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Plasma alpha-fetoprotein in neonatal foals affected by prematurity, sepsis and neonatal encephalopathy

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ABSTRACT

Alpha-fetoprotein (AFP) concentrations have been reported in healthy foals and proposed as a biomarker of sepsis in foals born from mares with experimentally induced placentitis. This study aimed to describe the diagnostic and prognostic value of plasma AFP in foals spontaneously affected by different diseases. The study included all foals less than 72 h old that were diagnosed with either: (1) prematurity (PRE), when born prior to 320 days of gestation with immature physical characteristics; (2) sepsis (SEP), in the presence of both positive blood culture and SIRS or (3) neonatal encephalopathy (NE), with evidence of hypoxic-ischemic injury. Data from healthy foals (H; n=20) were obtained from a previous study. Foals received a complete physical and hematochemical evaluation and blood culture sample collection at hospital admission. Forty-six foals with an average age of 16 h were enrolled and divided into: PRE group (n=7); SEP group (n=14); NE group (n=25). AFP was measured in plasma collected at admission using a commercially available immunoassay validated for horses. AFP was increased in foals in PRE, SEP and NE groups compared with healthy ones (P<0.001) but was not able to discriminate between different diseases and outcomes. Overall, AFP was negatively correlated with foal age (r=−0.6; P<0.001), foal weight (r=−0.3; P=0.048), monocytes count (r=−0.4; P=0.011) and SAA concentration (r=−0.4; P=0.011). AFP appears to be a useful but non-specific indicator of neonatal health, since it upregulates not only in the presence of SIRS and bacteremia, but also during prematurity and hypoxic-ischemic injury.

1. Introduction

Alpha-fetoprotein (AFP) has been known in human medicine since the 1960s [1], yet has only recently received scientific attention in equine medicine, in both clinical [2–11] and experimental [12–14] settings. It is a secretory glycoprotein belonging to the albuminoid superfamily [15] and is produced in the developing fetus by the yolk sac and the fetal liver in equal amounts, then the yolk sac degenerates and the fetal liver, together with the gastrointestinal tract, becomes the main site of synthesis [16]. AFP acts as a growth regulator, immunoregulator and antioxidant, and has been suggested as a positive acute phase protein [17].

Circulating AFP concentration has been found to increase following various pregnancy-related complications in people, including inflammation, preterm premature rupture of membranes, and preterm labor, and is assumed to increase as an indicator of placental leakage [18]. Elevated maternal serum AFP concentrations were found in many different fetal disorders, including neural tube defects, omphalocele, gastroschisis and fetal bowel obstructions, whereas low maternal serum AFP concentration is associated with an increased risk of fetal Down syndrome [19].

In horses, AFP is expressed in high concentrations in fetal plasma and in amniotic and allantoic fluids. Its concentration in maternal plasma increases in normal mid-pregnancy (20–30 weeks) and then decreases towards parturition, although considerable individual variations has been noted among mares [3,6]. Twin pregnancies, placentitis and impending abortion were associated with high mare serum AFP concentrations [2]. During experimentally induced ascending placentitis, AFP concentrations in maternal circulation increase, likely due to an increased permeability of placental vessels, leakage from fetal fluids, and/or an increased protein expression [12,20]. Some studies have identified circulating AFP concentration as a useful marker for

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spontaneous ascending and focal mucoid placentitis [8] and for conditions of embryonic loss, abortion and preterm birth [11], while others have not found it useful for non-infectious idiopathic abortions [8] and for high-risk pregnancies, including infectious and non-infectious placental diseases and mare's systemic illness [9].

In equine neonatal medicine, information on AFP is still scarce. Healthy neonatal foals are born with high circulating concentrations of AFP, and a dramatic reduction is noted in the first week of life [5,6,9]. Foals that become sick during the first week of life have increased AFP concentrations at 24 h post-birth [5] and sick foals born from mares with high-risk pregnancy have significantly increased concentrations of AFP compared with healthy control foals during the first 72 h of life [9]. Premature foals born from mares with experimentally induced ascending placentitis and classified as septic based on sepsis score [21] demonstrated high concentrations of AFP [14]. However, AFP concentration was not a useful prognostic marker in hospitalized foals requiring intensive care [5].

