UNIVERSIDADE FEDERAL DE PELOTAS
Faculdade de Veterinária
Programa de Pós-Graduação em Veterinária

Tese

Marcadores bioquímicos como preditores de sepse e sobrevivência em potros nascidos de éguas com placentite

Luciana de Araujo Borba

Pelotas, 2019

Luciana de Araujo Borba

Marcadores bioquímicos como preditores de sepse e sobrevivência em potros nascidos de éguas com placentite

Tese apresentada ao Programa de Pós-Graduação em Veterinária da Faculdade de Veterinária da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências (área de concentração: Sanidade Animal).

Orientadora: Prof.ª Dr.ª Bruna da Rosa Curcio

Coorientador: Prof. Dr. Carlos Eduardo Wayne Nogueira

Pelotas, 2019

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

B726m Borba, Luciana de Araujo

Marcadores bioquímicos como preditores de sepse e sobrevivência em potros nascidos de éguas com placentite / Luciana de Araujo Borba ; Bruna da Rosa Curcio, orientadora ; Carlos Eduardo Wayne Nogueira, coorientador. — Pelotas, 2019.

69 f. : il.

Tese (Doutorado) — Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, 2019.

1. Sepse. 2. Potros. 3. Parâmetros bioquímicos. 4. Proteínas de fase aguda. 5. Sobrevivência. I. Curcio, Bruna da Rosa, orient. II. Nogueira, Carlos Eduardo Wayne, coorient. III. Título.

CDD : 636.1

Elaborada por Gabriela Machado Lopes CRB: 10/1842

Luciana de Araujo Borba

Marcadores bioquímicos como preditores de sepse e sobrevivência em potros nascidos de éguas com placentite

Tese aprovada como requisito parcial para obtenção do grau de Doutor em Ciências, Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas.

Data da Defesa: 26/02/2019

Banca examinadora:

Prof.ª Dr.ª Bruna da Rosa Curcio (Orientadora)
Doutora em Biotecnologia pela Universidade Federal de Pelotas.

Prof. Dr. Carlos Eduardo Wayne Nogueira
Doutor em Medicina Veterinária pela Universidade Federal de Santa Maria.

Prof. Dr. Augusto Schneider
Doutor em Biotecnologia pela Universidade Federal de Pelotas.

Dr.ª Luciana de Araujo Lins
Doutora em Ciências pela Universidade Federal de Pelotas.

Agradecimentos

Agradeço a Deus, pela vida e por me proporcionar saúde e força para superar as dificuldades e lutar pelos meus sonhos e ideais.

A Universidade Federal de Pelotas (UFPel), pela acolhida e formação.

A professora Dr.ª Bruna da Rosa Curcio, que além de orientadora se tornou uma amiga. Por toda amizade, apoio, conselhos, ensinamentos transmitidos e por horas dedicadas a este trabalho. Obrigada por tudo!

Ao professor Dr. Carlos Eduardo Wayne Nogueira pela amizade, conselhos, orientação, confiança, e pelos ensinamentos transmitidos.

Ao meu marido Felipe Calgarotto Borba, pelo amor, compreensão e, acima de tudo, pela amizade e incondicional apoio desde o início desta caminhada.

Aos meus pais, pelo amor, incentivo e apoio incondicional. De nada adiantaria minha caminhada se vocês não tivessem me ensinado os primeiros passos!

As colegas e amigas que fiz durante esse período, e que foram fundamentais para seguir em frente nesta jornada. Carolina da Fonseca Sapin, Fernanda Timbó Del'Rey Dantas, Gabriela Castro da Silva e Jemhally Dillenburg Hack. Muito obrigada pela amizade, por todas as horas de trabalho e companheirismo de vocês. Adoro vocês e levarei cada uma no coração!

Ao Grupo de Ensino Pesquisa e Extensão em Clínica Médica de Equinos (ClinEq), pelo auxílio na realização deste trabalho.

