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Postnatal colostrum management and its impact on IgG blood levels of neonatal foals

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Summary: There is ongoing need to investigate the quality, quantity, and timing of colostrum intake in neonatal foals as their ability to ward off infectious diseases largely relies on colostral maternal antibodies. The aim of the study was to evaluate whether postnatal colostrum management has an impact on plasma gamma globulin levels, on the incidence of failure of passive transfer of immunity (FPTI) and on the risk of disease. For this purpose, first of all the usefulness of plasma total globulin (TG) for the detection of FPTI was determined. The study was designed as a prospective cohort study with three groups and included 182 foals, which were randomly assigned to one of the three groups. The foals were assisted in standing and finding the udder if necessary. All foals were free to ingest colostrum by nursing from the udder. Foals in group 1 (n = 58) were not given any colostrum by bottle. Foals in group 2 (n = 66) received two times 150 ml of the mare's colostrum one and two hours postnatal by bottle-feeding. Foals in group 3 (n = 58) were two times bottle-fed 150 ml of pooled colostrum one and two hours postnatal. Pooled colostrum had been obtained from mares of the same stud and foaling season ahead of the study. Foal blood samples were taken 12 and 24 hours postnatal. Gamma globulin levels were determined using capillary zone electrophoresis and plasma total globulin was calculated by using an in-house dry chemistry analyzer. The different postnatal care practices did not result in significantly different average gamma globulin concentrations neither 12 hours postnatal (group 1: 1219 ± 472 mg/dl; group 2: 1191 ± 437 mg/dl; group 3: 1360 ± 446 mg/dl) nor 24 hours postnatal (group 1: 1109 ± 423 mg/dl; group 2: 1059 ± 406 mg/dl; group 3: 1230 ± 389 mg/dl). Feeding colostrum via bottle to group 2 and group 3 foals resulted in a delayed suckling from the udder (time between birth and first unassisted colostrum intake: group 1: 1.6 ± 0.8 hours; group 2: 2.6 ± 0.7 hours; group 3: 2.6 ± 0.7 hours). Neither the incidence of FPTI (IgG concentration < 800 mg/dl; group 1: 25.9 %, group 2: 21.2 %, group 3: 13.8 %) nor the morbidity (group 1: 19.0 %; group 2: 24.2 %; group 3: 19.0 %) did differ significantly between the three groups. 38 of 182 foals developed an infectious disease, but foals with FPTI were not more likely to become sick than foals with adequate IgG levels (p < 0.57). Results of the current study suggest that there is no general need in feeding additional colostrum to healthy neonatal foals to ensure a sufficient IgG level in plasma. No correlation was observed between FPTI and the development of infectious diseases. IgG concentrations determined by electrophoresis were highly correlated with plasma globulin levels.

Keywords: horse, foal, neonate, colostrum, IgG, FPTI, total globulin, electrophoresis

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Introduction

Neonatal foals are born hypogammaglobulinemic due to limited diaplacental transfer of immunoglobulins (*Jeffcott* 1974a, *Giguère* and *Polkes* 2005). Their immune system is considered as capable (immunocompetent) but immature (*Perkins* and *Wagner* 2015). Therefore, maternally derived colostral antibodies against specific antigens are needed to prevent infections during the neonatal phase. This results in a special importance of colostrum supply and colostrum management for the prevention of infectious disease in the young foal.

Postnatal colostrum management is well evaluated for calves, which also depend on the transfer of passive immunity. As in foals, sufficient supply with high-quality colostrum is considered essential for health and development of the newborn calf (*Conneely* et al. 2014). The uptake of ≥ 3 liter of high-quality colostrum within 6 hours after birth is recommended in calves to avoid failure of passive transfer of immunity (*Osaka* et al. 2014, *Godden* et al. 2009). The optimal amount of colostrum, depending on the quality and the timing of colostrum supply, is still under investigation in foals. It is commonly recommended that foals take up colostrum of sufficient quality within the first two hours after parturition. Colostrum should contain at least 30 g/l immunoglobulin G (IgG) and be administered in a period of 24 hours at a volume of 1 to 2 L to ensure an adequate level of IgG (*Giguère* and *Polkes* 2005).

