Colostral, Milk, and Serum Immunoglobulin G Concentrations in Quarter Horse Mares and their Foals from Birth to Weaning

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation with honors distinction from the College of Food, Agricultural, and Environmental Sciences at The Ohio State University

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Abstract

The failure of passive transfer (FPT) of maternal immunoglobulins, primarily IgG, to the neonatal foal predisposes the foal to potentially life-threatening illnesses. Sufficient protection against these infectious diseases is attained when serum IgG concentrations in a newborn foal are above 800mg/dL. Partial FPT occurs when serum IgG concentrations are between 400 and 800 mg/dL, and failure of passive transfer occurs when serum IgG concentrations are less than 400 mg/dL after 24 hours post-partum. The frequency of FPT occurrence in newborn foals is between 3% and 20%, and several factors have been thought to influence the passive transfer of IgG. The purpose of this study was to evaluate the colostral, milk and serum IgG concentrations in Quarter Horse mares and their foals from birth to weaning, and to determine the correlation, if any, between mare age, number of pregnancies, days of gestation, month foaled, foal sex and IgG concentrations. The majority of correlations found in this study are consistent with previous research in other horse breeds (Kohn et al., 1989; Erhard et al., 2001). However, unique to this study, both positive and negative correlations were found between both mare and foal IgG concentrations and both gestation length and month foaled.

Introduction

Antibodies are defined as Y-shaped proteins on the surface of B cells that are secreted into the blood or lymph in response to an antigenic stimulus, such as a bacterium, virus, parasite, or transplanted organ, and neutralize the antigen by binding specifically to it. They are also referred to as immunoglobulin. (The American Heritage Dictionary of the English Language, Fourth Edition.) There are five main classes of antibodies Immunoglobulin G (IgG), Immunoglobulin M (IgM), Immunoglobulin A (IgA), Immunoglobulin E (IgE), and

Immunoglobulin D (IgD). They each have their own specific functions within the body. Immunoglobulin G is considered the most versatile immunoglobulin because it can perform all the functions of immunoglobulin molecules. These functions include antigen binding, fixation of complement, and binding to various cells like phagocytic cells, lymphocytes, platelets, mast cells, and basophils. Immunoglobulin G constitutes about 75% of serum immunoglobulin. The other four classes of immunoglobulin also serve important functions in the body, but will not be focused on in this research project. Immunoglobulin M is the first immunoglobulin produced by fetuses. Immunoglobulin A is the major class of immunoglobulin found in secretions such as tears, saliva, and mucus. This makes it very important in local or mucosal immunity. Immunoglobulin E is the least common serum immunoglobulin and is involved in allergic reactions. The function of IgD is currently unknown and, like IgE, is found in very low doses in serum immunoglobulin. (Mayer, Immunology.) Immunoglobulin G will be the focus of this research project because of its importance in prenatal health; specifically in newborn foals.

When foals are first born, they have no immune system of their own. This is due to the fact that mares and foals have an epitheliochorial placental connection in utero, and there is no placental transfer of antibodies (Encyclopædia Britannica). Foals are completely dependent upon the antibodies they absorb from ingesting the dam’s colostrum. When the foal ingests the colostrum, specialized cells that line the small intestine absorb the antibodies and transfer them into the foal’s blood. This process is referred to as passive transfer. When antibodies are not supplied to a foal in sufficient amounts, this can cause many serious health issues. This problem is called failure of passive transfer (FPT). Foals are considered to have failure of passive transfer if, after 24 hours of age, circulating antibody levels are less than 400 mg/dl. If levels are below 200 mg/dl, this is termed 'complete' failure of passive transfer, which would imply that a newborn foal did not receive any colostral antibodies from her dam. A level of 400 to 800 mg/dl is considered partial failure of passive transfer and if blood antibody concentration is greater than 800 mg/dl, it is considered an adequate level. Failure of passive transfer is an especially prominent issue in the equine industry because it occurs in about 10-20% of newborn foals (McCue, 2007). This can have especially detrimental effects in this species because of the long generation interval and high costs involved in producing foals. One of the aspects that make studying failure of passive transfer so difficult is the fact that passive transfer occurs in a very short window of time. The specialized cells in the intestinal lining absorb antibodies rapidly between the first 6-8 hours after birth, but absorption completely stops by 24 hours post-partum. The most common causes of failure of passive transfer are poor quality colostrum from the dam and premature lactation. Failure of passive transfer can also occur as a result of many other causes including failure of colostrum production due to fescue toxicity, inability or lack of foal to nurse, prematurity or dysmaturity, foal rejection by mare, or failure of foal to absorb ingested antibodies. (McCue, 2007.)

Many studies have been conducted to determine the importance of passive transfer and colostrum in neonatal animals. A study in which Holstein calves were fed a commercial colostrum replacer showed that calves fed maternal colostrum had more IgG than calves fed a colostrum replacement (Swan et al., 2007). Calves fed maternal colostrum also showed much smaller percentages of failure of passive transfer than calves fed colostrum replacement; 28.0% vs. 93.1%, respectively (Swan, 2007.) Another study evaluated the association of neonatal serum immunoglobulin G1 concentration with health and performance in beef calves and found that in the preweaning period, lower perinatal IgG1 concentrations showed significant association with higher morbidity rates, higher mortality rates, and lower average daily gains (Dewell et al., 2006). It also showed that serum with a high concentration of IgG1 was important for optimal health and performance in calves.

