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Tumor necrosis factor-α is transferred to equine neonates via colostrum but is not associated with their health status

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ABSTRACT

We followed the hypothesis that equine neonates with reduced transfer of tumor necrosis factor-α (TNFα) are at increased risk of neonatal infection. We investigated TNFα concentrations in colostrum of healthy mares and blood of their neonates in a non-hospitalized population of Warmblood mares where delivery, neonatal adaptation and health was closely monitored by veterinarians. Concentration of TNFα and IgG was determined in colostrum respective milk and in neonatal blood collected immediately after delivery and 18 h thereafter in 97 foals that were assigned to groups failure of passive transfer (FPT; $n = 31$) and control (CON; $n = 66$) based on serum IgG concentration at 18 h of age. Foal health was assessed repeatedly during the first 24 h of life. Statistical analysis was done with $p < 0.05$ indicating significance. There were no significant differences between foal groups FPT and CON regarding age and parity of dams, gestation length (FPT 343 ± 10, CON 340 ± 8 days) and foal sex. Concentrations of TNFα in colostrum at birth and in foals at 18 h varied but did not differ between groups (colostrum FPT 6.1 ± 9.1, CON 9.9 ± 31.5 ng/ml; foal FPT 2.3 ± 5.9, CON 2.4 ± 5.3 ng/ml; n.s.). There was an increase in the mean serum TNFα concentration until 18 h in foals (n.s. between groups). Results of the present study confirm previous findings of TNFα transfer from the mare to the neonate via colostrum but do not suggest that transfer of TNFα via colostrum is important for protection of the neonate against infectious diseases.

1. Introduction

Timely and adequate colostrum intake is vital for the equine neonate because the epitheliochorial placenta does not allow for transfer of maternal immunoglobulins to the fetus (Perryman et al., 1980). Therefore, neonatal equine sepsis resulting from failure of passive transfer is still among the most important reasons for foal losses (Jeffcott, 1974; Giguère et al., 2017). Colostrum which contains immunoglobulins and additional factors, provides various bioactive substances to the neonate that may serve different purposes. For example, insulin like growth factor (IGF)-1 and its receptor have been identified to be involved in postnatal intestinal development in several species (pig: Widdowson et al., 1976; human: Heird and Hansen, 1977; cattle: Baumrucker et al., 1994). Such an association could not be confirmed in newborn foals despite the presence of large concentrations of IGF-1 in equine colostrum (Palm et al., 2013; Palm et al., 2015). The involvement of colostral cytokines in the development of adaptive immunity was suggested for the newborn horse (Burton et al., 2009; Secor et al., 2012; Mariella et al., 2017). To the best of our knowledge, scientific evidence in this regard, however, at best is preliminary because no information on the influence of cytokine intake via colostrum on the subsequent health of foals is available.

In the horse, the fetal liver and bone marrow are active sites of hematopoiesis from as early as 100 days of gestation (Battista et al., 2014). Newborn foals are, however, still immunologically naïve to antigens and their adaptive immunity is considered immature (Galan et al., 1986). Adaptive immunity depends on cytokines that are produced by a specific T helper-cell subpopulation or, in case of tumor necrosis factor α (TNFα), by activated macrophages. In several species, cytokines are also transferred to the neonate via colostrum. Their presence in colostrum has been determined in humans (e.g., Bocci et al., 1993; Takahata et al., 2003), cattle (Goto et al., 1997; Hagiwara et al., 2000; Hagiwara et al., 2008) and pigs (Nguyen et al., 2007). In horses, the present information available in this regard is very limited. Research on the abundance of peripheral cytokines so far focused on the identification of their possible prognostive value as markers for survival of septic foals (Burton et al.,

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2009). Because of colostral transfer of some cytokines like IGF-6 and TNFα, it was suggested that failure of passive transfer (FPT) in foals may cause susceptibility to infectious diseases, not only due to of a lack of IgG but also in proinflammatory cytokines. The lack in proinflammatory cytokines has been suggested to play a role in neonatal immune protection because of the naïve nature of the neonatal foal's immune system (Burton et al., 2009; Secor et al., 2012). TNF-α is known to stimulate inflammatory responses directly by inducing inflammatory gene expression and indirectly by inducing cell death (Van Loo and Bertrand, 2023).