In foals, transfer of passive immunity is usually assessed 12 to 24 hours postnatal, due to the peak of IgG levels in foal blood plasma (*Warko* and *Bostedt* 1993, *Tscheschlok* et al. 2016a). In general, enteral absorption of colostral antibodies ceases

24 hours after birth. Blood IgG levels higher than 800 mg/dl are considered adequate (*Koterba* et al. 1985) whereas IgG levels between 400 and 800 mg/dl are classified as PFPTI (partial failure of passive transfer of immunity) and IgG levels below 400 mg/dl are classified as TFPTI (total failure of passive transfer of immunity) (*Tyler-McGowan* et al. 1997, *Giguère* and *Polkes* 2005, *Liepman* et al. 2015). FPTI predisposes foals for infections and often leads to an increased susceptibility for neonatal foal infectious diseases (*Crawford* et al. 1977, *McGuire* et al. 1977, *Koterba* et al. 1985, *Raidal* 1996). The risk of disease for hypogammaglobulinemic foals is also governed by environmental factors like housing conditions and farm management (*Baldwin* et al. 1991).

The aims of this study were to evaluate the usefulness of plasma total globulin (TG) in predicting FPTI, and whether postnatal colostrum management has an impact on plasma gamma globulin levels, on the incidence of FPTI and on the risk of infectious disease.

Materials and methods

Study sample

182 foals were included in the study which were born on one breeding farm during one season and raised under the same environmental conditions. Foaling took place in separate boxes, where the mares and their newborns were closely monitored by experienced farm staff and clinically examined by veterinarians immediately after birth. Inclusion criteria were vigorous foals, born on term, without any malformations or severe limb deformities, from mares with a colostrum with a refractive index above 22 % respectively above 50 g/l IgG.

Study design

The study was performed as a prospective study during April to July of foaling season 2020. The group assignment was randomized.

All foals were assisted in standing and finding the udder if needed and were free to ingest colostrum by nursing from the udder. Before inclusion in the study, it was made sure that the foals had a decent sucking reflex and could stand on their own. Group 1 included 58 foals that did not receive any colostrum by bottle. Group 2 included 66 foals which were supplemented one and two hours postnatal with 150 ml of their mother's colostrum by bottle feeding. Group 3 included 58 foals which received pooled colostrum for supplementation. They got bottle-fed 150 ml of the pooled colostrum one and two hours postnatal. The pooled colostrum was prepared from colostrum of 95 mares of the same stud who had foaled earlier during the season. Each single colostrum sample used for the pool had a refractive index of ≥ 22 % respectively 50 g/l IgG. A maximum of 250 ml colostrum was collected after their own foal's first intake to ensure sufficient supply with maternal antibodies. The refractive index of the pooled colostrum was 29.5 %. It was stored in 150 ml aliquots at -20 °C until use.

Assessment of drinking at the udder

The parturition of each foal was supervised closely by veterinarians and experienced farm staff. As soon as the foals did their first attempts, they were assisted in standing and finding the udder. The time between birth and first unassisted intake of colostrum was determined when they were able to stand up, to find the udder and to nurse independently.

Blood sampling and blood parameters

To assess the transfer of passive immunity via colostrum, blood samples were routinely taken from the foals 12 and 24 hours postnatal. Venous blood was collected from the jugular vein into lithium-heparin tubes and centrifuged (10 min, 2000 g, room temperature). Total plasma protein and albumin was analyzed immediately after collection and centrifugation with a dry chemistry analyzer (FUJIFILM DRI-CHEM NX 500 PF)[1]. Plasma total globulin concentration was then calculated as the difference of total protein and albumin. Plasma aliquots (1.5 ml) were stored frozen at -18° C and within one month after the study used for the measurement of IgG levels by means of capillary zone electrophoresis by an external laboratory[2].

