Evaluation of IgG Concentration in Foals with Failure of Passive Transfer after Administration of Intravenous Serum or Plasma

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Plasma treatment in foals resulted in greater increases in post-transfusion serum IgG concentrations compared with serum treatment. The results of this study suggest that similar foal serum IgG concentrations can be achieved 3 days post-transfusion by administering 1 unit of plasma product or 2–3 units of serum product. Authors’ addresses: Avon Animal Hospital, 45 Morison Dr., Box 2406, Windsor, Nova Scotia B0N 2T0, Canada (DeLuca); Department of Health Management, Atlantic Veterinary College, 550 University Avenue, Charlottetown, Prince Edward Island C1A 1Y6, Canada (McClure and Miller); Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive West, Madison Wisconsin 53706 (Lunn). © 2001 AAEP.

1. Introduction

Foals are born virtually agammaglobulinemic because the epitheliocorial placenta precludes transfer of maternal immunoglobulins to the fetus prior to parturition. Ingestion and absorption of maternal colostrum must occur in order for a foal to obtain antibodies for protection against opportunistic and pathogenic organisms to which it is exposed shortly after birth. Colostral immunoglobulin, primarily IgG, is normally absorbed through specialized cells lining the small intestine for 18–24 h after birth.1–4 Foals that do not attain adequate quantity of colostral immunoglobulin are said to have failure of passive transfer (FPT). There are numerous circumstances in which FPT can result, such as premature lactation, orphaned foals, rejected foals, and those too weak to nurse. Other causes of FPT may be related to poor concentration of colostral antibody in the mare5 or poor absorption of antibody in the foal.6,7

In the past, serum IgG concentrations of >400 mg/dl were considered adequate in newborn foals.8 However, more recent work suggests IgG concentrations of >1000 mg/dl to be normal.9 FPT has been strongly associated with development of neonatal septicemia and an increased risk of infectious disease, especially in those foals with IgG <400 mg/dl.10–14 Therapy for FPT in foals older than 18–24 h includes administration of an exogenous source of immunoglobulin. In such situations, intravenous equine plasma has been the therapy of choice.7,15–18 However, finding a suitable donor and obtaining fresh plasma can be inconvenient and time-consuming. Frozen plasma can be purchased
commercially but is expensive and must be carefully thawed before administration. In recent years, several intravenous serum products labeled for treatment of FPT have become available. These products are less costly and, because they are pasteurized, can be conveniently stored refrigerated. However, there is currently minimal research verifying the effectiveness of such serum products. This study compares the efficacy of a commercial intravenous equine concentrated serum product\(^7\) with a commercial equine plasma product\(^6\) in the treatment of FPT. The main objective was to assess and compare the ability of each product to increase foal’s serum IgG concentration.

2. Materials and Methods

Animals

Fifty-one foals between 1 and 5 days of age were determined to have FPT based on serum IgG concentrations <800 mg/dl. Serum IgG concentrations were determined using a modified zinc sulfate turbidity test or a glutaraldehyde coagulation test. Foals admitted into the clinical trial had nursed normally from their dams and had not received exogenous IgG. Transfusions were performed in stalls where foals were handled as little as possible and allowed to nurse from their dams ad lib in order to minimize stress. All procedures were in compliance with an experimental protocol approved by the University of Prince Edward Island’s Animal Care Committee.

Experimental Protocol

Foals with FPT deemed to have a low risk of sepsis based on the history and physical examination were admitted into the clinical trial. Foal 1 was randomly assigned to a treatment group, with plasma (PG) or serum (SG), by a coin toss. Subsequent foals were assigned to a treatment group based on alternating rotation. Weights were obtained using the average of three girth tape measurements or a scale. A 16-gauge intravenous catheter was aseptically placed in the jugular vein of each foal to be transfused. Blood was immediately collected for a complete blood count, fibrinogen concentration, serum glucose concentration, and radial immunodiffusion (RID) equine IgG test kit.d All samples were tested in duplicate and the average of the zones of diameter were used to determine IgG concentration. Any sample whose concentration was greater than the upper standard for the kit (1600 mg/dl) was diluted and retested, and then multiplied by the dilution factor to determine the IgG concentration. IgG concentrations were also calculated for treatment product aliquots (IgGAliquot) after being appropriately diluted as described above.