Studies in horses regarding IgG concentrations have varying results. In a study done in Thailand in 2004 of Thoroughbred horses, researchers reported that parity, the occurrence of foal deaths or stillbirths, and sex of foal significantly influenced the serum IgG levels in the foals (Butudom et al., 2004). This contradicts the results of a study done in Germany in 2001 which found no significant influences on IgG as a result of breed, mare age, number of pregnancies, days of gestation, month foaled, foal sex, or location (Erhard et al., 2001). This proposes an interesting question: What factors, if any, affect serum Immunoglobulin G concentrations in newborn foals? In this research project, many of the factors in the two previously mentioned studies were evaluated in Quarter Horses. The Null Hypothesis in this case would agree with the second study; that factors such as breed, mare age, number of past pregnancies, length of gestation, month foaled and foal sex would have no effect. The Alternate Hypothesis would agree with the first study; that these factors will have an effect.

Materials and Methods

In order to test these hypotheses, the IgG concentrations of the eight Quarter Horse mares and their foals born at The Ohio State University Equine Center from March to June 2008 were evaluated. Foaling data and history were recorded and is shown in Table 1. Mare age ranged from 6 to 18 years old with an average of 10.625 years old. The number of previous pregnancies for each mare ranged from 0 to 9 with an average of 2.4 pregnancies. The length of gestation ranged from 323 days to 359 days with an average of 342.9 days. Five foals were born in April and three foals were born in May. Fifty percent of these foals were male and fifty percent were female. Each foaling was attended to insure that the initial blood and colostrum samples were collected prior to the foal nursing. Blood (via jugular venipuncture) and milk collections occurred at 0 hours, 12 hours, 24 hours, 7 days, 14 days, 21 days, 28 days, 56 days, 84 days, and 112 days post-partum. These collections followed an approved animal care and use protocol. Blood samples were centrifuged at 1,200 x g for 10 minutes and the serum was collected. Both serum and colostrum samples were stored at -80°C until assayed. To measure the IgG concentrations separately in the blood serum of the mares and foals as well as in the colostrum and milk, a Single Radial Immunodiffusion (SRID) Test was used. The SRID Kits were provided by the Veterinary Medical and Research Development (VMRD), Inc.

Three microliters of each reference standard and each serum and colostrum sample were pipetted into the plates provided in the SRID kits. Due to the time and temperature dependence of these kits, the plates were then left at room temperature for 24 hours. The SRID plates contain an agarose gel. An antiserum specific for the Immunoglobulin G protein is incorporated into this gel, and this antiserum reacts with the antigen in each sample to form a ring of precipitation. This ring is proportional in size to the concentration of Immunoglobulin G within each sample. After 24 hours, the ring diameters of each sample were then measured in millimeters. When plotted on the semi-log graph paper provided by VMRD, a linear relationship can be seen between the ring diameters and the IgG concentrations. This relationship can be used to determine concentration of each sample based on ring size (VMRD).

Results

IgG concentrations were not detectable in foal serum immediately after foaling and prior to nursing (Day 0). At this time, no correlation was found between the mare serum IgG concentrations and the colostrum concentrations (r= 0.27). Figure 1 displays the concentration data for Day 0 in graph form. After nursing (Day ½), 50% of the foals had a serum IgG concentration of 400-800 mg/dL, and 50% had a concentration above 800mg/dL. At this time, no correlation was found between the mare serum IgG concentrations and the colostrum IgG concentrations, nor the mare serum IgG concentrations and the foal serum IgG concentrations (r= -0.25 and r= -.024, respectively). A slight positive correlation (r= 0.61) was found between the foal serum IgG concentrations and the colostrum IgG concentrations. Figure 2 displays the concentration data for Day ½ post partum in graph form. By 24 hours post partum, 50% of the mares stopped secreting IgG in their colostrum. No correlations were found between the mare serum IgG concentrations and the colostrum IgG concentrations, nor the foal serum IgG concentrations and the colostrum IgG concentrations (r= 0.58 and r= -0.56, respectively). Additionally, no correlation was found between the mare serum IgG concentrations and the foal serum IgG concentrations (r= -0.45). Figure 3 displays the concentration data for Day 1 post partum in graph form. By Day 7 post partum all eight mares had ceased secreting IgG in their milk, therefore no more milk data was able to be collected from this point on. No correlation was found between the mare serum IgG concentration and foal IgG concentration for Day 7, Day 14, Day 21, or Day 28 post partum (r= -0.25, r= 0.07, r= -0.37, and r= -0.30, respectively). Figures 4, 5, 6, and 7 display the serum IgG levels for Days 7-28 post partum. At Day 56 post partum a slightly negative correlation (r= -0.56) was found between the mare serum IgG concentrations and the foal serum IgG concentrations. Figure 8 displays the concentration data for Day 56 post partum in graph form. This negative correlation between the mare serum IgG concentrations and the foal serum IgG concentrations continued to Day 84 post partum to a slightly higher degree (r= -0.89). This trend represents the foals' exposure to new environmental antigens causing a subsequent immune response. Figure 9 displays the concentration data for Day 84 post partum in graph form. On Day 112 post partum, the negative correlation between the mare serum IgG concentrations and the foal serum IgG concentrations continued to a slightly lesser degree (r= -0.60). Figure 10 displays the concentration data for Day 112 post partum in graph form. Table 2 shows the mean values and standard deviations of the IgG concentrations in mg/dL of all colostrum, mare serum, and foal serum samples.