Statistical methods

The statistical analysis was done using SAS Software, version 9.4, and SAS Enterprise Guide, version, 7.15 (SAS Institute Inc., Cary, NC). P-values < 0.05 were assumed to indicate statistically significant results. Kruskal-Wallis test, with post-hoc Wilcoxon rank-sum tests, was used to compare duration of gestation, colostrum quality of the mares and time until unassisted colostrum intake of the foals between the groups. The IgG-levels were analyzed using a mixed model with foal

Table 1 Comparison of baseline values between foal groups (group 1: n = 58, no supplementation with colostrum; group 2: n = 66, supplementation with 300 ml of colostrum from the mare; group 3: n = 58, supplementation with 300 ml of pooled colostrum). | *Vergleich der Grunddaten, gruppenvergleichende Darstellung (Gruppe 1: n = 58, keine Supplementation mit Kolostrum; Gruppe 2: n = 66, Supplementation mit 300 ml Kolostrum der Mutterstute; Gruppe 3: n = 58, Supplementation mit 300 ml Pool-Kolostrum).*

	All foals	Group 1	Group 2	Group 3
Number of foals	182	58	66	58
Duration of gestation (days; mean ± SD)	336 ± 7.6	337 ± 7	335 ± 8	337 ± 7
Colostrum quality (Brix index in %; mean ± SD)	27.2 ± 3.3	26.4 ± 3.0	27.8 ± 3.4	27.4 ± 3.5

group, time point (12 h and 24h) and their interaction, i.e., the combined effect of group and time, as fixed effects, and individual effects with autoregressive covariance structure over time. Tukey-Kramer adjustment was used for the post-hoc analysis. Normality of the residuals was checked visually using histograms and Q-Q-plots. A Chi-square-test was used to test the association of categorical variables, e.g., for differences of the incidence of FPTI between groups.

Results

The mean duration of gestation was 336.7 ± 7.6 days. The mean colostrum quality (Brix values) was 27.2 ± 3.3 % (min: 22.1 %, max: 38.4 %) and did not differ significantly between the groups (Table 1). Pooled colostrum was used without any incidents of neonatal isoerythrolysis on all 58 foals of group 3.

The time between birth and the first unassisted colostrum intake from the udder in the 182 foals was 2.3 ± 0.9 hours. In group 1 (no colostrum supplementation) the foals needed 1.6 ± 0.8 hours after birth to drink at the mare's udder. In each of the other groups, group 2 (supplemented with 300 ml of colostrum from the mare) and group 3 (supplemented with 300 ml of pooled colostrum), foals nursed unassisted after 2.6 ± 0.7 hours postnatal (Fig. 1). Group 1 foals nursed significantly earlier than group 2 and group 3 foals ($p < 0.0001$ for the Kruskal-Wallis and the post-hoc test to compare group 1 with the other groups).

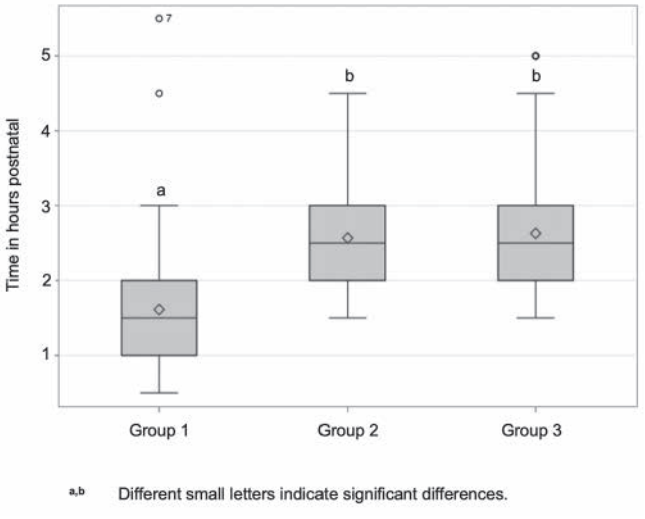

Fig. 1 Time in hours postnatal to first unassisted colostrum intake compared between the groups (group 1: n = 58, no supplementation with colostrum; group 2: n = 66, supplementation with 300 ml of colostrum from the mare; group 3: n = 58, supplementation with 300 ml of pooled colostrum). | *Zeit in Stunden zwischen Geburt und selbstständiger Kolostrumaufnahme, gruppenvergleichende Darstellung (Gruppe 1: n = 58, keine Supplementation mit Kolostrum; Gruppe 2: n = 66, Supplementation mit 300 ml Kolostrum der Mutterstute; Gruppe 3: n = 58, Supplementation mit 300 ml Pool-Kolostrum).*

