

# Colostrum and foal serum immunoglobulin G levels and associations with perinatal abnormalities in heavy draft horses in Japan

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*The purpose of this study was to elucidate the colostrum and foal serum immunoglobulin G (IgG) concentration values in heavy draft horses in Japan and to examine the effects of peripartum mare condition on colostrum immunity. Colostrum was obtained 1 hr after foaling (pre-suckling; n=178). Blood was collected from the jugular vein of the foals (n=147) at 24 to 48 hr after birth. The foaling statuses of 73 mares were recorded. The average colostrum IgG concentration was 10,540 ± 3,190 mg/dl (median=10,928; range 1,434–17,514 mg/dl). The average serum IgG concentration obtained from neonatal foals 24 to 48 hr after birth was 1,750 ± 919 mg/dl (median=1,890; range 0–3,510 mg/dl). Although colostrum IgG did not differ between the normal foaling mare (n=59) and dystocia mare (n=14), foal serum IgG was lower in foals born in dystocia than in foals in normal foaling (P<0.05). This study demonstrates reference values for colostrum and foal serum IgG specific to heavy draft horses in Japan and suggests that dystocia may interfere with the acquisition of colostrum immunity in neonatal foals.*

**Key words:** colostrum, dystocia, foal, heavy draft horse, immunoglobulin G

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Foals are born essentially agammaglobulinemic and obtain protective immunity through the absorption of ingested colostrum antibodies [16]. Horses have a diffuse epitheliochorionic placenta that does not allow for immunoglobulin transfer from the dam to the fetus during gestation [29]. Newly born foals have specialized cells in their small intestinal mucosa that indiscriminately and actively absorb large molecules, including immunoglobulins and bacteria, through pinocytosis [11]. The foals' intestinal

mucosa capacity to absorb immunoglobulins disappears by approximately 24 hr after birth. This “gut closure” is caused by the replacement of specialized enterocytes with more mature cells [7]. Most immunoglobulins in the colostrum are of the immunoglobulin G (IgG) type, with the rest comprising very small amounts of IgM and IgA [12]. Immunoglobulins are selectively aggregated from the dam's blood into colostrum over the last few weeks of gestation under hormonal influences [11]. Timely ingestion of high-quality colostrum is essential to provide the foal with protective immunity. Foals that do not acquire sufficient immunoglobulins from colostrum are considered to have failure of passive transfer (FPT), and these foals are at higher risk of developing neonatal infection and death [13, 17, 21]. Although a serum IgG concentration of at least 400 mg/dl has been considered evidence of adequate passive transfer, most normal foals attain values of more than 800

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mg/dl [17, 18]. Serum IgG greater than 800 mg/dl may be required for adequate immunity [13]. Therefore, serum IgG between 400 and 800 mg/dl is called partial FPT.

The concentration of serum IgG in foals varies by report [5, 15, 24, 27], and the variability of colostral IgG between studies is even greater [3, 5, 9, 12, 14, 17, 20, 22]. In cattle, calf serum IgG levels after ingestion of colostrum vary greatly depending on the breed [25]. Furthermore, another report suggested that the level of passive immunity required for prevention of infectious diseases differs depending on the surrounding environment [30].

Heavy draft horses have a unique history in Japan. After the Sino-Japanese War (1894–1895) and Russo-Japanese War (1904–1905), Japanese domestic horses were crossbred with some foreign draft breeds to create a horse suitable for both military and industry uses [19]. After World War II, heavy draft horses were crossbred with Percheron, Breton, and Belgian draft horses to improve their ability to compete in draft horse racing (Ban'ei horse racing) [23]. Although some reference values for hematological and serum biochemical properties of heavy draft horses in Japan have been reported [1], the reference values for colostral immunity remain unclear.

Perinatal abnormalities such as dystocia and retained fetal membranes are important problems in equine reproduction and occur more frequently in heavy draft horses than in light breed horses [8, 26, 28]. Previous studies reported that dystocia causes negative effects on neonatal foals [4, 10]. These perinatal abnormalities may have a negative impact on passive transfer of colostral immunity in foals. However, to the best of our knowledge, the influence of the perinatal abnormalities on the passive transfer of immunity has not been reported in heavy draft horses in Japan.

The purpose of this study was to elucidate the colostral and foal serum IgG levels in heavy draft horses in Japan and to examine the effects of perinatal abnormalities on colostral and foal serum IgG.