As minhas colegas da Secretaria da Agricultura do Estado do Rio Grande do Sul, pela acolhida, amizade e apoio. A participação de vocês foi muito importante para que eu pudesse conciliar o trabalho e o doutorado! Obrigada meninas!

A todos que, direta ou indiretamente fizeram parte da minha formação e crescimento pessoal e profissional, muito obrigada!

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudos.

Muito Obrigada!

“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê.”
(Arthur Schopenhauer)

Resumo

BORBA, Luciana de Araujo. **Marcadores bioquímicos como preditores de sepse e sobrevivência em potros nascidos de éguas com placentite.** 2019. 67f. Tese (Doutorado em Ciências) – Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2019.

O desenvolvimento de sepse neonatal é uma consequência comum da placentite, sendo considerada a maior causa de morte em potros com menos de sete dias de vida, assumindo importância clínica e econômica em todo mundo devido aos custos com o tratamento e a baixa taxa de sobrevivência. Considerando a importância e necessidade da detecção precoce da sepse neonatal e do estabelecimento de um prognóstico para esses potros, se faz necessária a investigação de marcadores que apresentem alta sensibilidade e facilidade para mensuração na prática clínica. Desta forma, objetivamos avaliar a utilidade de marcadores sanguíneos como preditores precoces de sepse e de não sobrevivência em potros neonatos. Foram utilizados 35 potros, divididos em três grupos: sete potros saudáveis serviram como grupo controle, e 28 potros nascidos de éguas com placentite ascendente induzida experimentalmente foram divididos em dois grupos de acordo com o escore de sepse, em potros não sépticos (n=19) e potros sépticos (n=9), e de acordo com a sobrevivência, em: sobreviventes (n=19) e não sobreviventes (n=9). Em um primeiro estudo foram comparados os marcadores sanguíneos em potros de acordo com o escore de sepse, verificando-se que potros que apresentam sepse neonatal demonstraram acentuadas alterações no metabolismo energético, com hipoglicemia ao nascimento e redução da atividade da enzima GGT, e aumento do lactato e ureia, além de apresentarem elevadas concentrações de fibrinogênio, AFP e SAA. Colesterol e lactato demonstraram serem bons marcadores para detectar a sepse nas primeiras 48 horas de vida. No segundo estudo, foi avaliada a relação entre os marcadores bioquímicos e inflamatórios com a não sobrevivência, e sua utilidade como preditores de não sobrevivência nos potros durante o primeiro mês de vida. A mensuração dos níveis de glicose, triglicerídeos e GGT ao nascimento demostraram ser bons marcadores para predizer a não sobrevivência, com elevada sensibilidade e especificidade, podendo ser utilizados para auxiliar na tomada de decisões sobre as chances de sobrevivência de potros em rotina clínica. Com os resultados obtidos, ficou demonstrado que potros sépticos e não sobreviventes apresentam metabolismo energético e lipídico alterados, e intensa resposta inflamatória e que marcadores sanguíneos periféricos, como colesterol e lactato, são marcadores adequados para detectar precocemente a sepse e, glicose, triglicerídeos e GGT são úteis na rotina clínica para prever a não sobrevivência em potros.

Palavras-chave: sepse; potros; parâmetros bioquímicos; proteínas de fase aguda; sobrevivência

Abstract

BORBA, Luciana de Araujo **Biochemical markers as predictors of sepsis and survival in foals born from mares with ascending placentitis.** 2019. 67f. Thesis (Doctor degree in Sciences) – Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2019.