In the present study, we followed the hypothesis that equine neonates with reduced transfer of TNFα are more prone to neonatal infection than those with early abundance of TNFα. In addition, we aimed to elucidate factors that might explain the wide variation in TNFα concentrations in colostrum and the neonatal circulation that have been previously reported in preliminary investigations by Secor et al. (2012). We therefore investigated TNFα concentrations in colostrum of healthy mares and the blood of their neonatal foals in a non-hospitalized population where delivery, neonatal adaptation and health was closely monitored by veterinarians.

2. Material and methods

2.1. Experimental animals

The study was approved by the competent authority for animal experimentation in Lower Saxony State, Germany (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit; license number 33.19–42,502-05-20A483).

A total of 100 healthy Warmblood brood mares with singleton pregnancies monitored for foaling in a veterinary clinic in Northern Germany were available for this study. Mares were between 4 and 21 years of age (average 11.6 years). From these mares, 52% (52/100) were brought to the clinic on average 17.5 days (range 1–55 days) before parturition. The other 48% (48/100) lived on the property that belonged to the veterinary clinic for 6 months to 5 years prior to foaling. Mares were housed in individual box stalls (4x7m with two windows) on straw and shavings. The mares were fed concentrates and hay or haylage twice daily, mineral supplements and water was always available. Mares had daily access to an outdoor paddock or pasture for at least two hours. All mares were vaccinated against EHV 1,4 (Equip EHV, Zoetis, Berlin, Germany) in months 5, 7 and 9 of their pregnancy.

Transrectal ultrasound examinations of the genital tract including the fetus and placenta were performed at regular intervals throughout pregnancy. At least 2 weeks before the calculated birth date (day 336 after ovulation), the mammary gland was checked for development and the presence of secretions twice daily. Routine stable controls were performed 5–10 times daily and all stables were equipped with cameras for continuous monitoring. A birth control system (Sigloo Stable Alarm System, Equirep, Scomor, Hungary) was used for monitoring of parturition. The transmitter of the system was fixed to the vulva of each mare under local anesthesia at least five days before the calculated birth date.

2.2. Experimental design

All parturitions were attended. Complications during stages 2 and 3 of parturition were noted and – if required – obstetrical assistance was provided. Concentrations of TNFα and IgG were determined in colostrum respective milk and in neonatal blood (serum) collected immediately after delivery from the Vena (V) umbilicalis and 18 h thereafter from the V. jugularis externa. Milk samples were immediately frozen at −20 °C. Blood samples were collected into evacuated tubes containing EDTA as an anticoagulant (Vacuette 9 ml K3EDTA; Greiner bio-one, Kremsmünster, Austria) and evacuated tubes for serum sampling (Vacuette CAT Serum Fast Separator; Greiner bio-one). Samples were immediately centrifuged (2000 ×*g*, 5 min). One aliquot of the supernatant from each serum and EDTA plasma, respectively, was sent to the Antech Lab Germany GmbH (former Synlab Vet GmbH) laboratory (Branch office Geesthacht, Germany) for further analysis, the other aliquots were frozen at −20 °C. Analysis of TNFα in milk and plasma was performed in the authors' laboratory in Vienna. Three foals (2 male, 1 female) were excluded from the study because one of the two blood samples was lacking. For analysis of data, the remaining 97 foals were retrospectively assigned to two groups: i) failure of passive transfer (FPT; $n = 31$) and ii) control (CON; $n = 66$) based on their serum IgG concentration being below or above 0.8 g/l at 18 h of age, respectively.

Foals that were at risk of inadequate colostral IgG intake because of dystocia, compromised behavior or if foals were determined to have FPT based on IgG testing (SNAP Fohlen IgG; IDEXX, Hoofddorp, The Netherlands) with inadequate IgG concentration in the circulation, they received one or two liters of plasma after the 18 h blood sample was collected. Plasma was produced from veterinary clinic owned horses (between 4 and 15 years old, mares and geldings, over 500 kg body weight, healthy general examination, not been to foreign countries/stays abroad, vaccinated against influenza, tetanus, herpes, tested negative EVA and EIA). For collection a 12-gauge intravenous catheter was placed into the V. jugularis externa and sterile collection bags containing anticoagulant (sodium citrate) were filled by gravity. Every donor provided 5 l of blood every 4 weeks. Separation of cells and plasma was achieved by sedimentation. After 18 h when sedimentation was complete, the plasma was filled in collection bags and frozen at −20 °C).

2.3. Monitoring of neonatal behavior

Foal health was assessed via a neonatal behavior score repeatedly during the first 24 h of life (Gorlt, 2004; Supplementary file 1). Scoring was made by the veterinarian in charge of the foaling unit within the first 60 min after birth (score 1) and between 2 and 24 h after birth (score 2). Foals were scored as vital (score 1: 8–9 points; score 2: 18 points), slightly compromised (score 1: 6–7 points; score 2: 15–17 points) or compromised (score 1: <6 points; score 2: <15 points).