## Materials and Methods

### *Animals*

This study was conducted at three private farms in Tokachi, Hokkaido, Japan. The study included 212 heavy draft mares (36 Percherons and 176 crossbreeds between Percheron, Belgian, and Breton heavy draft horses) that foaled between 2009 and 2015. The mean age of the mares was 9.2 years (range: 3–19 years). Only dry hay was fed *ad libitum* before foaling, and commercial concentrates (wheat bran and oat: digestible energy 5.6–8.4 Mcal/day) were fed after foaling. The mares were put out to graze with other mares during the daytime and kept in individual stalls (approximately 4 m × 8 m) at night at farms A and B. The

mares were kept in paddocks (approximately 20 m × 30 m) with 3–4 other mares throughout the day at farm C. Foals had *ad libitum* access to their dam's udder. No supplements were given. The farms did not routinely measure serum IgG in foals or colostrum quality and were blinded to the results of the IgG measurements. Therefore, no interventions such as colostrum supplementation or plasma transfusion were provided to any foals before the foals reached 1 day of age. Some mares leaked colostrum prior to foaling. Their foals were fed on frozen good quality colostrum obtained from other mares, and the data of these foals were excluded from this study. Foaling events and foal health status during the first three months of life were recorded for 73 mares and their foals foaled between 2013 and 2015. Parturient dams showing signs of foaling were monitored with a wireless camera. Foaling events such as rupture of the chorioallantoic membrane, appearance of the amniotic sac, and delivery of foals were recorded. The samples used in the study were collected during the routine clinical evaluations conducted on client-owned neonatal horses presented to the mobile clinic service of the Veterinary Medical Center of Obihiro University of Agriculture and Veterinary Medicine.

### *Mare and foal conditions*

Dystocia was defined as prolonged labor with strong fetal traction by more than 3 persons or mechanical tools with or without correcting fetal displacement. If stage II (interval between rupture of the chorioallantois until delivery of the foal) was >30 min and labor did not progress, traction was applied to the fetus [10]. Retained fetal membranes was defined as the failure to pass fetal membranes by 3 hr post-foaling [26], and postpartum septic metritis was defined as an elevated rectal temperature of >38.5°C within the first 4 days post-foaling [2]. Foals were examined and scored for APGAR within 5 min of birth as described in Knottenbelt *et al.* [11]. Foals that had an APGAR score of ≤ 10/14 were considered to have a low APGAR score. The farm staff observed all foals within 24 hr after birth and subjectively evaluated the foal suckling behavior. Farm staff also recorded the health status of the foal within 3 months after birth. Foal heat diarrhea was considered normal.

### *Sample collection*

Approximately 10 ml of colostrum was obtained 1 hr after foaling (pre-suckling). The initial colostrum samples were centrifuged for 30 min at 5,000 g, and the whey was separated and stored at –30°C for IgG analyses at a later date. Blood was collected from the jugular vein of the foals using 21G×1 ½” needles (Venject II MN-2138MS, Terumo Corp., Tokyo, Japan) and placed into 5 ml plain vacuum tubes (Venject II VP-P050K, Terumo Corp.) at 1 day (24–48 hr) after birth. The blood samples were allowed

to clot at 37°C for 90 min and then centrifuged at 1,000 g for 12 min. The serum was withdrawn and frozen at -30°C for IgG analyses at a later date.

### *Sample analysis*

Colostrum and serum IgG concentrations were determined using a commercially available single radial immunodiffusion kit (ECOS check for Equine IgG, Institute for Metabolic Ecosystem, Miyagi, Japan) according to the manufacturer's recommendations. The inter-assay coefficient of variation of this kit was less than 4%. The IgG quantification assays were performed at the end of each breeding season.

### *Data verification method*

First, we calculated the mean values of colostrum and foal serum IgG concentrations and compared them with data reported in previous studies that examined various breeds. We also examined the incidence of FPT and the correlation between colostrum and foal serum IgG. Next, we examined the effects of maternal perinatal abnormalities on colostrum and foal serum IgG levels. Finally, we studied the relationship between foal serum IgG levels and foal health status.

### *Statistical analysis*

All statistical analyses were performed using JMP Pro version 13 (JMP Pro version 13, SAS Institute, Cary, NC, U.S.A.). Significance was defined as a *P* value of <0.05, and a tendency was reported when the *P* value was <0.10 but not <0.05. Analyses of colostrum IgG and foal serum IgG were performed using linear regression analysis. The association between mare and peripartum conditions was examined using logistic regression analysis. Covariates were tested individually and offered to the final model built if *P*<0.20. The model was built using a backwards-stepwise method with a threshold of *P*<0.05 for retention in the final model. The association between foal health statuses with covariates was performed using ANCOVA. The differences in foal serum IgG concentrations at 1 day of age between different foal health statuses were evaluated using Dunnett's *post hoc* test with foals without any recorded health complications being used as the control.