No correlations were found between any of IgG concentrations and mare age, number of previous pregnancies, or foal sex. On Day ½, Day 7, and Day 21 post partum, slightly negative correlations were found between the foal serum IgG concentrations and the length of gestation (r= -0.65, r= -0.60, and r= -0.76, respectively). On Day ½, Day 7, and Days 21-56 post partum, positive correlations were found between the foal serum IgG concentrations and the month foaled (r= 0.88, r= 0.69, r= 0.63, r= 0.73, and r= 0.70, respectively). Table 3 displays all of the found correlation coefficients for the foal serum samples. Mare serum IgG concentrations were typically positively correlated with the length of gestation and negatively correlated with the month foaled. Table 4 displays all of the found correlation coefficients for the mare serum samples. On Day ½ post partum, a slightly negative correlation (r= -0.57) was found between the mare milk IgG concentration and the length of gestation. Additionally, a slightly positive correlation (r= 0.69) was found between the mare milk IgG concentration and the month foaled. On Day 1 post partum, a slightly positive correlation (r= 0.58) was found between the mare milk IgG concentration and the length of gestation, while a negative correlation (r= -0.77) was found between the mare milk IgG concentration and the month foaled. Table 5 displays all of the found correlation coefficients for the mare milk samples.

Discussion

The majority of correlations found in this study are consistent with previous research in other horse breeds (Kohn et al., 1989; Erhard et al., 2001). The number of pregnancies has been shown to have a significant effect on immunoglobulin levels in other livestock species (Burkey et al., 2007). However, previous studies in horses found no significant influences on IgG levels in foals could be demonstrated due to mare age, number of pregnancies, days of gestation, month foaled or foal sex. In contrast, in this study of Quarter Horse mares and their foals from birth to weaning, both positive and negative correlations were found between both mare and foal IgG concentrations and both gestation length and month foaled. Possible reasoning behind these found correlations for month foaled could be nutrition or length of daylight. In this study, however, all eight mares were kept on the same diet throughout their gestations, so nutrition is, therefore, negligible. Length of daylight is known to affect hormones and breeding cycles in the equine species, however, further studies would need to be done in order to confirm a link between length of daylight and Immunoglobulin G concentrations in horses. A possible reason behind the found correlations and gestation length could be the Ohio State Equine Facility's vaccination schedule. All eight mares were vaccinated on day 300 of their pregnancy, so it makes sense that there would be a positive correlation between the mare serum IgG concentrations and the days of gestation due to the vaccination-induced immune response in the mare. It is also logical that there would be a negative correlation between days of gestation and the foal serum IgG concentrations because the further the foals are born from the vaccination date, the less likely they are to reap the benefits of that vaccination in their dam's colostrum. Further studies would need to be completed in order to confirm the results of this study and to further explore the physiological reasons behind these results.

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Tables and Figures

Figure 1. Serum IgG concentrations at 0H post partum.

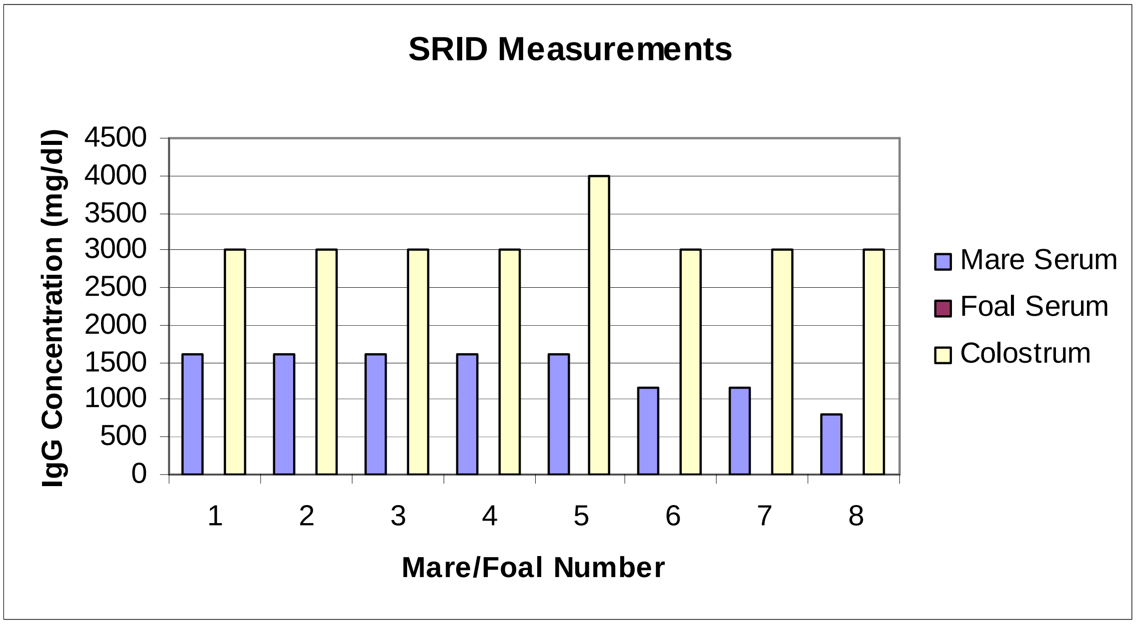

Figure 2. Serum IgG concentrations at 12H post partum.