2.4. Laboratory analysis

The concentration of TNFα in colostrum and in foal blood was determined as described previously (Secor et al., 2012) with an equine TNFα ELISA kit (Equine TNFα duo set, R&D Systems) validated in the authors' laboratory. For samples of the present study, the interassay and intra-assay coefficient of variation was 19.9 and 18.8%, respectively. The minimal detectable concentration was 2.1 pg/ml.

IgG concentration in colostrum was determined by sugar refractometer as described previously (Chavatte et al., 1998). All blood analyses were processed in the same laboratory (Antech Lab Germany). White blood cell count was determined by flow cytometry with an automated blood cell counter (Advia2120i, Siemens, Forchheim, Germany). The SAA concentration was determined by immunoturbidimetry and iron and total protein concentration by photometry on an automated biochemical analyser (AU5800; Beckman Coulter, Krefeld, Germany). Immunoglobulin G was determined by electrophoresis (Minicap; Sebia, Mainz, Germany). Reference values were 4.0–14.8 G/l for leukocytes, <50 mg/l for SAA, 5–68 μmol/l for iron, 40–65 g/l for total protein and 2–22 g/l for IgG. The intra-assay and inter-assay coefficient of variation were 3.8% and 3.5% for leukocytes, 1.6–2.5% and 2–3.4% for SAA, 0.4–1.3% and 2.1–5% for iron, 0.3–0.5% and 0.8–2.2% for total protein, respectively. For electrophoresis, the intra-assay coefficient of variation for IgG was 2.2–2.4%. The minimal detectable concentration was 0.05×10^3/ μl for leukocytes, 2 mg/l for SAA, 0.3 μmol/l for iron, 0.77 g/l for total protein and 0.19 g/l for IgG.

2.5. Statistical analysis

Statistical analysis was done with the IBM SPSS statistics software (version 27.0). Data were analysed for normal distribution by Shapiro Wilk test. If data were not normally distributed, log-transformation was performed. Differences of frequencies between groups were compared by chi^2 test. Differences in mean gestation length between groups were analysed by *t*-test. Differences between groups for Brix in colostrum, TNFα concentrations, and all other blood parameters were compared by the general linear model for repeated measures with time (0 and 18 h) as intra subject factor, and group (CON and FPT) as between subject factor. Correlations were calculated by Spearman Rho test. Only significant correlations with an r value >0.5 were considered. All values are given as mean ± SD. A *p*-value <0.05 was considered significant.

3. Results

There were no significant differences between foal groups FPT and CON with regard to age and parity of dams, gestation length (FPT 343 ± 10, CON 340 ± 8 days) and foal sex (Table 1). Milk loss from the mammary gland during the last 12 h before parturition was noted in 8/31 FPT and 5/66 CON mares, respectively ($p < 0.01$). All foals were born mature. The mean concentration of serum IgG in neonates at 18 h after birth was 5.0 ± 1.7 g/l in group FPT and 13.4 ± 4.5 g/l in group CON ($p < 0.001$).

Information on health of neonatal foals in groups FPT and CON within their first hour of life, their first 24 h of life as well as any specific treatment is provided in Table 2 and for a subset of foals with increased SAA concentration at 18 h in Table 3. For the whole foal population, there were no differences between groups in the behavior score. Two foals in group FPT and four foals in group CON had problems to stand within the first hours of life and therefore received special assistance. A total of 17 foals in group FPT and four foals in group CON, respectively, received plasma transfusions.

Brix in colostrum was determined in 28 of 31 FPT and 53 of 66 CON foals. Brix was <20% (i.e., inadequate) in 11 of the 28 FPT (39%) and 9 of the 53 (17%) CON foals ($p < 0.05$). Brix in milk at 18 h after birth in mares of both groups was reduced (Fig. 1a; time $p < 0.001$, group x time $p < 0.05$). As expected, there was an increase in IgG concentration in serum of foals from 0 h to 18 h that was larger in group CON than in group FPT (group $p < 0.001$, time $p < 0.001$, group x time $p < 0.001$). A similar development was detected for total protein concentration in serum (group $p < 0.001$, time $p < 0.001$, group x time $p < 0.001$). At 18 h after birth, total protein concentration was above 4 g/l in all foals of group CON, but only in 12 of the 31 foals in group FPT. There was a marked positive correlation ($R = 0.935$, $p < 0.001$) between the serum IgG concentration and total protein concentration in foals at 18 h.