Circulating AFP could be a useful screening tool for foals requiring intensive care in the first week of life, but its diagnostic and prognostic value in different pathological conditions is still under investigation. This study aimed to investigate the diagnostic and prognostic value of plasma AFP concentrations at the time of hospital admission in foals spontaneously affected by different diseases.

2. Materials and methods

2.1. Population

This study, approved by the Animal Care and Use Committee of the University of Bologna (Approval number, 358469; Approval date, 1 December 2023), was carried out on client-owned foals admitted for perinatal disease to the Equine Perinatology and Reproduction Unit of the Department of Veterinary Medical Sciences (University of Bologna), Italy, from January to June, 2024. Sick foals were included in the study if they met the following inclusion criteria: (i) age at admission less than 72 h; (ii) single diagnosis of prematurity, sepsis or neonatal encephalopathy; (iii) clinical conditions requiring level 1–3 of intensive care [22].

The following data were recorded for each foal admitted to the hospital after birth: breed, sex, age at admission (hours), weight (kg), mare's history (age, parity, gestational length, diagnosis of high-risk pregnancy, dystocia or placental insufficiency), complete clinical evaluation, venous blood glucose concentration at admission (mg/dL) (Medisense Optium, Abbott Laboratories Medisense Products, Bedford, MA, USA), venous blood lactate concentration at admission (mmol/L) (Lactate SCOUT+, Leipzig, Germany), hematochemical parameters (ADVIA 2120 analyzer, Siemens Healthcare srl, Milan, Italy for complete blood cell count, AU 400 analyzer, Olympus/Beckman Coulter, Lismeehan, Ireland for serum biochemistry and BCS XP coagulation analyzer, Siemens Healthcare srl, Milan, Italy for plasma fibrinogen concentration), serum IgG concentration (by immunoturbidimetric method; DVM Rapid Test II, MAI Animal Health, Elmwood, WI, USA), presence of a systemic inflammatory response syndrome (SIRS), positive or negative blood culture, level 1–3 of intensive care [22], length of hospitalization (days), diagnosis and outcome (survived or not survived).

High-risk pregnancy was defined as a history of premature udder development/lactation, an increase of the combined thickness of the uterus and placenta (CTUP), purulent/serosanguineous vulvar discharge, or systemic illness in the mare [23,24]. Dystocia was defined as any impediment of stage II parturition resulting from maternal, fetal or fetal membranes causes [25,26]. Diagnosis of placental insufficiency was performed retrospectively following macroscopic and histopathological examination of the placenta [24,27].

Foals were defined as premature when they were born prior to 320 days of gestation with immature physical characteristics: low body weight, inability to maintain body homeostasis and to suckle, hyperextension of flexor tendons, incomplete carpal and tarsal bone ossification [28]. Foals were classified as septic in the presence of both infection and SIRS [29]. Infection was confirmed on the basis of positive blood culture, while SIRS diagnosis required the presence of at least three of the following criteria, one of which must be abnormal temperature or leukocyte count [30]: fever (>39.2°C) or hypothermia (<37.2°C); tachycardia (>115 beats/min); tachypnea (>56 breaths/min); leucocytosis (>14.4 × 10^3 cells/mm^3), leukopenia (<6.9 × 10^3 cells/mm^3) or >5% band neutrophils; venous blood lactate concentration >5.0 mmol/L; venous blood glucose concentration <50 mg/dL. Foals were classified as affected by neonatal encephalopathy based on their history and clinical signs, especially those of neurological dysfunction [28], with the exclusion of other neurological diseases such as meningitis or trauma; typical historical events included high-risk pregnancy, dystocia, and/or placental insufficiency, and common clinical signs included loss or absence of the suckle reflex, inappropriate teat-seeking behaviour, dysphagia, hyperreactivity, and weakness [31,32].