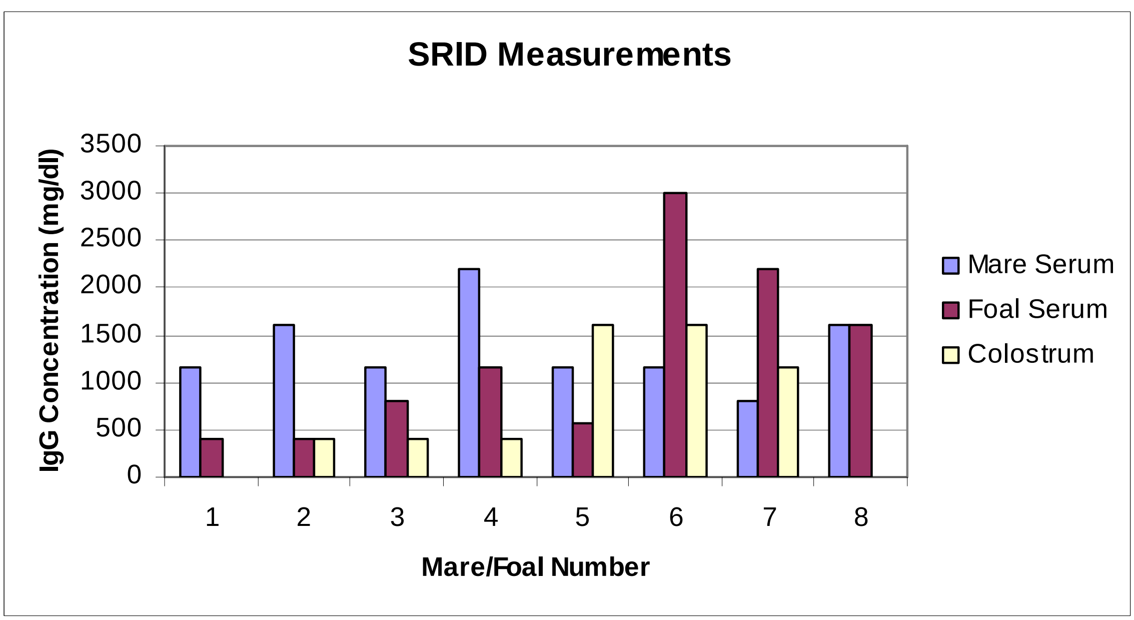

Figure 3. Serum IgG concentrations at 24H post partum.

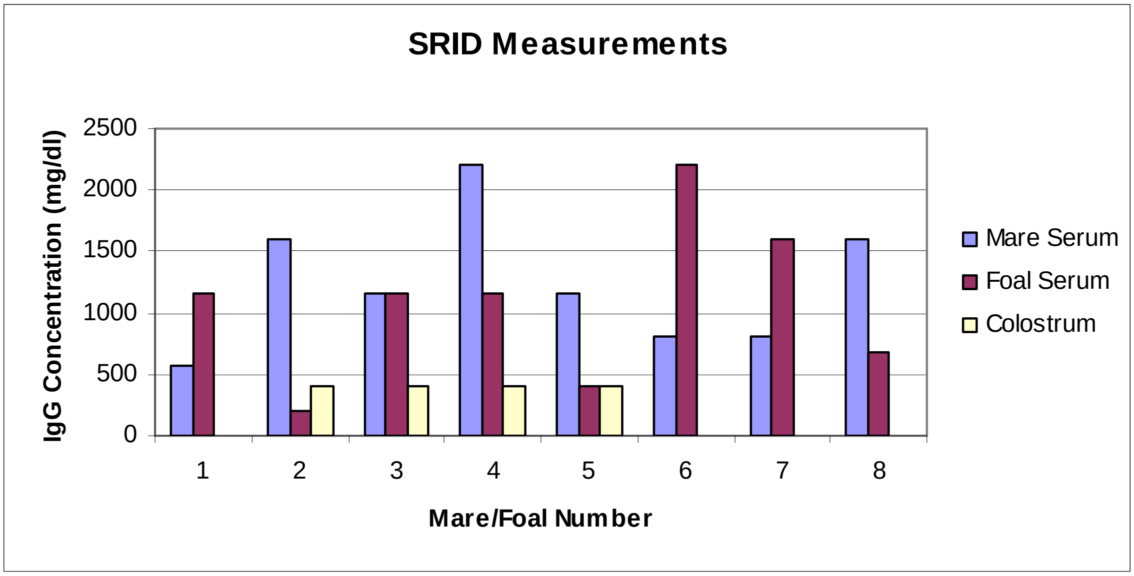

Figure 4. Serum IgG concentrations at D7 post partum.