There were no differences in TNFα concentration in mammary gland secretions collected from dams of the foal groups FPT and CON at 0 and at 18 h after birth (Fig. 1b). Irrespective of group, TNFα concentration in mammary gland secretions declined during this time ($p < 0.05$). The concentration of TNFα in colostrum did not differ between mares with or without milk dripping before foal delivery (without milk loss: 8.9 ± 27.8 ng/ml; with milk loss: 7.4 ± 9.6 ng/ml, n.s.). In all foals, serum TNFα concentration was below the lower detection limit of the assay immediately after birth (0 h) irrespective of group. Concentrations of TNFα in colostrum at birth and in foals at 18 h varied considerably but did not differ significantly between groups (colostrum FPT 6.1 ± 9.1, CON 9.9 ± 31.5 ng/ml; foal FPT 2.3 ± 5.9, CON 2.4 ± 5.3 ng/ml). There was an increase in the mean serum TNFα concentration until 18 h of age with no differences between groups (Fig. 1c). There was a positive correlation between the TNFα concentrations in colostrum (0 h) and milk collected at 18 h ($r = 0.613$, $p < 0.001$) as well as between TNFα concentrations in colostrum and in serum of foals at 18 h ($R = 0.827$, $p < 0.001$). There was also a positive correlation between the TNFα concentrations in milk at 18 h and foal serum collected at 18 h ($r = 0.597$, $p < 0.001$).

There was an increase in serum SAA (Fig. 2c) and a decrease in serum iron concentrations (Fig. 2d) in foals between 0 h and 18 h of life irrespective of group. There was also an increase in peripheral PMN numbers without differences between groups (Fig. 2e). Lymphocyte numbers decreased slightly in FPT but increased in CON foals (time x group $p < 0.05$; Fig. 2f). High concentrations of SAA (> 322 mg/l; the mean + 2 x SD of all foals was calculated as cut off value) were detected in eight foals at 18 h of age (Table 3). Three foals belonged to the FPT group, the other five to the CON group. No specific pattern of TNFα concentration in colostrum and blood was present in this subpopulation. Three foals (two assigned to group FPT and one assigned to group CON) received plasma because they were considered at risk of FPT: one of them was born with premature milk loss and placental separation, in another one premature milk loss occurred and in a third foal, the dam suffered from retained fetal membranes and colic during stage 3 of parturition. In one additional foal where rupture of the urinary bladder was diagnosed and treated by surgery at 36 h of age, FPT was present but not diagnosed at that time. All foals presented in Table 3 were discharged at 7 days of age and no further problems were reported by the owners.

Table 1
Information on gestation, foaling outcome, occurrence of premature lactation and characteristics stages 2 and 3 of parturition in the dams ($n = 98$) of the foals included into the analysis.

Characteristic	Group FPT	Group CON	Statistics
N (male/female foal)	31 (18/13)	66 (34/32)	n.s.
Parity of dam (primiparous vs. pluriparous)	4 vs. 27	12 vs. 54	n.s.
Age of dam (years)	12.8 ± 4.9	11.6 ± 4.7	n.s.
- age range	4–19	4–21	
Gestation length (days)	342 ± 10	341 ± 8	n.s.
Premature lactation			
≥ 10 days before delivery	0	2 (3%)	n.s.
≥ 12 h before delivery	8 (28%)	5 (8%)	P < 0.05
Stage 2 of parturition			P < 0.05
No complications	21 (68%)	59 (89%)	
Fetal malposition	8 (26%)	4 (6%)	
Red bag	1 (3%)	0	
Other complications	1 (3%)	3 (4.5%)	
Stage 3 of parturition			
Retained placenta	0	1 (1.5%)	n.s.

Table 2
Information on foal health on the first day of life.