The mean plasma IgG level of all foals was 1253 ± 455 mg/dl at 12 hours postnatal (Table 2). The values dropped to 1130 ± 411 mg/dl at 24 hours postnatal. While this effect of time on the mean plasma IgG level was statistically significant ($p < 0.0001$; Fig. 2), neither the differences in mean of the three foal groups ($p < 0.1$) nor the combined effect of time and group ($p < 0.8$) were significant. The non-supplemented group 1 foals and group 2 foals supplemented with 300 ml of colostrum from the mare displayed slightly but not significantly lower mean values than group 3 foals supplemented with 300 ml of pooled colostrum at both times.

The mean plasma total globulin (TG) concentration at 24 hours postnatal was 30 ± 5 g/l (range: 13–43 g/l) for all foals. Plasma TG concentrations were highly positively correlated (r = 0.9) with plasma IgG levels 24 hours postnatal measured by electrophoresis ($p < 0.0001$; Fig. 3). IgG levels ≥ 800 mg/dl were predicted with TG concentrations of ≥ 25 g/l with a sensitivity of 97.9 % and a specificity of 67.6 %. Detailed sensitivities and

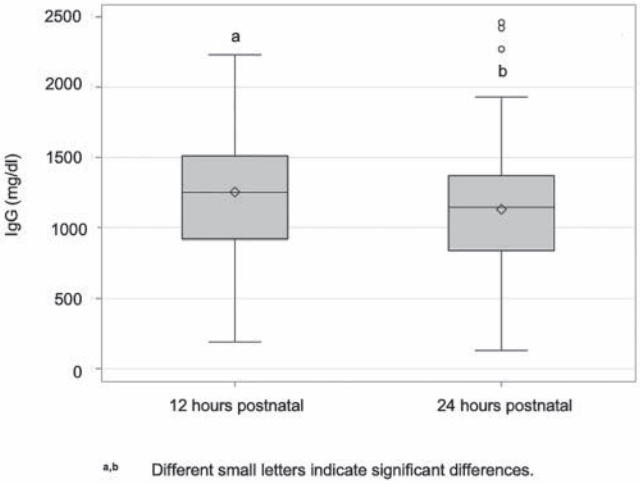

Fig. 2 Mean IgG level of all foals (n = 182) 12 and 24 hours postnatal. | *Mittlere IgG-Konzentration aller Fohlen (n = 182) 12 und 24 Stunden postnatal.*

Table 2 IgG concentrations (mg/dl) in foal plasma (group 1: n = 58, no supplementation with colostrum; group 2: n = 66, supplementation with 300 ml of colostrum from the mare; group 3: n = 58, supplementation with 300 ml of pooled colostrum). | *IgG-Konzentrationen (mg/dl) im Plasma der Fohlen (Gruppe 1: n = 58, keine Supplementation mit Kolostrum; Gruppe 2: n = 66, Supplementation mit 300 ml Kolostrum der Mutterstute; Gruppe 3: n = 58, Supplementation mit 300 ml Pool-Kolostrum).*

		All foals (n = 182)	Group 1 (n = 58)	Group 2 (n = 66)	Group 3 (n = 58)
12 hours postnatal	Mean ± SD mg/dl	1253 ± 455 [a]	1219 ± 472 [a]	1191 ± 437 [a]	1360 ± 446 [a]
	Range mg/dl	190–2440	300–2210	230–2440	190–2230
24 hours postnatal	Mean ± SD mg/dl	1130 ± 411 [b]	1109 ± 423 [b]	1059 ± 406 [b]	1230 ± 389 [b]
	Range mg/dl	130–2460	240 – 2270	130–2460	210–2420

[a,b] Different small letters within columns indicate significant differences. SD: standard deviation

specificities for different cut-offs for plasma TG concentrations to assess an IgG level ≥ 800 mg/dl are shown in Table 3.