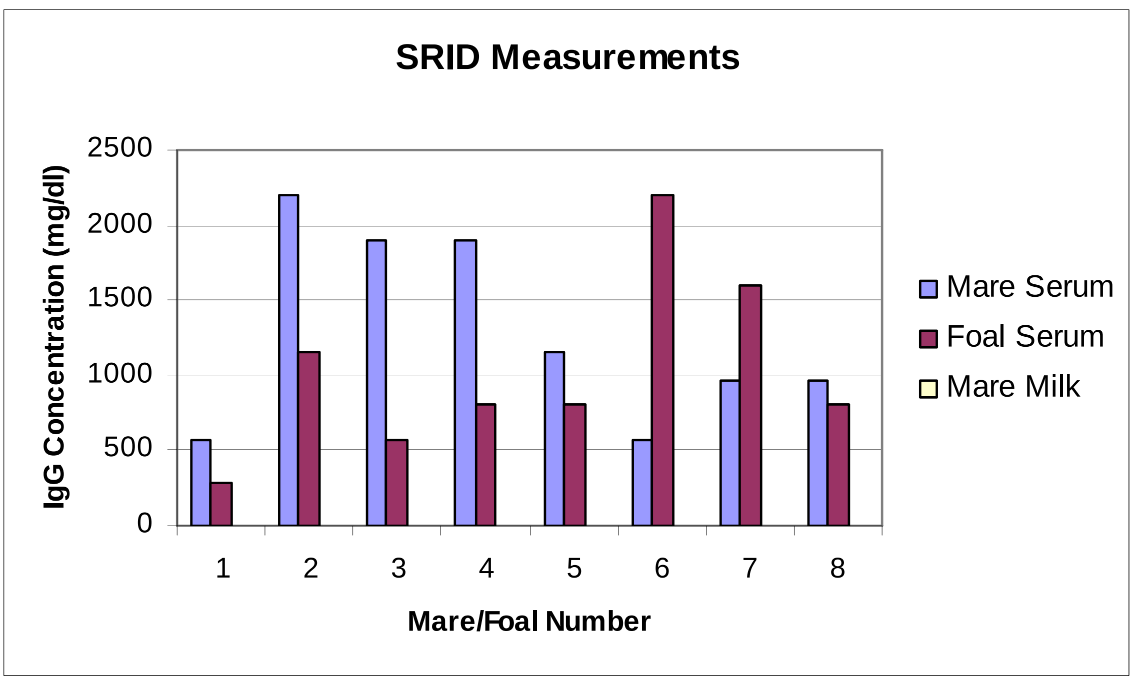

Figure 5. Serum IgG concentrations at D14 post partum.

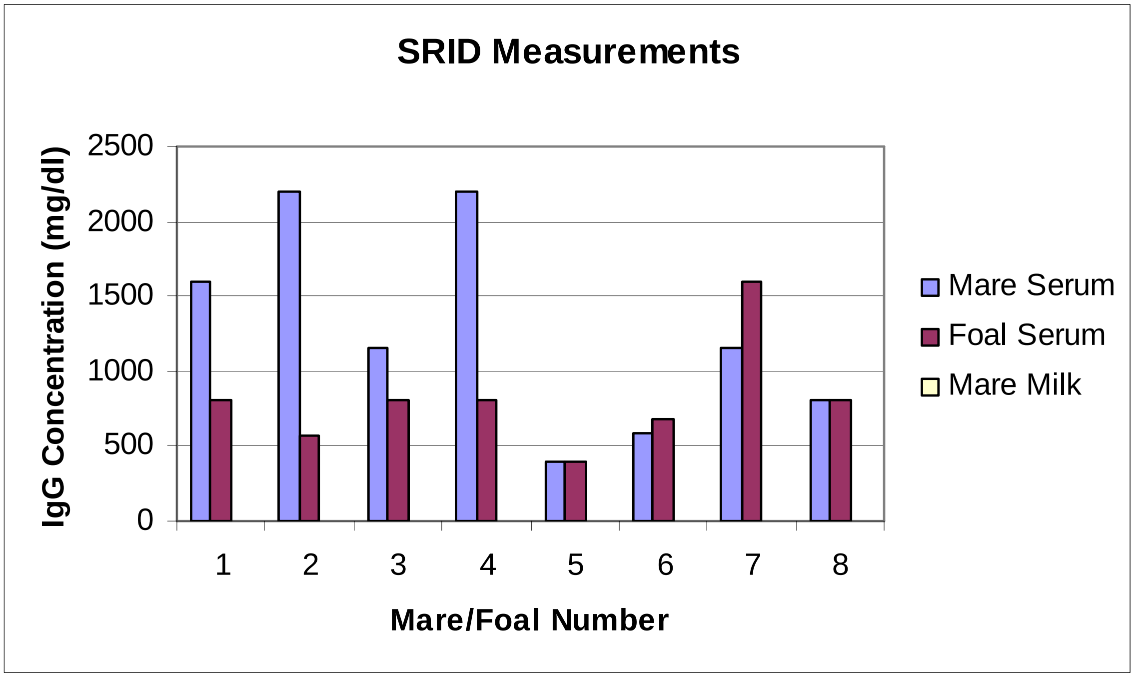

Figure 6. Serum IgG concentrations at D21 post partum.