Characteristic	Group FPT	Group CON	Statistics
Number (n)	31	66	
Behavior Score I (< 60 min)			
Vital neonate	29 (94%)	63 (96%)	n.s.
Slightly compromised neonate	2 (6%)	3 (4%)	
Compromised neonate	0	0	
Behavior Score II (< 18 h)			
Vital neonate	27 (87%)	64 (97%)	n.s.
Slightly compromised neonate	4 (13%)	2 (3%)	
Compromised neonate	0	0	
Extra care/treatment			
Not required	12 (39%)	58 (88%)	p < 0.001
Support at nursing	2 (6%)	4 (6%)	
Plasma transfusion	17 (55%)	4 (6%)	

4. Discussion

Previously published research on the abundance of peripheral cytokines in foals mainly focused on the identification of possible prognostic markers for the survival of septic foals (Burton et al., 2009). While some cytokines are already produced by the newborn foal, others are transferred to the neonate via colostrum, among them TNFα, IFN, IL4, IL6,

Table 3
Characteristics of a subpopulation of foals ($n = 8$) where concentration of serum SAA at the time of determination of serum IgG concentration was increased (>322 mg/ml = mean + 2xSD of all foals; $n = 97$). Cells presenting deviations from normal are highlighted in beige colour (Abbreviations: a.p.: ante partum; n.a.: not available).

Foal Number	62/20	65/20	70/20	7/21	14/21	19/21	20/21	37/21
Group	CON	CON	FPT	CON	CON	FPT	CON	FPT
Age of dam (years)	7	15	15	10	14	5	8	17
Parity of dam	pluripa-rous	pluripa-rous	pluripa-rous	pluripa-rous	pluripa-rous	maiden	pluripa-rous	pluripa-rous
Mammary gland	Normal	Normal	Milk loss 12h ap	Normal	Normal	Normal	Normal	Milk loss for 1 day ap
Parturition	une-ventful	Une-ventful, colic during stage 3	une-ventful	une-ventful	une-ventful	une-ventful	une-ventful	Red bag
Retained fe-tal mem-branes	No	Yes	No	No	No	No	No	No
Brix in colos-trum (%)	26	16	20	25	27	28	n.a.	19
Gestation length (d)	333	342	334	347	334	346	337	333
Foal sex	Female	Female	Male	Male	Male	Male	Male	Male
Score 1 (<1h of life)	9	7	9	6	9	9	9	6
Score 2 (<24 h of life)	19	18	19	19	19	19	18	18
Special vet-erinary at-tention and treatment	No	Plasma (1L), support at nurs-ing (for 8h)	Plasma (2L)	Large foal, support at nurs-ing (for 6h)	No	No	No	Plasma (1L), support at nurs-ing (for 8h)
TNF colost-rum (ng/ml)	1.9	0.4	0	1.8	2.2	0.1	0.1	0.3
TNF serum 18h (ng/ml)	0.2	0	0	1.7	0.8	0	0	0
IgG serum 18h (g/l)	15.1	12.1	4.8	12.1	15.8	4.9	14.6	2.1
Total protein 18h (g/l)	53.4	53.6	43.3	50.3	59.5	40.9	55.7	35.4
SAA 0h (mg/l)	<5	<5	<5	<5	<5	<5	<5	<5
SAA 18h (mg/l)	583	421	749	551	721	455	413	452
Iron 18h (5-68 µmol/l)	38.4	55.8	46.4	38.5	33.3	59.5	34.1	42.2
PMN 0h (G/l)	3450	3520	950	1530	2809	4453	3150	1802
PMN 18h (G/l)	6142	7912	3360	12100	5236	8289	12727	4736
Final out-come	Une-ventful recov-ery	Une-ventful recovery	Une-ventful recov-ery	Une-ventful recov-ery	Une-ventful recov-ery	Rupture of the urinary bladder diag-nosed 36h af-ter birth, surgery, une-ventful recov-ery	Une-ventful recov-ery	Inten-sive care for 2 days after birth (antibi-otic and NSAID treat-ment), blood count and be-havior normal on D3, une-ventful recov-ery