Sick foals were divided into three groups: (1) prematurity (PRE group); (2) sepsis (SEP group); (3) neonatal encephalopathy (NE group). Exclusion criteria included the presence of more than one pathological condition; the presence of a positive blood culture or localized sites of infection in PRE and NE groups; and the presence of neurological signs in NE group attributable to meningitis or trauma.

A population of 20 healthy foals, born from mares with normal pregnancy and eutocic parturition hospitalized for *peripartum* monitoring, examined by the research team in a previous study, represented the healthy control group (H group) [9]. All data on these healthy foals were collected under the same conditions and using the same materials and methods as in the present study. Foals were classified as healthy when they had an Apgar score ≥9 at birth [33] and a normal clinical evaluation during hospitalization, including a normal complete blood count, serum biochemistry at birth and an IgG serum concentration >800 mg/dL at 12–24 h of life [9,24].

2.2. Sample collection and analysis

All blood samples were collected at admission by jugular venipuncture into plastic tubes containing anticoagulant for routine hematological and biochemical analyses and determination of fibrinogen concentration. An aliquot of the plasma sample for each subject was centrifuged at 3000 g/10 min within 30 min of collection, stored at −20°C and then analyzed at the end of the foaling season.

AFP was measured in plasma collected at admission using a heterologous commercially available immunoassay on a chemiluminescence platform (AF Immulite 2000 Systems, Immulite 2000 XPi, Siemens Healthineers), previously validated for horses as described elsewhere [9,12]. The AFP assay had a range of 0.2 to 300 IU/mL. Samples above the upper detection limit were diluted with the diluent of the commercial kit. In accordance with the manufacturer, a conversion factor of 1.21 was applied for the conversion of IU/mL to ng/mL human AFP. The analytical sensitivity of the AF Immulite 2000 assay is 0.2 IU/mL. Specificity data provided by the manufacturer did not identify any clinically significant cross-reactivity.

2.3. Statistical analysis

The Kolmogorov–Smirnov test was used to assess data for normal Gaussian distribution. Since data were non-normally distributed, they were assessed with non-parametric tests and expressed as median, interquartile range (IQR). Differences in plasma AFP concentrations, rapid determinations (glucose and lactate venous blood concentrations), hematochemical parameters and serum IgG concentration between groups (H, PRE, SEP, NE groups) were analyzed with Kruskal-Wallis test. Differences in clinical parameters (foal's age and weight at admission, mare's age and parity, gestation length, hospitalization length and

outcome) between groups were analysed with Kruskal-Wallis test. Foal's age at admission, hospitalization length and outcome were analysed only among sick foals.

The Mann-Whitney test was used in sick foals to assess differences in plasma AFP concentrations between colts and fillies, foals with a history of acute onset of the disease and foals with a history of chronic placental insufficiency, foals with level 2 or 3 of intensive care, foals with or without SIRS and foals survived or not. The Spearman's rank correlation coefficient was evaluated in the entire population between plasma AFP concentrations and the variables: age, weight, mare's age and parity, gestation length, rapid determinations and hematochemical parameters at admission. Significance was set at $P<0.05$. All the data analysis was performed with the statistic software SPSS.

3. Results

Forty-six sick foals, with an average age at hospital admission of 16 ±13 h (0–53), were enrolled and divided into: PRE group ($n=7$); SEP group ($n=14$); NE group ($n=25$).

AFP concentrations were increased in foals at hospital admission in PRE, SEP and NE groups compared with healthy ones at each time point, as shown in Table 1 and depicted in Fig. 1, but no differences in AFP concentrations were found among the three groups.