At 24 hours postnatal, 37 of 182 foals (20.3%) had IgG concentrations < 800 mg/dl, the majority of these foals (n = 32) had values between 400 and 800 mg/dl (PFPTI) and five foals had IgG concentrations < 400 mg/dl (TFPTI) (Table 4). The incidence of FPTI in the three groups did not differ significantly (group 1: 25.9%, group 2: 21.2%, group 3: 13.8%; p < 0.26).

38/182 foals (20.9%) developed an infectious disease (omphalitis, diarrhea, or bronchopneumonia) during the first month of life. The morbidity did not differ significantly between the foal groups (Table 5; group 1: 19.0%; group 2: 24.2%; group 3: 19.0%). Nine of the 38 sick foals (23.7%) had IgG concentrations < 800 mg/dl at 24 hours postnatal. There was no significant association between an IgG concentration < 800 mg/dl and developing an infectious disease in the first four weeks postnatal (p < 0.57).

Discussion

The aim of the current study was to evaluate the advantages of an additional intake of colostrum for the newborn foal.

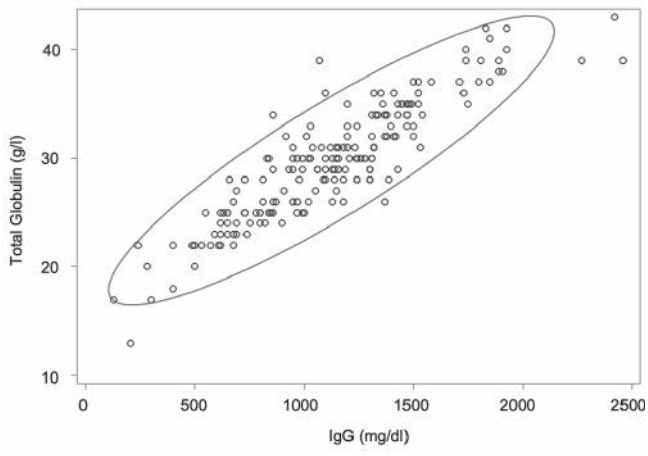

Fig. 3 Correlation of plasma total globulin and IgG concentration 24 hours postnatal. Elypse markes 95% prediction. | *Korrelation von Gesamtglobulin (g/l) und IgG-Konzentration (mg/dl) 24 Stunden postnatal. Streuungsdiagramm mit 95% Vorhersageellipse.*

Table 3 Sensitivity and specificity for detecting IgG ≥ 800 mg/dl with different cut-off values for plasma total globulin (TG). | *Sensitivitäten und Spezifitäten der Cut-off Werte für Gesamtglobulin (TG) zur Vorhersage eines IgG-Wertes ≥ 800 mg/dl.*

Cut-off TG (g/l)	Sensitivity (%)	Specificity (%)
29	75.9	100.0
28	83.5	94.6
27	85.5	91.9
26	91.7	89.2
25	97.9	67.6
24	100.0	56.8

Studies comparing different postnatal management practices are rare in foals (*Tscheschlok* et al. 2016a). More research has been done in cattle, where it is common to transfer colostrum via bottle or nasogastric tube, thus ensuring a sufficient supply of maternal antibodies (*Godden* et al. 2009). In calves, the timing and recommended amount of colostrum intake has been clearly defined (*Jaster* 2005; *Conneely* et al. 2014, *Osaka* et al. 2014). Newborn calves are usually being kept separately and do not nurse any colostrum from the udder which allows calculation of the total IgG uptake. In contrast, foals are being kept with the mare to ensure the establishment of a dam-foal bond. All foals in the current study were free to nurse from the udder and foals in group 2 and group 3 were additionally supplemented with colostrum via bottle feeding. This consideration limits verifying the total IgG uptake by the foal but allows evaluating the advantages of an additional colostrum feeding. Unlike in horses, the administration of pooled colostrum to calves is a common practice which allows a standardization of the IgG uptake (*King* et al. 2019). The use of pooled colostrum for foals is generally limited by the fact that foals usually nurse from their dam and are merely supplemented with foreign colostrum if the dam's colostrum is limited in volume and/or concentration of antibodies. In the current study, the use of pooled colostrum ensured the supply of all foals of group 3 with the exact same amount of additional IgG. The colostral quality of the pooled colostrum with a refractive index of 29.5% can be considered high containing more than 50 g/L IgG (*Chavatte* et al. 1998, *Cash*