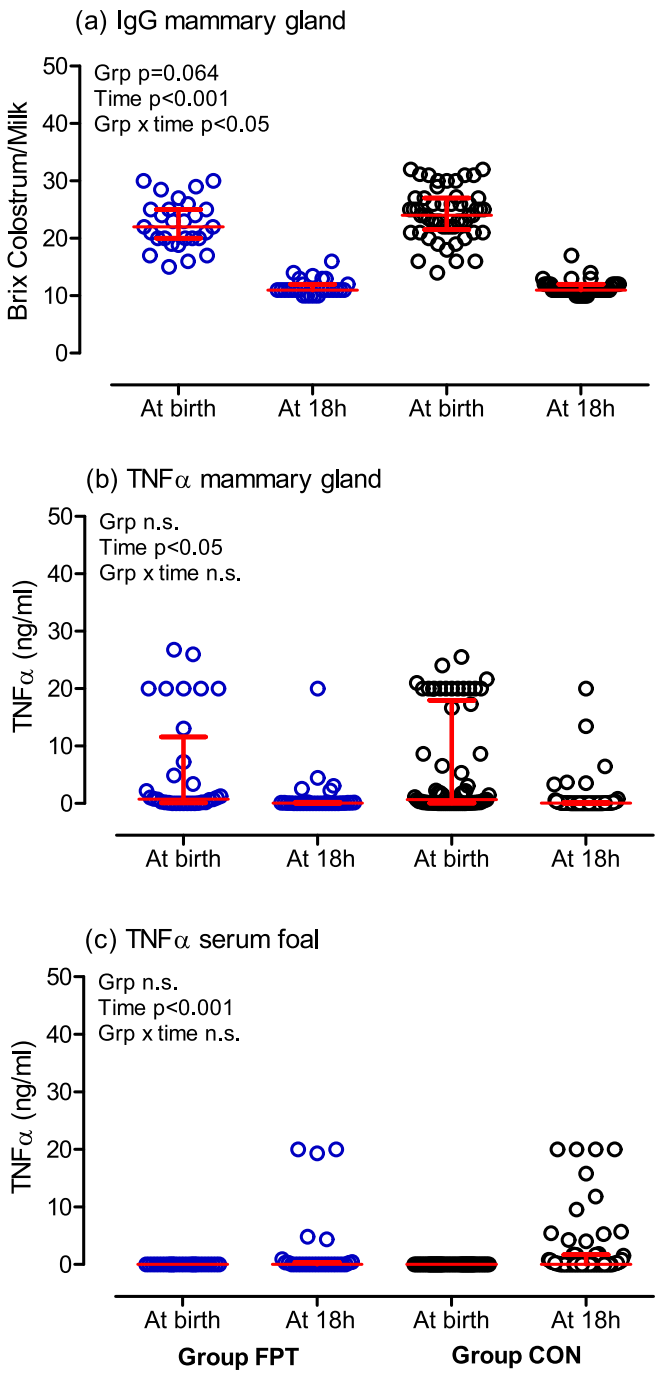

Fig. 1. Concentrations of (a) IgG (Brix) and (b) TNFα (ng/ml) in mammary gland secretions (colostrum, milk) of mares; (c) TNFα (ng/ml) in serum of their foals at birth (0 h) and 18 h after birth for groups FPT (blue circles; n = 31) and CON (black circles; n = 66). Symbols represent values of individual animals at different times. Statistical differences are indicated in the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and IL-8 (Secor et al., 2012; Mariella et al., 2017). A possible involvement in passive immunity and protection against infectious diseases has therefore been suggested (Secor et al., 2012). The results of the present study clearly confirm previous findings of TNFα transfer from the mare to the neonate via colostrum (Secor et al., 2012). The present investigation further extends these results to a larger foal population that was closely followed during the last weeks of gestation, parturition and the neonatal period. The present findings do, however, not support the suggestion of Secor et al. (2012) that transfer of TNFα via colostrum is important for protection of the neonate against infectious diseases.

In the present investigation, there was a wide range of TNFα concentration in colostrum of dams at parturition and TNFα concentration in the serum of their foals. The TNFα transfer via colostrum was not associated with transfer of IgG because no differences in TNFα concentration in colostrum at birth or in foals at 18 h of age were detected between neonates with or without FPT. More research is required to investigate the source of TNFα in the colostrum of mares at parturition. The cytokine TNFα is involved in the pathophysiology of ascending placentitis in mares (Fedorka et al., 2022; Fedorka and Troedsson, 2022). Serum TNFα concentration was low and steady in non-pregnant mares as well as in pregnant mares between days 120 and 330 after ovulation. In contrast, experimental induction of ascending bacterial placentitis between days 275 and 285 of pregnancy resulted in a small but significant increase of serum TNFα concentration (Fedorka et al., 2022). This increase was most likely due to the massive production of TNFα in the feto-placental unit. Unfortunately, there is no information on mammary gland development or milk dripping in mares of this study (Fedorka et al., 2022). Premature mammary gland development with or without milk dripping is, however, among the most common clinical signs of ascending bacterial placentitis in mares. In the present study, milk dripping occurred only in 15 out of the 97 mares in late in pregnancy, i.e., within 12 h before foal delivery. It was most likely associated with preparation of the mare for parturition (stage 1 of parturition) but not with ascending placentitis. Only in one mare, presence of ascending placentitis was assumed because of premature milk loss and premature placental separation ("red bag") at parturition. In this mare, however, TNFα concentration in colostrum was low and not detectable in serum of her foal. Because TNFα has been identified as a key regulator in morphogenesis of the mammary gland in several species (Varela and Ip, 1996; Lee et al., 2000), respective processes in their mammary gland tissue must be considered as reasons for an increase of TNFα in the mammary secretions of mares in the present study. The large variation of colostrum TNFα concentration among mares may reflect differences in morphogenesis of the mammary gland still present at parturition, but this requires further investigations.