The clinical data collected in all groups are shown in Table 2. Differences were found between groups in foal's age and weight at admission and in mare's gestation length. A history of high-risk pregnancy was present in 6/46 foals (13%): 3/7 foals in PRE group (increased CTUP), 1/14 foals in SEP group (premature udder development/lactation) and 2/25 foals in NE group (mare's systemic illness). In the PRE group, a history of chronic fetal distress due to placental insufficiency was recorded in 5/7 foals, whereas the history was not available in 2/7 foals. In the SEP group, sepsis was reported as acquired after birth in 11/14 foals, whereas the history was not available in 3/14 foals. In NE group, acute hypoxia (history of premature placenta separation, dystocic parturition, caesarean section) was reported in 10/25 foals, and chronic hypoxia in 4/25 foals (history of placental insufficiency: placental villous hypoplasia, placental thickening), whereas the history was not available in 11/25 foals.

Considering only foals with a reported medical history (30/46 foals, 65%), the disease was associated with an acute onset in 21/30 foals (70%) and with a chronic onset in 9/30 foals (30%). SIRS [30] was present at admission in 3/7 foals (43%) in PRE group, in all foals in SEP group and in 9/25 foals (36%) in NE group, for a total of 26/46 foals (57%). Overall, 1/46 foals (2.2%) required level 1 of intensive care, 24/46 foals (52.2%) level 2 and 21/46 foals (45.7%) level 3 [22]. Thirty-four/46 foals (74%) survived hospital discharge, while 12/46 foals (26%) died or were euthanized because of the moribund condition.

No differences in AFP concentrations were found between survivors and non-survivors, between colts and fillies, between foals that required level 2 or 3 of intensive care, between foals with a history of acute disease onset or a history of chronic fetal distress and between foals with or without SIRS.

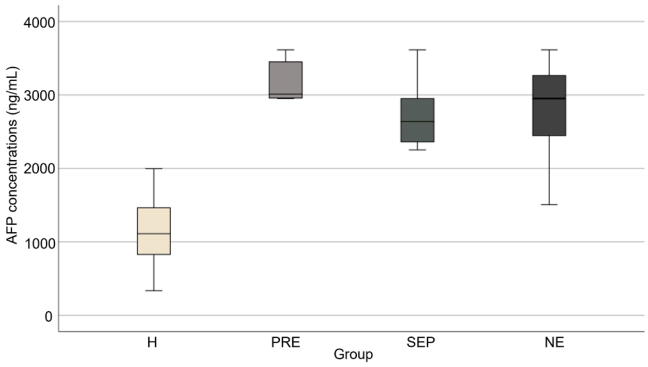

Fig. 1. Alpha-fetoprotein (AFP) concentrations (ng/mL) in foals' plasma were increased in PRE, SEP and NE groups compared with group H ($P<0.001$). H, healthy control group, PRE, prematurity group ($n=7$), SEP, sepsis group ($n=14$) and NE, neonatal encephalopathy group ($n=25$).

Rapid determinations, hematochemical parameters and serum IgG concentrations in H, PRE, SEP and NE groups are shown in Table 3. Differences were found between groups in blood lactate concentration, serum creatine kinase, total bilirubin, plasma fibrinogen and serum SAA concentration. Overall, in sick foals, AFP concentrations were negatively correlated with clinical parameters, such as age ($r=-0.589$; $P<0.001$) and weight at hospital admission ($r=-0.296$; $P=0.048$), and with hematochemical parameters, such as monocytes count ($r=-0.374$; $P=0.011$) and SAA concentration ($r=-0.400$; $P=0.011$).

4. Discussion

The present study was conducted to determine for the first time the usefulness of AFP as a biomarker in the most representative diseases in a population of newborn foals less than 3 days old, in which the diagnosis was strictly defined. AFP appears to be a useful but non-specific indicator of neonatal health, as it increases not only in the presence of SIRS and bacteremia, defining a septicemic status, but also during prematurity and hypoxic-ischemic injury. Therefore, the biomarker was not able to discriminate between different pathological conditions and outcomes.