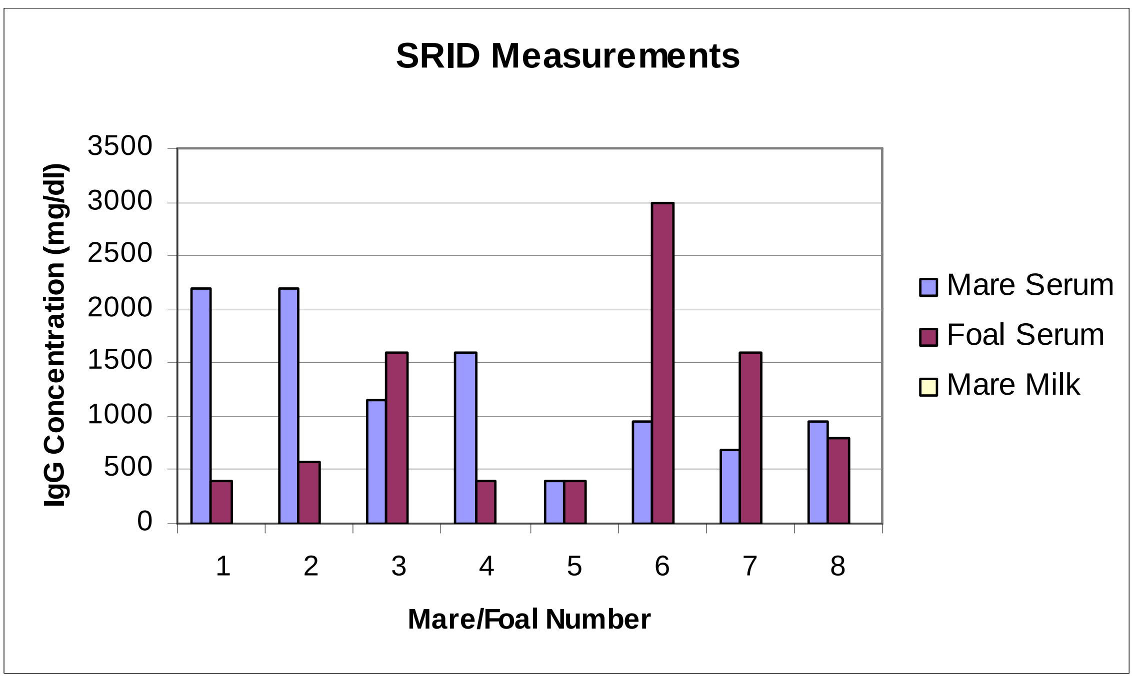

Figure 7. Serum IgG concentrations at D28 post partum.

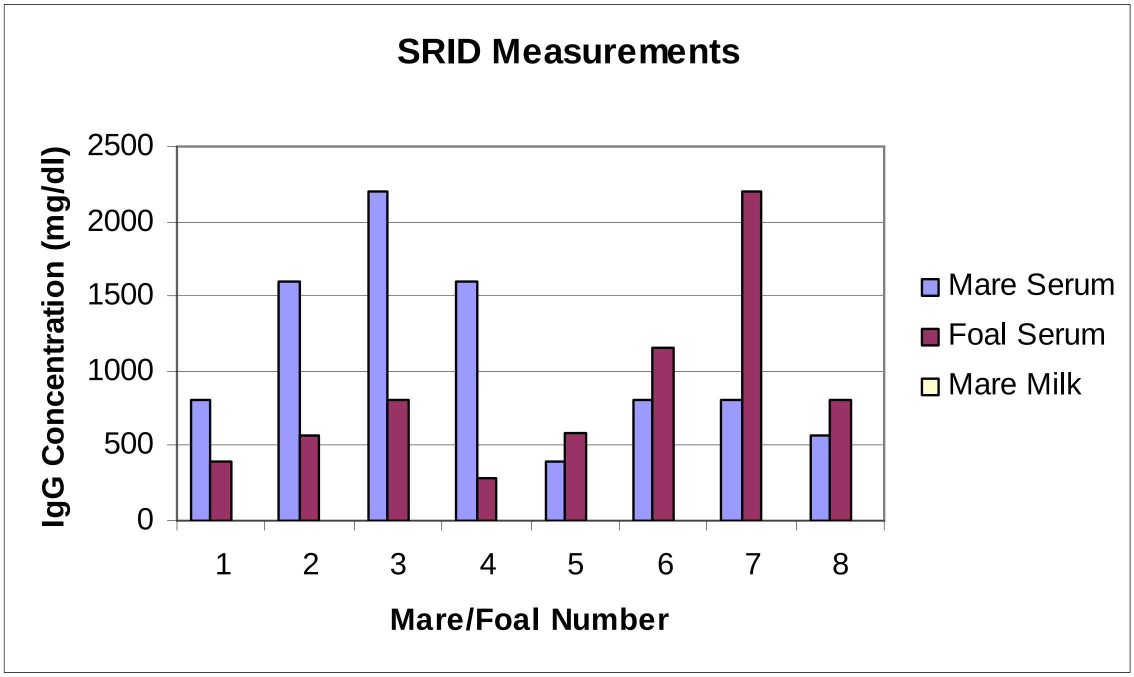

Figure 8. Serum IgG concentrations at D56 post partum.