It is interesting to note that a large proportion of the foals in the present study, i.e., 30%, suffered from FPT although closely supervised at birth. The diagnosis was based on retrospective determination of IgG in blood by electrophoresis. No difference in age of dams between groups was detected although the risk for FPT is increased in foals of mares >15 years of age (LeBlanc et al., 1992). Although all mares were considered healthy, IgG concentration in colostrum was inadequate (Brix ≤20%) in almost 40% of mares of the FPT foals. In eight mares of this group, milk loss started approximately 12 h before delivery of the foal and in 10 mares of the FPT group, complications during stage 2 of parturition occurred that required slight to moderate obstetrical intervention. It is therefore feasible that either an inadequate quality or amount of colostrum or impaired nursing behavior were the main reasons for FPT in foals (LeBlanc et al., 1992; Sellon, 2000; Giguère and Polkes, 2005). Also, prolonged duration of stage 2 of labor has been identified an important reason of increased morbidity and mortality of the equine neonate (McCue and Ferris, 2012).

The low incidence of neonatal sepsis in the present study agrees with the rule that early diagnosis of FPT and timely supplementation of IgG via infusion of plasma is a highly efficient measure to prevent clinical manifestation of sepsis (LeBlanc et al., 1992; McClure et al., 2001). Only 55% of the foals later diagnosed as suffering from FPT received plasma because a lack of IgG supply was suspected. Determination of colostrum quality at birth may be misleading because it does not provide information on the volume of colostrum still available to the foal. Despite the presence of high-quality colostrum at birth, an insufficient amount may still result in FPT. The behavior score used in our study (Gorlt, 2004) could not reliably detect foals that were at risk fo FPT. Although a close association between IgG and total protein concentrations in blood of

