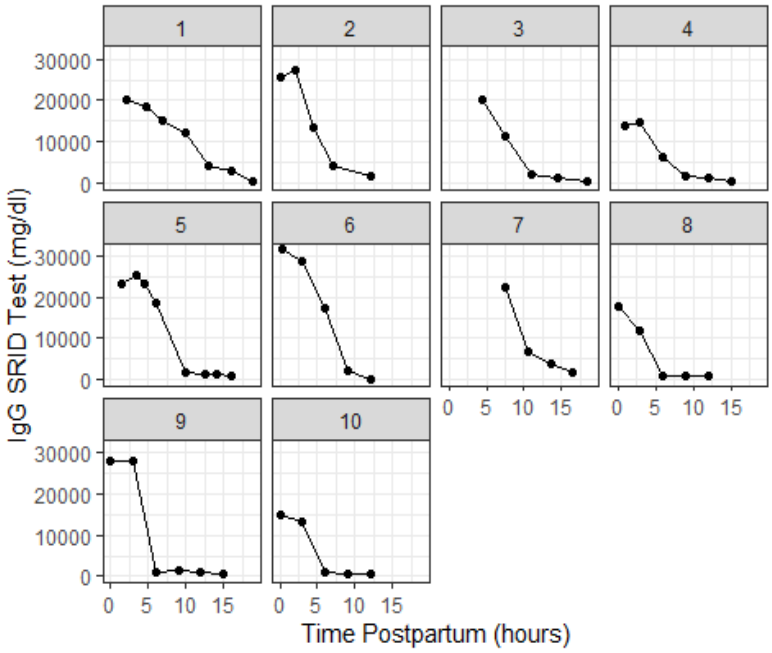

Figure 2. IgG concentrations in colostrum (mg/dl) for 10 individual postpartum mares as determined by SRID test

Average Brix refractometry score of colostrum samples collected immediately after foaling was 27.9 ± 3.7%. Refractometry score declined rapidly over time as foals were nursed (Figures 3 and 4). The Brix percentage decreased to < 15% by 9.3 ± 3.0 hours postfoaling and was 11.4 ± 1.6% at 12 hours postfoaling.

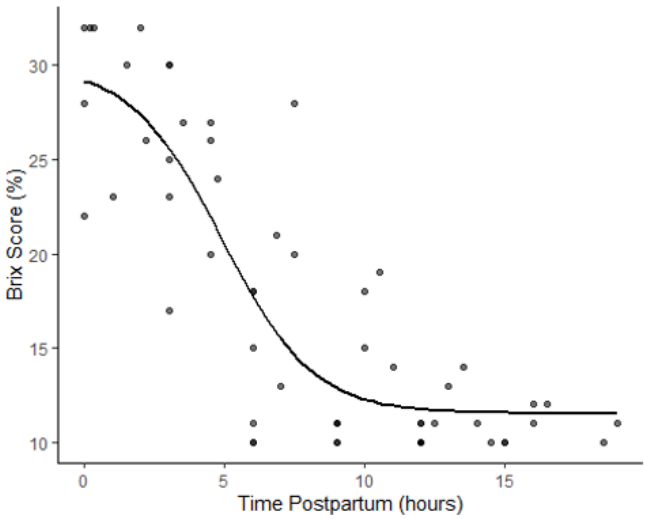

Figure 3. Brix refractometry score (%) for equine colostrum from immediately after birth to 12 - 18 hours in 10 mares; note: a nonlinear logistic regression curve was fit to data.