Data recorded for each foal included foaling season, body weight in kg (bwt), method of weaning, sepsis score, treatment product, number of units transfused (units), IgG\(_0\), IgG\(_{20\text{min}}\), and IgG\(_{3\text{day}}\), using a commercial radial immunodiffusion (RID) equine IgG test kit. All samples were tested in duplicate and the average of the zones of diameter were used to determine IgG concentration. Any sample whose concentration was greater than the upper standard for the kit (1600 mg/dl) was diluted and retested, and then multiplied by the dilution factor to determine the IgG concentration. IgG concentrations were also calculated for treatment product aliquots (IgGAliquot) after being appropriately diluted as described above.

The total grams of IgG per unit transfused (Gm-IgG\(_{\text{total}}\)) was calculated using the formula Gm-IgG\(_{\text{total}}\) (mg/dl) \times 0.01 g/mg/dl/unit (dl/unit = 9.5 per unit plasma or 2.5 per unit serum). Total grams of IgG transfused (Gm-IgG\(_{\text{total}}\)) was calculated by Gm-IgG\(_{\text{unit}}\) \times units. The change in serum IgG concentration for each unit transfused was determined for the time period between IgG\(_0\) and IgG\(_{20\text{min}}\). Changes in the foal’s serum IgG concentration for each unit transfused was calculated...
similarly for the time period between IgG₀ to IgG₃day and IgG₂₀min to IgG₃day.

Statistical Analysis

A RID IgG standard curve was generated using 4 known standards and a least squares linear regression equation was estimated using a statistical computer software program. When standards failed to achieve a linear equation with a $R^2$ value of $\geq 95\%$, all samples and standards were repeated in duplicate. Using the least squares regression equation, serum sample RID IgG concentrations were predicted from the zone of inhibition diameter of the serum sample. Multiple regression was used to determine significant predictor variables for the outcome, IgG₃day. Variables were selected by a backward elimination procedure. Odds ratios were estimated for treatment product and transfusion reactions using $\chi^2$ analysis. Differences between seasonal variations in IgG Aliquot and (ΔIgG₃day – IgG₀min)/units were evaluated using an ANOVA. Differences identified by the ANOVA were examined using least squares differences. Separate statistical software programs were used for multiple regression and $\chi^2$ analysis and ANOVA. Significance was assessed at the 95% confidence level.

3. Results

Fifty-one foals received transfusions over a two-year period. Five foals were excluded from the study due to their high risk of septicemia based on a foal sepsis score of $\geq 11$. (1 PG, 4 SG). In total, 46 foals remained for analysis (25 PG, 21 SG). Body weights between the two treatment groups were similar (Table 1). One or 2 units of treatment product was transfused depending on the severity of FPT as determined by modified zinc sulfate turbidity test or a glutaraldehyde coagulation test. In total, 54% (13/24) of foals transfused with plasma received 2 units and 67% (14/21) of foals transfused with serum received 2 units. Additionally, one foal received 1.26 units of plasma. The mean number of units transfused was similar between both treatment groups (Table 1). Minor transfusion reactions were noted in 50% (12/24) of foals transfused with serum and in 33% (9/27) of foals transfused with plasma. There was no significant difference in the occurrence of transfusion reactions between the two treatment groups (probability > $\chi^2 = 0.227$). None of the reactions were severe or required discontinuation of the transfusion.

A significant increase in IgG₃day occurred in plasma treated foals as compared to serum treated foals ($p = 0.005$). The number of units transfused was positively associated with IgG₃day ($p = 0.004$). IgG₀ also was positively associated with IgG₃day ($p < 0.001$). Foal bwt was found to be inversely associated with IgG₃day ($p < 0.001$), but the method used to determine foal weight was not significant ($p = 0.26$). The regression model for IgG₃day using the predictors treatment, units, IgG₀, bwt, and season, had an $R^2 = 71.3\%$.

When Gm-IgG/Total replaced units in the regression model ($R^2 = 71.9\%$), it was positively associated with IgG₃day ($p < 0.001$) and it forced treatment product out of the model as a significant predictor. The number of units used in a transfusion and Gm-IgG/unit explained most of the variation in Gm-IgG/Total ($R^2 = 78.7\%$). The Gm-IgG/unit was significantly greater in the plasma product versus the serum product ($p = 0.0001$; Table 2).