Table 4 IgG concentrations of the foals 24 hours postnatal (group 1: n = 58, no supplementation with colostrum; group 2: n = 66, supplementation with 300 ml of colostrum from the mare; group 3: n = 58, supplementation with 300 ml of pooled colostrum). | *IgG-Konzentrationen der Fohlen 24 Stunden postnatal (Gruppe 1: n = 58, keine Supplementation mit Kolostrum; Gruppe 2: n = 66, Supplementation mit 300 ml Kolostrum der Mutterstute; Gruppe 3: n = 58, Supplementation mit 300 ml Pool-Kolostrum).*

IgG (mg/dl)	Group 1 (n = 58)	Group 2 (n = 66)	Group 3 (n = 58)
≥ 800	43	42	50
< 800	15	14	8
400–800	14	12	6
< 400	1	2	2

Table 5 Number of healthy foals and foals who developed an infectious disease (group 1: n = 58, no supplementation with colostrum; group 2: n = 66, supplementation with 300 ml of colostrum from the mare; group 3: n = 58, supplementation with 300 ml of pooled colostrum). | *Anzahl an gesunden Fohlen und Fohlen, die eine Infektionserkrankung entwickelten (Gruppe 1: n = 58, keine Supplementation mit Kolostrum; Gruppe 2: n = 66, Supplementation mit 300 ml Kolostrum der Mutterstute; Gruppe 3: n = 58, Supplementation mit 300 ml Pool-Kolostrum).*

	All foals (n = 182)	Group 1 (n = 58)	Group 2 (n = 66)	Group 3 (n = 58)
Healthy foals	144	47 [a]	50 [a]	47 [a]
Sick foals	38	11 [a]	16 [a]	11 [a]

[a,b] Different small letters within columns indicate significant differences.

1999, *Luft* 2000). Although there is no published data on the frequency of neonatal isoerythrolysis induced by foreign colostrum intake in foals, the risk of neonatal isoerythrolysis by providing multiple colostrum sources must be considered. In the current study, however administration of the pooled colostrum resulted in no cases of isoerythrolysis in 58 foals.

The foal's ability to absorb immunoglobulins from the colostrum is limited by time due to the rapid turnover of the specialized enterocytes in the small intestine, which are capable to absorb macromolecules into the bloodstream. The absorption rate is highest after birth and subsequently declines. At approximately 20 hours postnatal the amount of absorbed macromolecules becomes negligible (*Jeffcott* 1974c). Thus, early colostrum intake results in a greater quantity of absorbed immunoglobulins (*Raidal* et al. 2005). In the present study, group 1 foals, assisted in standing and finding the udder without receiving additional colostrum by bottle, nursed unassisted 1.6 ± 0.8 hours after birth. For foals supplemented with colostrum by bottle (group 2 and 3) the duration was 2.6 ± 0.7 hours from birth to unassisted nursing. This might suggest that additional bottle-feeding could have reduced the efforts of supplemented foals to learn how to suckle from the udder compared to non-supplemented foals. Nevertheless, healthy foals are supposed to nurse independently after one to three hours which was observed in all foals of this study and which is in line with previous observations (*Jeffcott* 1974b, *LeBlanc* 2001, *Tscheschlok* et al. 2016a).