## **Results**

### *Colostrum and foal serum IgG*

Results for colostrum and foal serum IgG were expressed as the mean ± standard deviation, median, and range. The average colostrum IgG concentration (n=178) was 10,540 ± 3,190 mg/dl (median=10,928 mg/dl; range 1,434–17,514 mg/dl). The average serum IgG concentration (n=147) obtained from neonatal foals at 1 day after birth was 1,750 ± 919 mg/dl (median=1,890 mg/dl; range 0–3,510 mg/dl).

The results for colostrum and foal serum IgG concentrations in this study and previous studies that examined other breeds are shown in Tables 1 and 2. The incidences of FPT and partial FPT were 5.4% (n=8/147) and 4.1% (n=6/147), respectively. Both colostrum and foal serum were obtained from 113 dams and their neonates in this study. There was a tendency toward a weak linear association between colostrum and foal serum IgG concentrations (*r*=0.20, *P*=0.055).

### *Mare peripartum conditions*

The incidence of dystocia was 19.2% (n=14/73 of foaling mares). Three mares needed correcting fetal displacement (posterior, anterior dorsoilial, and lateral deviation of the head), and the other 11 mares needed strong traction (maternal-fetal disproportion). The incidence of retained fetal membranes was 8.2% (n=6/73), and the incidence of postpartum septic metritis was 17.8% (n=13/73). When tested individually using univariate analysis, mares with dystocia were more likely to develop postpartum septic metritis (OR=5.57, 95% CI 1.49–20.86, *P*=0.012) and tended to be more likely to have retained fetal membranes (OR=5.09, 95% CI 0.91–28.60, *P*=0.07). Foal sera were collected from all 73 newborns, but no colostrum samples were obtained from 9 mares. No effects of retained fetal membranes (*P*=0.209) and postpartum septic metritis (*P*=0.809) on foal serum IgG at 1 day of age were found in this study. Only dystocia was significantly associated with foal serum IgG at 1 day of age when evaluated as a continuous variable (*P*=0.039). Although colostrum IgG did not differ between the normal foaling mare and dystocia mare (normal foaling, 10,175 ± 438 mg/dl; dystocia, 10,309 ± 840 mg/dl; *P*=0.448), foal serum IgG was lower in foals born in dystocia than in foals in normal foaling (normal foaling, 1,815 ± 111 mg/dl; dystocia, 1,263 ± 196 mg/dl; *P*=0.016).

### *Foal health for the first 3 months*

Overall, 82.2% (n=60/73) foals did not have any health complications during the first three months of life. One foal that was born from a mare with dystocia and was observed by the owners to exhibit poor suckling died between 24 and 48 hr after birth. This foal had high serum concentrations of IgG at 1 day of age (1,236 mg/dl). Two foals had neonatal isoerythrolysis, and two foals had diarrhea. All four of these foals had high serum IgG concentrations (≥800 mg/dl) at 1 day of age that were numerically higher than the average for foals without any health complications. One foal had a congenital inguinal hernia, and one foal had a limb deformity and difficulty standing; both foals had serum IgG concentrations ≥800 mg/dl at 1 day of age. Two foals had low APGAR scores, and one of them had FPT, while the other had adequate passive transfer. Four

**Table 1.** Mean colostral IgG concentrations in various breeds at the time of parturition

Horse breed	Sample size	Colostral IgG (mg/dl)	Reference
Thoroughbred	32	2,354	McGuire <i>et al.</i> 1977 [17]
Quarter horse	8	3,125	Kenzig <i>et al.</i> 2009 [9]
Shetland pony	9	4,600	Rouse and Ingram 1970 [22]
Thoroughbred	22	4,608	Pearson <i>et al.</i> 1984 [20]
Various breeds	33	5,450	Erhard <i>et al.</i> 2001 [5]
Crossbreed	6	6,155	Krakowski <i>et al.</i> 1999 [14]
Warmblood and trait Breton	39	7,060	Chavatte <i>et al.</i> 1998 [3]
Standardbred	36	8,912	Kohn <i>et al.</i> 1989 [12]
Arabian	14	9,691	Pearson <i>et al.</i> 1984 [20]
Heavy draft horse	178	10,540	Present study