4. Discussion

Using a multivariate regression model, factors found to significantly affect a foal’s IgG₃day included IgG₀, treatment product (PG or SG), units transfused, bwt, and foaling season (1999 or 2000). Foals treated with plasma had significantly greater increases in serum IgG concentrations measured at IgG₃day than foals receiving serum treatment. This difference can be attributed to the amount of IgG that is present in a unit of transfusion product. The plasma product label indicates that one unit should contain a minimum of 25 g IgG and the manufacturer of the serum product claims that one unit should contain at least 28 g of IgG. Results of this study found that the Gm-IgG/unit of plasma was consistent with the manufacturer’s claims, however only half of the Gm-IgG/unit was detected in the serum product by our testing methods (Table 2). The number of units used to treat each foal was based on pre-transfusion IgG concentrations obtained using the modified zinc sulfate turbidity test or glutaraldehyde coagulation test. The importance of early transfusion in IgG deficient foals precluded the use of RID testing, since it can take 24–48 h before obtaining results. Compared to the RID IgG results, both screening tests utilized had limited specificity resulting in a high level of false positives. Consequently, 16 foals that were identified as having FPT and subsequently transfused, were later found to have IgG₀ concentrations of

| Table 1. Recorded Data for Foals in Plasma and Serum Treatment Groups |
|-----------------|-----------------|-------------|-------------|
| Parameter       | Treatment       | Mean        | SD          | Range         |
| bwt (kg)        | Plasma          | 51.8        | 7.4         | 38.6–66.4     |
|                 | Serum           | 52.2        | 5.7         | 43.6–64.1     |
| units           | Plasma          | 1.53        | 0.50        | 1–2           |
|                 | Serum           | 1.67        | 0.48        | 1–2           |
| IgG₀ (mg/dl)    | Plasma          | 653         | 410         | 0–1424        |
|                 | Serum           | 772         | 561         | 213–2638      |
| IgG₃day (mg/dl) | Plasma          | 1070        | 442         | 331–2249      |
|                 | Serum           | 979         | 436         | 356–1926      |

Plasma treatment group n = 25, Serum treatment group n = 21.

Bwt = body weight of foal in kg; One unit plasma = 955 ml/bag, one unit concentrated serum product = 250 ml/bottle; IgG₀ = pre-transfusion serum IgG concentration; IgG₃day = 3 day post-transfusion serum IgG concentration.
Plasma products are usually prepared from individual donor horses, whereas the concentrated serum product used in this study contains pooled serum from several horses. Although this may allow for more consistency between units of serum product, this type of preparation may increase the risk of introducing endotoxin into the product. A relatively larger percentage of serum-transfused foals had mild reactions; however, the difference between plasma and serum treatment was not significant. It is possible that pre-treatment with flunixin meglumine in serum treated foals decreased the incidence of clinical signs associated with transfusion reactions. The overall health of all 46 foals up to 3 months of age was not different between treatment groups, although the small number of foals limited a critical evaluation. Reported illnesses included, transient “foal heat” diarrhea which was observed in 10/46 foals (6 PG, 4 SG). Severe nasal discharge was noted in 6/46 foals (2 PG, 4 SG). Other respiratory illness including pneumonia occurred in 5/46 foals (3 PG, 2 SG). Umbilical complications including infection or patent urachus developed in 6/46 foals (3 PG, 3 SG).

Presently, there are many options for treatment of FPT in foals that conveniently bypass the logistics involved in finding and obtaining plasma from a suitable donor. This study focuses on a commercial serum product and a commercial plasma product, since these treatments are most commonly used. The advantages of using intravenous serum over plasma products are mainly lower costs and convenient storage, however, the quantity and quality of immunoglobulin in the product should be of foremost concern. In this study, plasma treatment in foals resulted in greater increases in post-transfusion serum IgG concentrations as compared with