Premature foals may present abnormalities of hepatic, gastrointestinal, renal, bone and immune metabolism in the adaptation of postnatal life [28]. In most of the few cases reported in the present study, the disease often resulted from conditions of placental insufficiency and fetal/neonatal inflammatory response syndrome (FIRS/SIRS). In the absence of bacteremia (negative blood culture) and local sites of infection in the small group examined, hematochemical alterations and increased inflammatory markers should be traced back to multiorgan immaturity and dysfunction, including immune capacities [28], and FIRS/SIRS, although the SIRS criteria have never been adapted to premature foals [30]. Regarding inflammatory markers, the increased plasma fibrinogen concentration in premature foals compared with healthy ones was in line with the positive outcome of most foals included in the PRE group, being considered a positive prognostic factor [28]. It could be hypothesized that the elevated plasma concentrations of AFP in premature foals may result from conditions of placental and fetal circulatory dysfunction, upregulation of AFP in the liver and/or gastrointestinal tract of the equine fetus, fetal/neonatal inflammation, altered liver metabolism, and reduced renal clearance due to immature body systems [9,34]. AFP acts as a growth regulator able to stimulate cellular growth in a concentration-dependent manner [35–39] and it has already been suggested as an acute-phase protein in the fetus [34].

In the present study, septic foals had bacteremia and SIRS, often resulting in multi-organ dysfunction, and the condition was likely to be considered acquired after birth due to the absence of a history of

Table 1
Alpha-fetoprotein (AFP) concentration (ng/mL) in foals' plasma in healthy foals (group H) at three different time points (at birth - T0; at 24 h of life - T24; at 72 h of life - T72), as previously reported [9], and at the time of hospital admission (TA) affected by prematurity (PRE group; $n=7$), sepsis (SEP group; $n=14$) and neonatal encephalopathy (NE group; $n=25$) hospitalized within 72 h after birth. Foal age is expressed as mean ±SD (min-max). Data are expressed as median, interquartile range (IQR). *Indicates a significant difference between groups in row ($P<0.001$).

Group H			Group PRE	Group SEP	Group NE
T0	T24	T72	TA 15 ±16 h (2–48)	TA 25 ±16 h (5–53)	TA 11 ±8 h (0–32)
1111.4 (621.0)	811.9 (505.8)	643.7 (414.1)	3012.1 (494.0) *	2638.6 (542.2) *	2951.8 (819.3) *

Table 2
Clinical data collected from healthy control foals (H group; *n*=20), previously reported [9], and 46 foals affected by prematurity (PRE group; *n*=7), sepsis (SEP group; *n*=14) and neonatal encephalopathy (NE group; *n*=25) hospitalized within 72 h after birth. SB = Standardbred; QH = Quarter Horse; SD = Saddlebred; AH = Arabian Horse; N = Normal pregnancy and eutocic parturition; H-R = High-risk pregnancy; PI = Placental insufficiency; NA = data not available; Sv = survived to hospital discharge; NSv = not survived; n = number of animals. Data are expressed as median, interquartile range (IQR). Different superscript letters in column indicate differences ($P<0.05$) among groups. Regarding foal's age at admission/birth, hospitalization length and outcome statistical analysis was only performed among PRE, SEP and NE groups because these parameters were inclusion criteria for the control group (H group). Age at admission was higher in SEP group compared to PRE and NE groups (P=0.032), weight at admission was lower in PRE group compared to H, SEP and NE groups ($P<0.001$) and gestation length was lower in PRE and SEP groups compared to H and NE groups ($P<0.001$).