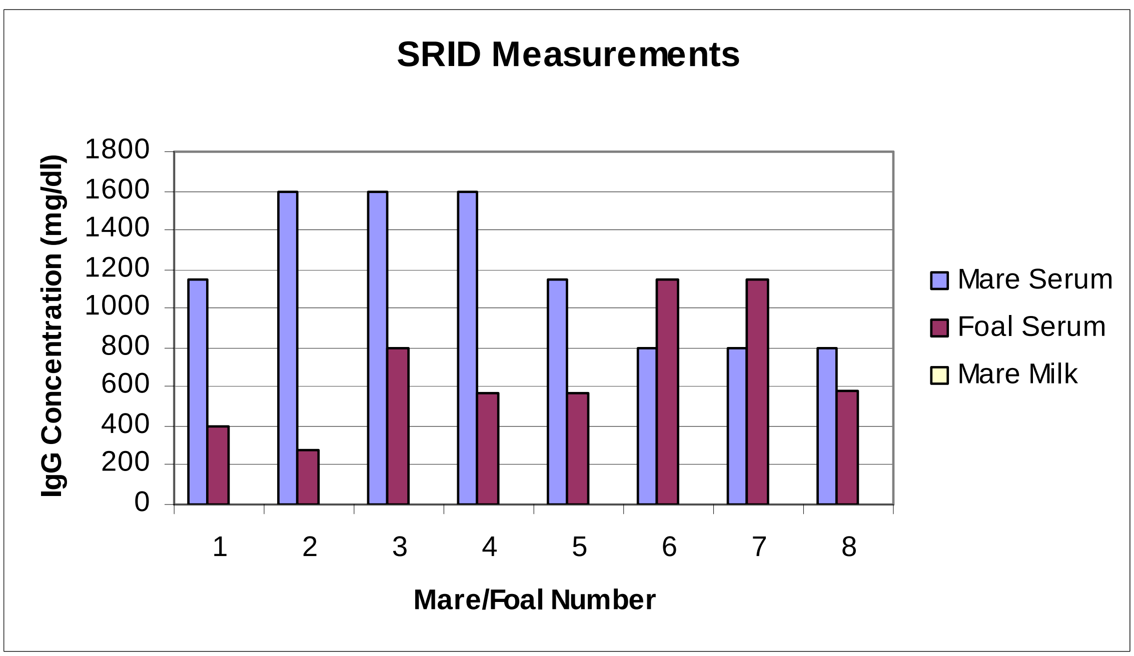

Figure 9. Serum IgG concentrations at D84 post partum.

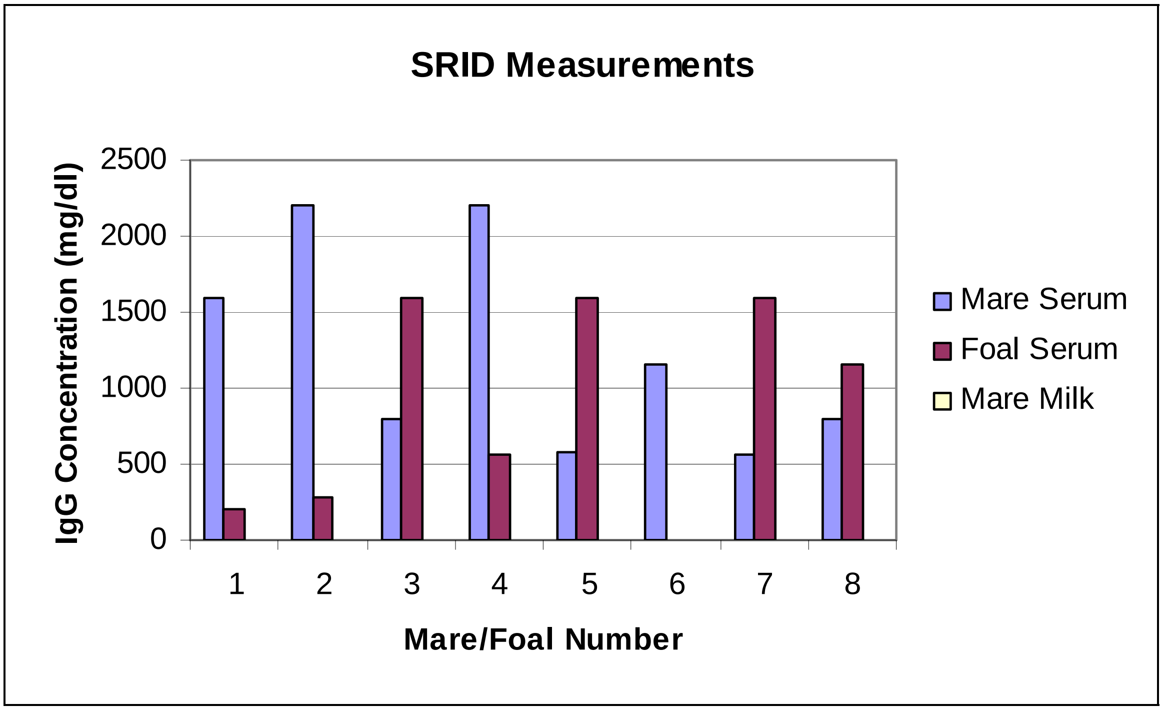

Figure 10. Serum IgG concentrations at D112 post partum.