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Table 2. Calculated Values for Foals in Plasma and Serum Treatment Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gm-IgG&lt;sub&gt;Un&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Plasma</td>
<td>30.42</td>
<td>6.18</td>
<td>14.25–46.64</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>15.53</td>
<td>3.77</td>
<td>6.39–22.54</td>
</tr>
<tr>
<td>Gm-IgG&lt;sub&gt;Total&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Plasma</td>
<td>46.79</td>
<td>18.63</td>
<td>14.25–93.27</td>
</tr>
<tr>
<td>(ΔIgG&lt;sub&gt;3day&lt;/sub&gt;–IgG&lt;sub&gt;0&lt;/sub&gt;)/unit (mg/dl/unit)</td>
<td>Plasma</td>
<td>351</td>
<td>146</td>
<td>(−)107–551</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>145</td>
<td>287</td>
<td>(−)988–514</td>
</tr>
<tr>
<td>(ΔIgG&lt;sub&gt;20min&lt;/sub&gt;–IgG&lt;sub&gt;0&lt;/sub&gt;)/unit (mg/dl/unit)</td>
<td>Plasma</td>
<td>−79</td>
<td>149</td>
<td>(−)347–179</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>−48</td>
<td>136</td>
<td>(−)333–150</td>
</tr>
</tbody>
</table>

Plasma treatment group n = 25, Serum treatment group n = 21.
Gm-IgG<sub>Un</sub> = total grams of IgG per unit transfused; Gm-IgG<sub>Total</sub> = total grams of IgG transfused; (ΔIgG<sub>20min</sub>–IgG<sub>0</sub>)/unit = change in serum IgG concentration from pre-transfusion to 20 minutes post-transfusion per unit of treatment product transfused; (ΔIgG<sub>3day</sub>–IgG<sub>20min</sub>)/unit = change in serum IgG concentration from 20 minutes post-transfusion to 3 days post-transfusion per unit of treatment product transfused.

<sup>a</sup>Plasma treatment resulted in a significantly higher value than serum product, p < 0.0001.
<sup>b</sup>Plasma treatment resulted in a significantly higher value than serum product, p < 0.001.

Other parameters in this chart were not statistically analyzed.

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>800 mg/dl based on RID results. Interestingly, when these foals were excluded, the variables in the regression model remained unchanged, indicating that IgG<sub>0</sub> concentrations of >800 mg/dl did not significantly influence the product effect on IgG<sub>3day</sub>. In an attempt to achieve adequate serum IgG concentrations, foals with serum IgG of 400–800 mg/dl were given one unit of treatment product and those with serum IgG <400 mg/dl were given two units. This was based on previous reports that estimated that a unit of plasma, containing 25 g of IgG, should increase a 50-kg foal’s serum IgG concentration by approximately 400 mg/dl. As expected, the number of units transfused had a significant effect on IgG<sub>3day</sub>, but the number of units transfused per foal was similar between treatment groups (Table 1). The increase in foal serum IgG concentration from pre-transfusion to 20 minutes post-transfusion on a per unit basis (ΔIgG<sub>20min</sub>–IgG<sub>0</sub>/unit) was 351 ± 146 mg/dl and 145 ± 287 mg/dl for plasma and serum treatment groups, respectively. However by day 3 post-transfusion for ΔIgG<sub>3day</sub>–IgG<sub>20min</sub>/unit had declined by 23% and 33% for plasma and serum products, respectively (Table 2). The increase seen in foal serum IgG concentrations with plasma immediately after transfusion and the gradual decline in IgG concentrations by 3 days is similar to previous reports. Transfusion with serum product increased foal serum IgG concentrations to less than half that seen with plasma. This is consistent with the aforementioned finding that IgG concentrations per unit of serum product were about half that of plasma. The cause of the decrease in post-transfusion IgG concentrations may be due to elimination of IgG that is denatured or bound to antigen, catabolism, or redistribution into the extracellular fluid compartment.
serum treatment. This is primarily due to Gm-IgG units in each product. Based on these results, it would take 2–3 units of intravenous concentrated serum product to match the increase in IgG3 day that occurs with 1 unit of plasma product.

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References and Footnotes


aPolymune-Plus®, Veterinary Dynamics, Templeton, CA 93465
bSeramune®, Sera Inc., Shawnee Mission, KS 66285-5866
cBanamine®, Schering-Plough Animal Health Corp., Kenilworth, NJ 07033-0530
dEquine RID kits, VMRD Inc., Pullman, WA 99163
*eS* tata 7.0, Stata Corp., College Station, TX 77845.