					Mare's History						
Group	Breed	Sex	Age (Hours)	Weight (Kg)	Age (Years)	Parity	Gest. Length (Days)	Diagnosis	Care Level (1–3)	Hosp. Length (Days)	Outcome
H (*n*=20)	SB (*n*=18) SD (*n*=2)	Males (*n*=10) Females (*n*=10)	0	50 (8) [a]	10 (4)	4 (3)	340 (15) [a]	N, (*n*=20)	/	/	Sv (*n*=20)
PRE, (*n*=7)	SB (*n*=4), AH (*n*=2), SD (*n*=1)	Males (*n*=1), Females (*n*=6)	10 (12) [a]	40 (9) [b]	13 (6)	3 (3)	315 (15) [b]	H-R (*n*=3), Dystocia (*n*=0), PI (*n*=5, NA (*n*=2)	1 (*n*=0), 2 (*n*=4), 3 (*n*=3)	25 (20)	Sv (*n*=6), NSv (*n*=1)
SEP, (*n*=14)	SB (*n*=3), QH (*n*=3), AH (*n*=1), SD (*n*=7)	Males (*n*=7), Females (*n*=7)	19 (27) [b]	46 (11) [a]	13 (7)	2 (1)	326 (5) [b]	H-R (*n*=1), Dystocia (*n*=0), PI (*n*=0), NA (*n*=3)	1 (*n*=1), 2 (*n*=5), 3 (*n*=8)	8 (8)	Sv (*n*=8), NSv (*n*=6)
NE, (*n*=25)	SB (*n*=10), QH (*n*=7), AH (*n*=1), SD (*n*=7)	Males (*n*=16), Females (*n*=9)	10 (11) [a]	44 (8) [a]	12 (7)	3 (3)	338 (14) [a]	H-R (*n*=2), Dystocia (*n*=10), PI (*n*=4), NA (*n*=11)	1 (*n*=0), 2 (*n*=15), 3 (*n*=10)	9 (12)	Sv (*n*=21), NSv (*n*=4)

Table 3
Results of the complete blood count, serum biochemistry, plasma fibrinogen concentration, serum IgG concentration, and venous blood lactate and glucose concentration (measured through rapid methods) in H, PRE, SEP and NE groups. Data are expressed as median, interquartile range (IQR). (a-b) Different superscript letters in row indicate differences ($P<0.05$) among groups. Blood lactate concentration was increased in SEP and NE groups compared with H and PRE groups (SEP vs. H, $P=0.004$; NE vs. H, $P=0.008$; SEP vs. PRE, $P=0.011$; NE vs. PRE, $P=0.025$), but it was not different between SEP and NE groups. Serum creatine kinase and total bilirubin were increased in PRE, SEP and NE groups compared with H group (PRE vs. H, $P=0.026$; SEP vs. H, $P<0.001$; NE vs. H, $P=0.001$ for creatine kinase and PRE vs. H, $P<0.001$; SEP vs. H, $P<0.001$; NE vs. H, $P=0.001$ for total bilirubin), but both markers were not different between PRE, SEP and NE groups. Plasma fibrinogen concentration was increased in PRE group compared with H group ($P=0.015$), whereas SAA concentration was increased in SEP group compared with H group ($P=0.046$), but both inflammatory markers were not different between PRE, SEP and NE groups. No differences were found between PRE, SEP and NE groups in serum IgG concentrations at admission.