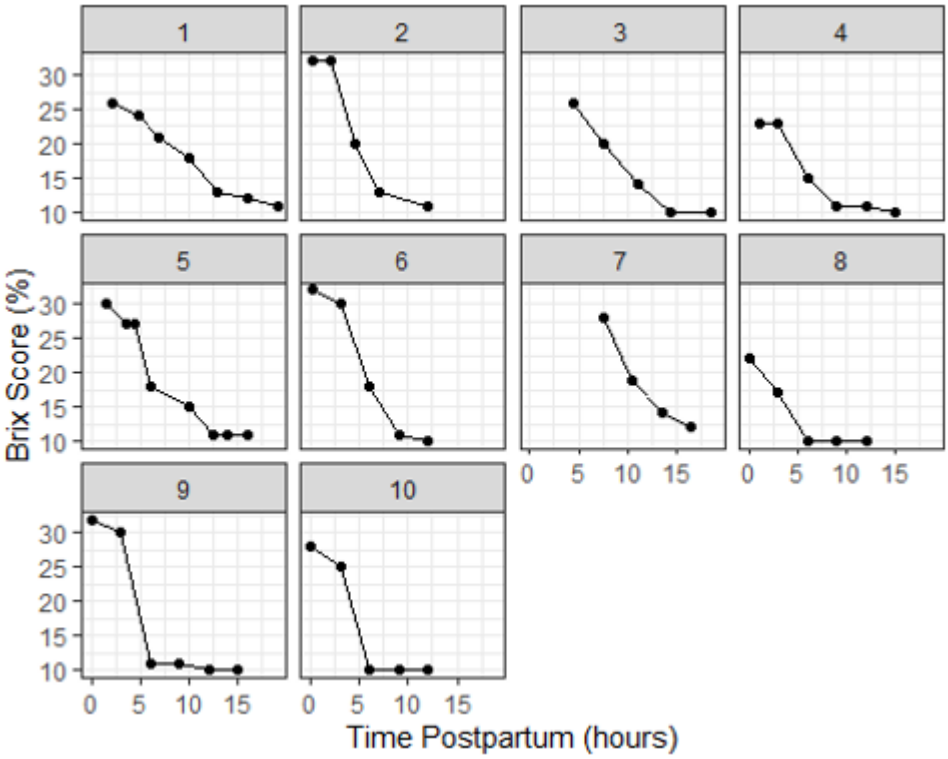

Figure 4. Brix refractometry score (percentage) for colostrum from 10 individual postpartum mares

The correlation coefficient (r) between SRID values and Brix scores for all 56 mammary fluid samples was 0.9661 (Figure 5).

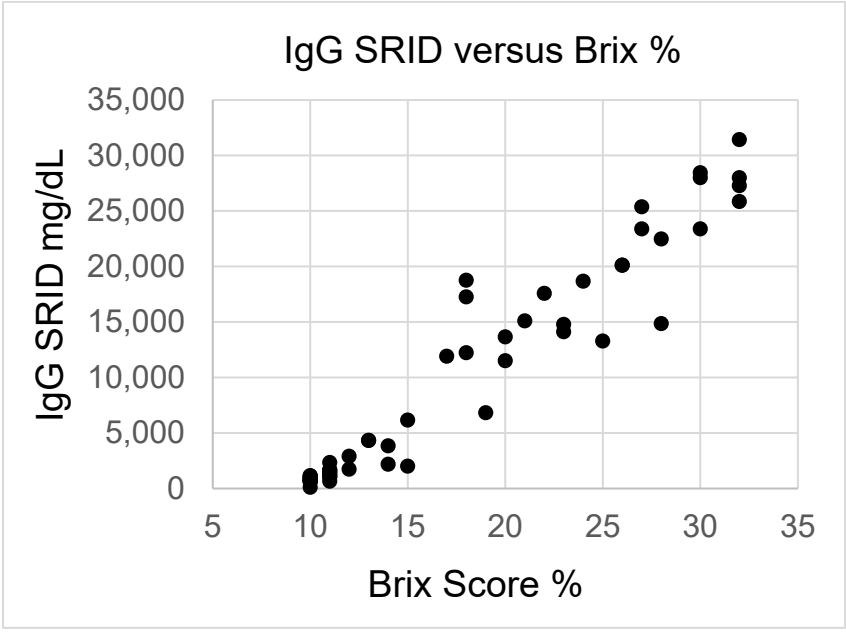

Figure 5. Relationship between IgG content measured by SRID test (mg/dl) and Brix refractometry score (percentage)

Discussion

Mares begin to produce colostrum during last 2 weeks of pregnancy by sequestering antibodies from their blood into fluid produced within alveoli of the mammary gland.[20] Production of new colostrum decreases or ceases at the time of foaling or shortly thereafter.[21] The high antibody content of colostrum is gradually depleted as the foal nurses and the fluid volume is replaced by production of milk. The transition of fluid within the mammary gland from colostrum to milk occurs over the first 24 hours in a normal postpartum mare.