To assess the absorbed amounts of colostral immunoglobulins, foal plasma IgG concentrations were determined at 12 and 24 hours postnatal. IgG levels peaked at 12 hours postnatal (mean: 1253 ± 455 mg/dl), which goes along with earlier findings (*Warko* and *Bostedt* 1993, *Tscheschlok* et al. 2016a). At 24 hours postnatal, we observed significantly lower IgG concentrations (Table 2). In all foal groups, IgG levels dropped consistently by about 10%. Whether this decline was due to metabolization and/or secretion of antibodies was not further studied. The rate of foal plasma IgG level decline between 12 and 24 hours may differ individually or between foal populations. *Tscheschlok* et al. (2016a) reported only a slight decline between 12 and 24 hours whereas *Warko* and *Bostedt* (1993) observed a similar decline as in our study (12.9%) but during the first three days of life.

Compared to group 1 foals, colostrum bottle-feeding of group 2 and group 3 foals did not result in significantly higher mean IgG values at 12 hours or 24 hours postnatal. However, bottle-fed group 3 foals displayed a substantially lower incidence of FPTI (13.8%) compared to foals of group 1 (25.9%) and group 2 (21.2%). Although the effect did not reach significance, this might suggest that supplementation with high-quality colostrum supply reduces the incidence of FPTI.

FPTI (IgG value < 800 mg/dl) was observed in 20.3% of the foals of the current study which is in the range of earlier reports (*Baldwin* et al. 1989, *Bublitz* et al. 1991, *Tyler-McGowan* et al. 1997, *Tscheschlok* et al. 2016a). The incidence of TFPTI (IgG value < 400 mg/dl) was 2.8% and similar to the findings of *Raidal* (1996). Other investigations reported higher TFPTI incidences (5.4% – 16%) for foal populations on breeding farms (*Baldwin* et al. 1989, *Baldwin* et al. 1991, *Tyler-McGowan* et al. 1997, *Aoki* et al. 2020). Under hospitalized conditions TFPTI incidence can reach 33%, probably associated with infectious disorders of sick foals (*Liepman* et al. 2015). In the current study, farm management, the exclusion of weak foals and mares with poor colostrum quality most likely promoted the observed low TFPTI incidence.

In our study, FPTI (IgG concentrations < 800 mg/dl) and the risk of infectious diseases were not significantly associated. This is similar to findings of *Baldwin* et al. (1991) and in contrast with other reports (*Raidal* 1996, *Tyler-McGowan* et al. 1997, *Liepman* et al. 2015). The reason why we did not observe an association between FPTI and neonatal infectious diseases might be the thorough health management on the farm ensuring an additional protection to undersupplied foals. It also needs to be considered that passive immunity by maternal colostral antibodies is only one facet of immunity transfer into newborn foals. Among other ingredients, colostral cytokines, growth factors and viable maternal immune cells favor the development of the newborn's immune system and may play an essential role in providing protection (*Perkins* and *Wagner* 2015).

Although capillary zone electrophoresis (CZE) measures the gamma globulin fraction and not directly IgG concentrations, it can be used to estimate the transfer of antibodies as most immunoglobulins are found in the gamma globulin fraction. Considering the delay between blood sampling and electrophoresis results, there is a need for on-farm methods to assess more promptly the transfer of maternal antibodies. In our study, total plasma globulin (TG) correlated very strongly with IgG concentrations determined by electrophoresis, which confirms similar previous statements (*Hurcombe* et al. 2012, *Fouché* et al. 2014, *Tscheschlok* et al. 2016b). Cut-off values of 25 g/l or 26 g/l TG to rule out FPTI showed satisfying sensitivities of 97.9% and 91.2%, respectively. Thus, TG determination is feasible as a more rapid and still reliable alternative when used with analyzer-specific cut-offs.

Conflict of interest statement

No competing interests have been declared.

Animal welfare statement

Not applicable for this article.

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Authorship

All authors contributed to the study design, data analysis and interpretation and preparation and approval of the manuscript. A. L. Nissen contributed additionally to data collection and study execution. M. Venner contributed particularly to study design and data interpretation.

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