Parameters Rapid Determinations	Group H	Group PRE	Group SEP	Group NE
Venous Blood Lactate (mmol/L)	3.0 (3.0) [a]	2.6 (1.1) [a]	12.6 (15.1) [b]	6.4 (9.9) [b]
Venous Blood Glucose (mg/dL)	73 (23)	43 (76)	92 (111)	85 (56)
Complete Blood Count				
Hemoglobin (g/dL)	15.2 (1.5)	14.1 (3.6)	15.6 (2.0)	14.4 (3.5)
Hematocrit (%)	48 (4)	44 (11)	47 (8)	43 (11)
Erythrocytes (10^6/μL)	10.7 (1.6)	10.1 (1.5)	10.3 (1.1)	9.9 (1.9)
Platelets (10^3/μL)	197 (54)	205 (61)	153 (101)	183 (82)
Leucocytes (10^3/μL)	7.2 (1.9)	8.6 (4.4)	6.3 (3.4)	6.8 (6.6)
Lymphocytes (cells/μL)	1260 (285)	470 (545)	1060 (580)	1060 (1150)
Monocytes (cells/μL)	180 (125)	120 (150)	180 (190)	90 (110)
Neutrophils (cells/μL)	5970 (2020)	7190 (3930)	4920 (3920)	5720 (6790)
Eosinophils (cells/μL)	10 (10)	10 (10)	10 (0)	10 (20)
Basophils (cells/μL)	30 (20)	50 (30)	40 (40)	50 (70)
Serum Biochemistry and Plasma Fibrinogen Concentration				
Creatine Kinase (IU/L)	224 (123) [a]	885 (2461) [b]	1837 (3069) [b]	714 (2093) [b]
Total Bilirubin (mg/dL)	2.4 (0.8) [a]	6.6 (3.3) [b]	4.7 (1.6) [b]	3.9 (2.2) [b]
Total Protein (g/dL)	4.2 (0.5)	4.3 (0.7)	4.1 (0.8)	4.2 (0.9)
Albumin (g/dL)	3.3 (0.5)	2.9 (0.3)	2.8 (0.7)	3.1 (0.4)
BUN (mg/dL)	36.4 (7.5)	44 (21)	44 (12)	40 (11)
Creatinine (mg/dL)	2.4 (0.8)	2.6 (1.9)	2.0 (0.6)	2.1 (2.8)
Phosphorus (mg/dL)	5.3 (0.9)	5.5 (2.3)	4.8 (3.0)	5.0 (1.4)
Calcium (mg/dL)	13.1 (0.7)	12.8 (1.7)	12.3 (3.0)	12.2 (1.3)
Sodium (mg/dL)	144 (4)	142 (3)	142 (9)	142 (8)
Potassium (mg/dL)	4.7 (0.7)	3.9 (0.1)	4.0 (1.2)	3.9 (0.9)
Chlorine (mg/dL)	100.8 (1.7)	92.5 (2.4)	100.5 (6.5)	98.7 (7.3)
Magnesium (mg/dL)	1.8 (0.2)	2.1 (0.4)	1.8 (0.8)	2.1 (0.5)
Fibrinogen (g/L)	1.6 (0.3) [a]	2.6 (2.8) [b]	1.9 (1.0) [a]	1.8 (0.4) [a]
Serum Amyloid A (μg/mL)	5 (10) [a]	103 (212) [a]	178 (294) [b]	15 (180) [a]
Serum IgG Concentration				
IgG (mg/dL)	>800	551 (314)	142 (516)	460 (541)

H, healthy control group, PRE, prematurity group (*n*=7), SEP, sepsis group (*n*=14) and NE, neonatal encephalopathy group (*n*=25).

placentitis in all mares. Studies in human medicine have shown the importance of AFP concentration as an early indicator of neonatal infection, since an increase in AFP concentrations is observed in the maternal circulation [40]. Like humans, in an equine experimental model [12], there was an increase in serum AFP concentration above the physiological concentration with the induction of ascending placentitis. According to the authors, this increase may be due to AFP upregulation, increased vascular permeability across the placenta, and/or reduced fetal metabolism/clearance due to the disease.

The concentration of AFP in equine neonatal sepsis has only been investigated in a small group of foals born from mares with experimentally induced ascending placentitis and classified as septic based on sepsis score [21], but also premature based on reported gestational ages [14]. In contrast, sick foals in the present study were classified as septic at hospital admission based on the presence of both positive blood culture and SIRS, as indicated by the literature and the current equine best practice [29,30,41]. To note, SAA did not differ among the different diseases, although it was increased in septic foals compared with healthy ones. This result could be due to the small sample size and/or to the presence of SIRS in some foals in the other two categories. Based on all general results, AFP can be considered a non-diagnostic marker of infection or sepsis in the equine neonate [5,14], mainly reflecting an inflammatory state as a positive acute-phase protein and an immunoregulator able to suppress immune response through several mechanisms [42–46].