Results confirmed that Brix refractometry scores are highly correlated to a quantitative measurement of IgG in colostrum as determined by SRID test. Brix refractometry had the advantages of being ultra-rapid, simple to perform, low cost, and easy to interpret. Primary clinical indication for routine use of the Brix refractometer to evaluate mare colostrum is in the prevention and management of passive transfer of immunoglobulins.[12,16] Early identification of poor-quality colostrum in a postpartum mare allows for early intervention through oral supplementation with frozen-thawed colostrum from an on-site colostrum bank within the first few hours of life. Routine evaluation of colostrum quality and therapeutic intervention has largely eliminated the need for intravenous plasma transfusions on well managed equine breeding farms. Observations regarding the health and development of the neonatal foal are also critical in the prevention of failure of passive transfer. Foals generally stand within 1 hour after birth and begin to nurse within 2 hours.[22,23]

Another clinical indication for use of the Brix refractometer for immediate evaluation of colostrum in a postpartum mare is the determination of quality of the colostrum for banking.[12,24,25] In general, it is recommended that colostrum be harvested for frozen storage if the Brix score is ≥ 20 to 23%.[8,12,17] Colostrum with a Brix score of < 20% is of poorer quality and should not be banked for future use. Our study results suggest that it is best to harvest colostrum for frozen storage very early in the postpartum period and not to wait even for a few hours since suckling significantly reduced IgG content. Furthermore, IgG content in mammary fluid of mares being suckled by healthy foals are low by 9 - 12 hours postpartum and are essentially just milk and no longer considered to be colostrum.

Finally, monitoring colostrum quality using a Brix refractometer can be used as 1 component of a management plan for the prevention of clinical cases of neonatal isoerythrolysis or 'jaundice foal syndrome'. Clinical neonatal isoerythrolysis may occur if a late-term pregnant mare has antiRBC antibodies directed against her foal's red blood cell antigens (i.e. Aa, Qa, etc.) and the newborn foal ingests a substantial quantity of high-titer antiRBC colostrum.[26,27] Serotesting late-term pregnant mares during last 2 weeks prior to their due date can be used to determine if there are antiRBC antibodies present and if the foal is at risk of clinical disease.[27] Seronegative pregnant mares are at no risk of having a foal develop clinical NI. In contrast, a foal born to a seropositive mare may be at risk, depending on the red blood cell antigen type of the foal and the type and titer of antiRBC antibodies in the mare.

Laboratory tests used to identify foals born from seropositive mares and therefore at risk of developing NI include hemolytic and agglutination cross-matching assays.[28] The jaundice foal agglutination (JFA) test is performed by mixing red blood cells from the foal into serial dilutions of the mare's colostrum followed by centrifugation. Agglutination of RBCs in the bottom of the centrifuge tube at a dilution of 1:16 or higher is considered significant.

If a JFA test is not available, prevention of clinical disease in at-risk foals is based on prevention of foal from nursing for 24 - 36 hours and harvesting and discarding the potentially harmful colostrum from the mare.[18] The former is usually accomplished by muzzling the foal, administration of supplemental colostrum and subsequently feeding a commercial equine milk substitute. The latter is accomplished by repeatedly stripping and discarding colostrum from the mare's udder every 2 hours for 24 hours. This timeframe is based on the physiological assumption that the foal cannot absorb any significant quantity of antibodies after 24 hours post birth.

Conclusion

Brix refractometry score for equine colostrum was highly correlated with the IgG concentration as determined by the single radial immunodiffusion assay. Evaluation of colostrum quality in the

immediate postpartum period is valuable in determination of which colostrum to harvest for banking and which newborn foals are at potential risk of failure of passive transfer. Monitoring colostrum quality over time may also be valuable in the management of foals at risk of neonatal isoerythrolysis. Additional laboratory and field studies are needed to confirm the relationship between the Brix refractometer score and specific antiRBC antibody concentration in mare colostrum.

Conflict of interest

None to declare.

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