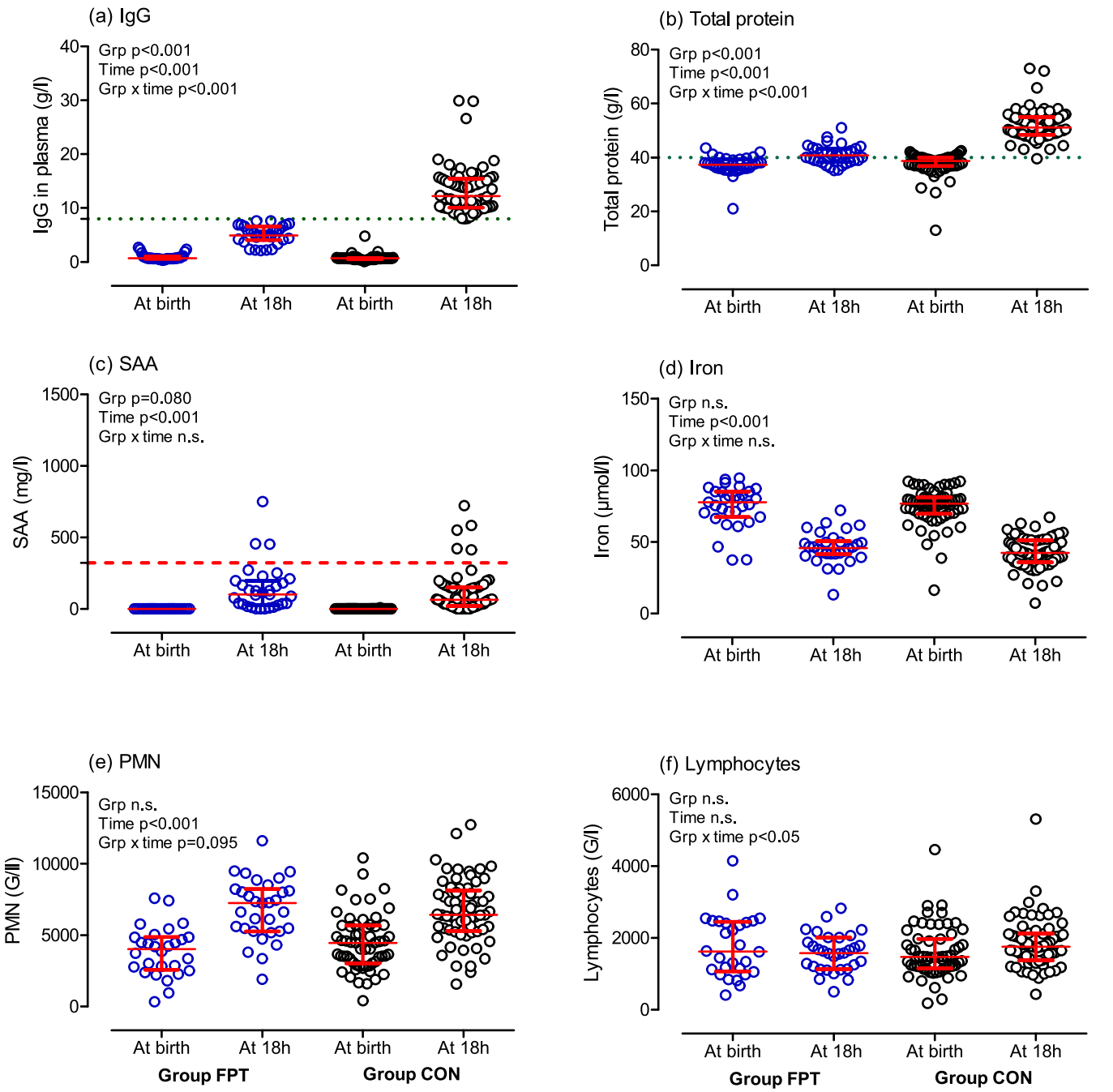

Fig. 2. Serum concentrations of (a) IgG (g/l), (b) total protein (g/l), (c) SAA (mg/l) and (d) iron (µmol/l) as well as (e) PMN and (f) lymphocyte numbers in serum at birth (0 h) and 18 h after birth in foals of groups FPT (blue circles; $n = 31$) and CON (black circles; $n = 66$). Symbols represent values of individual animals at different times. Statistical differences are indicated in the figure. Green dotted lines in (a) and (b) indicate the minimal reference value, the red dashed line in (c) indicates the mean + 2 x SD of SAA concentration determined at 18 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

neonatal foals has been described previously (Koterba et al., 1984), the relevance of total protein to indicate FPT has been questioned in a more recent study because of the wide variation of this parameter in foals hospitalized at an age between 1 and 18 days (Metzger et al., 2006). The present data, however, confirm a good reliability of total protein concentration in blood for indication of FPT when assessed at an early age, i.e., 18 h after birth in still clinically healthy neonates.

In addition to the determination of IgG and total protein concentrations as routine diagnostic procedures, assessment of SAA blood concentration in compromised neonatal foals is recommended (Stoneham et al., 2001; Hulten and Demmers, 2002; Hoeberg et al., 2022). In foals within the first 3 days after parturition, concentrations of SAA >100 mg/l were previously considered highly indicative for infections (Stoneham et al., 2001). In a more recent study, however, the optimal cut off for sepsis detection among a group of hospitalized sick foals was 1050 mg/l. In the same study, an SAA concentration < 300 mg/l was still considered low (Hoeberg et al., 2022) which agrees with the threshold for increased SAA concentration calculated in the present study. This threshold allowed to indicate a total of eight foals as having an increased SAA concentration that was, however, always below the cut off indicating sepsis (Hoeberg et al., 2022). All these foals recovered uneventfully and only two received surgical and antibiotic treatment,

respectively. This suggests that a slightly increased SAA concentration in blood of foals at 18 h of age that are otherwise uncompromised does not necessarily indicate that antibiotic treatment is required, especially if these foals are otherwise closely monitored.

5. Conclusion

The present study does not provide any evidence for an involvement of TNFα in prevention or pathogenesis of neonatal sickness in horses. The results do therefore not support the hypothesis that the intake of this cytokine via colostrum is of importance for health and development of the newborn foal. Further research in horses to investigate a likely role of TNFα in morphogenesis of the mammary gland is recommended. Results of this study clearly support and highlight the importance of early veterinary attention to the equine neonate especially when complications such as premature lactation and loss of milk or dystocia may increase the risk of FPT.

CRediT authorship contribution statement

Anna Vaske: Writing – original draft, Project administration, Methodology, Investigation, Data curation. **Camille Gautier:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Judith Winter:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology. **Christine Aurich:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors do not declare any conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2024.105273.

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