Equine neonatal encephalopathy can be the consequence of adverse peripartum events leading to ischemia/hypoxia/inflammation in the prepartum period ("chronic hypoxia", e.g., high-risk pregnancy, mares' systemic illness, placental insufficiency) and at parturition ("acute hypoxia", e.g., premature placenta separation, dystocia, caesarean section) [47]. In human neurology, there are at least four neurodegenerative disorders, all inherited as autosomal recessive traits and characterized by the presence of cerebellar ataxia, abnormal ocular movements, and neuropathy, for which an elevated concentration of serum AFP is an important diagnostic biomarker [19]. In the present study, AFP was not able to discriminate foals with hypoxic-ischemic injury from those with other diseases, but studies are reported in favour of its possible antioxidant role that would justify increased concentrations during NE [48,49]. As speculated in prematurity and confirmed in sepsis, also in NE upregulation of AFP may suggest neuroinflammation of feto-placental or neonatal origin and/or reduced fetal/neonatal metabolism/clearance due to hypoxia; again, foals affected by NE in the absence of systemic or localized infection could show increased concentrations of inflammatory markers.

As previously reported [5,9], AFP concentration was not associated with survival which could be related to the low number of non-surviving foals (12 *vs.* 34) or to the low prognostic potential of the marker. Similarly, elevated AFP concentrations were not associated with a higher level of intensive care, based on the classification proposed by Koterba [22]; indicative of the disease severity, or with the presence of SIRS at hospital admission. The metabolism of AFP in foals is unknown, but in people its half-life in serum is estimated to be approximately 5 days after parturition [50]. In the present study, when the entire population was divided according to the presence of an acute (premature placenta separation, dystocia, caesarean section, sepsis acquired after birth) or chronic (high-risk pregnancy, placental insufficiency, mare's systemic illness, placentitis) disease in the history, AFP showed no significant differences. In the present study, AFP concentration was a good indicator of a disease status in the neonatal foal, either in conditions that have arisen within a few hours, or in conditions resulting from weeks of fetal distress during intra-uterine life.

All sick foals in the present study showed increased creatine kinase activity and total bilirubin, as they are likely to have muscle damage due to prolonged recumbency, seizures and/or dystocia [51], as well as concomitant cholestasis and liver damage due to fasting and sepsis/hypoxia, respectively [52]. The negative correlation between plasma AFP and age at admission was not unexpected, as equine neonates are born with high AFP concentrations and then both healthy and sick foals showed a reduction in AFP concentrations up to 72 h after birth [9]. The negative correlation between plasma AFP and foal weight at hospital admission agrees with that reported in humans, where high AFP values were found in low-birth-weight infants and preterm births [50], and in horses, where there is a correlation between AFP and foal weight at birth [9].

Hypothetical and speculative conclusions concerning correlations with hematochemical parameters need to be critically evaluated, since none of these parameters were strongly correlated and statistical correlations do not prove biological relationships. The relationship between AFP and SAA should be further investigated considering the suppressive role of AFP on macrophages [46], which through IL-6 synthesis are the main promoters of hepatic SAA production [53].

The main limitations of the study design were the small representative sample size of each perinatal disease, and the inhomogeneous population of sick foals in terms of acute or chronic onset of the condition. Nevertheless, the population offers a spontaneous, as well as a rigorously clinically classified, model of equine neonatal disease.

5. Conclusions

In conclusion, plasma AFP concentration appears to be a useful screening tool for equine neonates requiring early and prompt intervention in the first three days of life, although it is neither diagnostic nor prognostic in the small population examined.

Ethical Statement

This study, approved by the Animal Care and Use Committee of the University of Bologna (Approval number, 358469; Approval date, 1 December 2023), was carried out on client-owned foals admitted for perinatal disease to the Equine Perinatology and Reproduction Unit of the Department of Veterinary Medical Sciences (University of Bologna), Italy. Owners provided informed consent, as part of the hospital consent form, for the use of foal data in the study.

CRediT authorship contribution statement

A. Lanci: Writing – review & editing, Visualization, Investigation, Data curation, Conceptualization. **N. Ellero:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **C. Castagnetti:** Writing – review & editing, Visualization, Supervision, Investigation, Conceptualization. **F. Perina:** Writing – review & editing, Visualization, Investigation. **F. Dondi:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **J. Mariella:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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