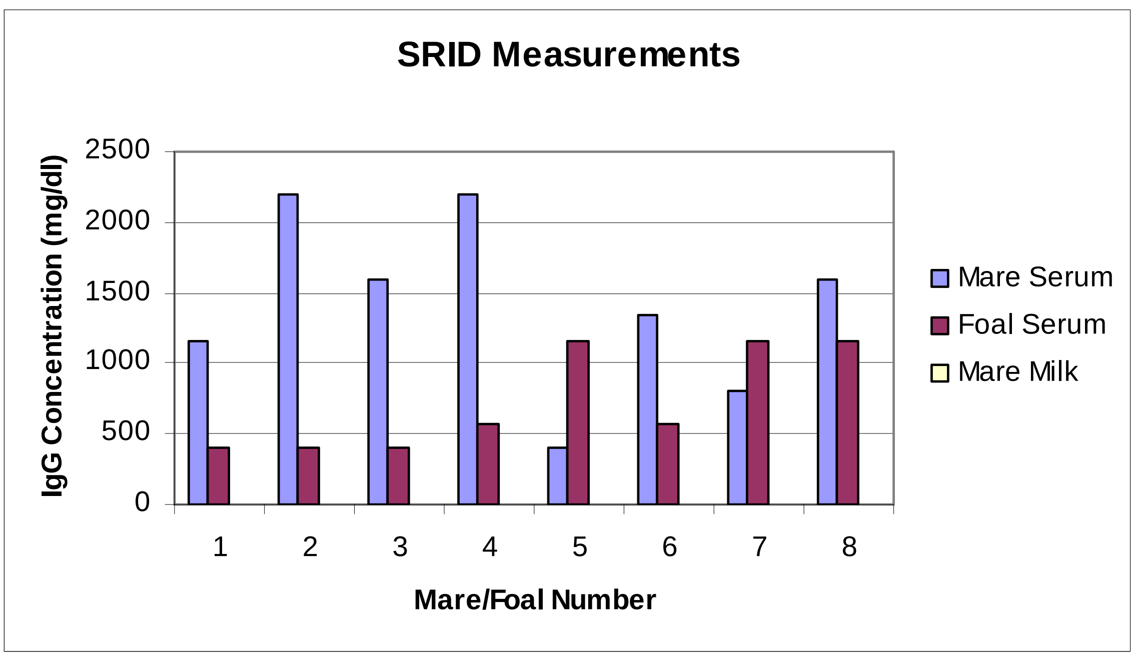

Table 1. Mare Foaling Data

Mare #	Mare Age	Previous Pregnancies	Days of Gestation	Month Foaled	Foal Sex
1	6	0	347	April	M
2	9	4	344	April	F
3	15	1	343	April	M
4	11	1	359	April	M
5	7	2	349	April	F
6	8	1	323	May	F
7	11	1	345	May	M
8	18	9	333	May	F

Table 2.

IgG concentration (mg/dL) of colostrum and serum from mares and foals.									
Source	0H	12H	24H	D7	D14	D21	D28	D56	D84
Colostrum									
Mean ± SD	3125±354	694±663	200±214	ND	ND	ND	ND	ND	ND
Range	3000-4000	ND-1600	ND-400	NA	NA	NA	NA	NA	NA
Mare Serum									
Mean ± SD	1388± 566	1350± 427	1233± 477	1275± 585	1260± 690	1269± 556	822± 550	1188± 412	1237± 670
Range	800-1600	800-2200	565-2200	565-2200	400-2200	400-2200	400-2200	400-2200	565-2200
Foal Serum									
Mean ± SD	ND	1264± 942	1066± 647	1024± 615	806± 352	1096± 922	847± 609	686± 323	999± 639
Range	NA	400-3000	200-2200	280-2200	400-1600	400-3000	280-2200	280-1150	200-1600
ND = Not detectable, NA= Not Applicable									

Table 3. Foal Serum Correlations

	Mare Age	Previous Pregnancies	Days of Gestation	Month Foaled	Foal Sex
FS0	-	-	-	-	-
FS1/2	0.15	0.01	-0.65	0.88	0.14
FS1	-0.11	-0.49	-0.44	0.55	-0.31
FS7	-0.15	-0.07	-0.60	0.69	0.37
FS14	0.24	-0.16	0.05	0.52	-0.59
FS21	0.06	-0.21	-0.76	0.63	0.11
FS28	0.14	-0.08	-0.34	0.73	-0.13
FS56	0.11	-0.29	-0.47	0.70	-0.14
FS84	0.40	0.04	-0.25	0.40	0.02
FS112	0.33	0.43	-0.07	0.53	0.28

Table 4. Mare Serum Correlations

	Mare Age	Previous Pregnancies	Days of Gestation	Month Foaled	Foal Sex
MS0	-0.54	-0.63	0.67	-0.94	-0.34
MS1/2	0.23	0.31	0.38	-0.32	0.06
MS1	0.42	0.42	0.41	-0.26	0.11
MS7	0.25	0.06	0.50	-0.58	-0.09
MS14	-0.09	-0.15	0.58	-0.50	-0.41
MS21	-0.25	-0.11	-0.76	-0.50	-0.22
MS28	0.02	-0.18	0.41	-0.45	-0.29
MS56	-0.04	-0.25	0.62	-0.86	-0.29
MS84	-0.29	-0.12	0.36	-0.48	-0.09
MS112	0.33	0.24	0.09	-0.21	-0.04

Table 5. Mare Milk Correlations

	Mare Age	Previous Pregnancies	Days of Gestation	Month Foaled	Foal Sex
MM0	-0.36	-0.05	0.23	-0.29	0.38
MM1/2	0.18	0.42	-0.57	0.69	0.65
MM1	-0.03	-0.14	0.58	-0.77	0.00
MM7	-	-	-	-	-
MM14	-	-	-	-	-
MM21	-	-	-	-	-
MM28	-	-	-	-	-
MM56	-	-	-	-	-
MM84	-	-	-	-	-
MM112	-	-	-	-	-