**Table 2.** Serum IgG concentrations of foals of various breeds at 1 to 2 days after birth

Horse breed	Sample size	Foal serum IgG (mg/dl)	Reference
Standardbred	138	998 *	LeBlanc <i>et al.</i> 1992 [15]
Thoroughbred	70	1,046 *	LeBlanc <i>et al.</i> 1992 [15]
Arabian	45	1,105 *	LeBlanc <i>et al.</i> 1992 [15]
Thoroughbred	29	1,200 *	Erhard <i>et al.</i> 2001 [5]
Standardbred	16	1,250 *	Erhard <i>et al.</i> 2001 [5]
Warmblood	60	1,430 **	Tscheschlok <i>et al.</i> 2017 [27]
Thoroughbred	52	1,635 **	Sedlinská <i>et al.</i> 2006 [24]
Heavy draft horse	147	1,750 *	Present study

\*Mean value, \*\* median value.

foals were observed to exhibit poor suckling by the owner, and two foals had FPT at 1 day of age. At 1 day of age, foals observed to exhibit poor suckling had significantly lower serum IgG concentrations compared with foals with no observed complication ( $P=0.037$ ), and foals with low APGAR scores tended to have lower serum IgG concentrations ( $P=0.089$ ).

## Discussion

This study reports the colostral and foal serum IgG concentrations from a large number of heavy draft horses in Japan. Previous studies have reported that colostral and foal serum IgG levels vary among studies (Tables 1 and 2). The reference values for both colostral and foal serum IgG were comparable or relatively higher in our study than in previous studies. The timing of collecting colostrum samples (immediately or within hours after birth), the IgG quantification method (single radial immunodiffusion or enzyme-linked immunosorbent assay), the method for managing the horses, and the surrounding environment differ between reports. Therefore, careful consideration is needed when discussing the effects of breed differences on colostral immunity. There is a report that breed differences significantly affect colostral IgG concentrations [20]. In that study, the mean concentra-

tion of IgG in the colostrum of the Arabian mares at the time of parturition was significantly higher than the average for the Thoroughbreds. Foal serum IgG levels vary widely even in studies examining the same breed (Thoroughbred) [5, 15, 24].

It is essential for successful transfer of colostral immunity that the foals drink a sufficient quantity of colostrum as early as possible after birth [11]. However, information on the time from birth to colostral ingestion and quantity of colostrum ingested within 1 day after birth was not available in the present study. In future studies, careful examination of these factors will provide a better understanding of characteristics of heavy draft horses in Japan.

Dystocia was associated with foal serum IgG when evaluated as a continuous variable. The dystocia rates in draft horses are higher than those in light breed horses [6]. The colostral IgG concentration did not differ between the normal foaling mare and dystocical mare in the present study. Previous studies reported that dystocia causes negative effects on neonatal foals, such as anemia, hypercortisolemia, acidemia, and hyperlacticacidemia [4, 10]. It was speculated that dystocia possibly affects the quantity or timing of colostrum intake by the foal and the efficiency of IgG absorption in the intestine and that it results in a decline of foal serum IgG levels. In the present study, only one of 14 foals born

in dystocia was observed to exhibit poor suckling during the first day of life. Although more studies will be needed in the future, dystocia presumably interferes with the acquisition of colostrum immunity in neonatal foals.

Despite the high dystocia rate, only one foal died in the first three months of life in this study. None of the surviving foals developed septicemia, even those that had FPT. Foals that were observed to exhibit poor suckling had significantly lower serum IgG at 1 day of age compared with normal foals. The classification of poor suckling is based on a farmer's subjective observation. Future studies should incorporate a more objective method to determine suckle reflex, quantity of colostrum intake, and time to colostrum intake after birth to confirm this observation. Foals that have low APGAR scores may benefit from prophylactic treatment, as they are likely to have FPT. This study was conducted on farms that do not test for FPT. The farmers and researchers were blinded to the results of the foal and colostrum IgG measurements, which were conducted at the end of each foaling season. This was a unique opportunity to determine the health consequences in foals that do not receive adequate colostrum passive transfer. We found that the foals were generally healthy after the samplings at 1 day of age, even those that had FPT. Two foals developed mild diarrhea that was not treated. Both these foals had high serum IgG concentrations at 1 day of age. Due to the lack of foals that developed diseases associated with FPT, we could not determine the minimum serum IgG concentration that is protective in the present study.

In conclusion, our study demonstrates reference values for colostrum and foal serum IgG specific to heavy draft horses in Japan and suggests that dystocia may interfere with the acquisition of colostrum immunity in neonatal foals.

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