Neonatal sepsis is a common consequence of placentitis and has been considering the leading cause of neonatal death during the first week post-foaling. Assuming clinical and economic importance worldwide due to treatment costs and low survival rate. Considering the roll of early diagnosis of septicemia in neonatal foals and the establishing a prognosis for these foals, it is necessary to investigate markers that presenting high sensitivity that can be easily assessed in clinical practice. Thus, we aimed to evaluate the usefulness of blood markers as early predictors of sepsis and as predictors of non-survival in neonatal foals. Thirty-five foals were used, divided into three groups: seven healthy foals served as a control group, and 28 foals born from mares with experimentally induced ascending placentitis were divided into two groups according to sepsis score in: non-septic foals (n = 19) and septic foals (n = 9), and according to outcome in: survivors (n = 19) and non-survivors (n = 9). In the first study, it was found that foals with neonatal sepsis showed marked changes in the energy metabolism, with hypoglycemia at birth and reduced GGT enzyme activity, and increased lactate and urea levels, besides presenting high concentrations of fibrinogen, AFP and SAA. Cholesterol and lactate have been shown to be good markers for detecting sepsis within the first 48 hours of life. In the second study, we evaluated the relationship among biochemical and inflammatory markers with non-surviving, and their usefulness as death predictors. Measurement of glucose, triglyceride and GGT levels at birth have been shown to be good markers for predicting non-survival, with high sensitivity and specificity, and can be used to assist in making decisions about the chances of survival of foals in clinical routine. The results showed that septic and non-surviving foals presented several metabolic and inflammatory responses, so peripheral blood markers such as cholesterol and lactate are suitable markers for early detection of sepsis and glucose, triglycerides and GGT are useful in clinical practice for predicting non-survival in foals.

Keywords: sepsis; foals; biochemical markers; acute-phase proteins; survival

Lista de Figuras

Artigo 1

Artigo 2

Lista de Tabelas

Artigo 1

Artigo 2

Lista de Abreviaturas e Siglas

AFP	Alpha-fetoprotein
ALT	Altrenogest
CEEA	Ethical Committee on Animal Experimentation
ECP	Estradiol cypionate
EDTA	Ethylenediamine tetraacetic acid
EU	European Union
FM	Flunixin meglumine
GGT	Gama-Glutamiltransferase
Ig	Immunoglobulin
IgG	Immunoglobulin G
LR+	Likelihood ratio
LSD	Least significance difference
NPV	Negative predictive value
OR	Odds ratio
PFA	Proteínas de fase aguda
PMN	Polymorphonuclear leukocytes
PPT	Proteínas plasmáticas totais
PPV	Positive predictive value
SAA	Serum Amyloid A
SE	Standard error
SD	Standard deviation
SS	Sepsis score
TMS	Trimethoprim-sulfamethoxazole
TS	Total solids
TSZ	Sulphate turbidity test
UFPel	Universidade Federal de Pelotas

Lista de Símbolos

<	Menor
>	Maior
≤	Menor ou igual
≥	Maior ou igual
±	Mais ou menos
=	Igual
°C	Graus Celsius
%	Porcentagem
μg/dL	Microgramas por decilitro
H	Hora
Min	Minutos
mg/L	Miligramas por Litro
mg/Kg	Miligramas por quilograma
mg/dL	Miligramas por decilitro
mmol/L	Milimol por litro
Cells/μL	Cells per microlite
UI/L	Unidade Internacional por Litro

Sumário

1 Introdução

O crescimento e o desenvolvimento fetal dependem de um ambiente intrauterino saudável e da eficiência placentária (BUCCA, 2006), uma vez que este é responsável por suprir as necessidades nutricionais, metabólicas e endócrinas do feto, através da placenta (ROSSDALE et al., 1997). Qualquer deficiência na função e estrutura placentária pode refletir em déficit no crescimento e maturidade fetal, além de refletir em danos na vida pós-natal (WHITWELL, 1980). A insuficiência placentária decorrente da placentite resulta em comprometimento das trocas metabólicas e gasosas entre a mãe e o feto (MARCONI et al., 1999), sendo o comprometimento fetal dependente da natureza, duração, severidade e do estágio da gestação em que ocorre (BUCCA, 2006). Neste contexto, a placentite ascendente em éguas corresponde a mais de 30% dos partos prematuros e mortes neonatais dentro das primeiras 24 horas de vida (McKINNON, 2009).

Potros nascidos de éguas com placentite podem variar desde extremamente prematuros, com pequeno tamanho e imaturidade dos órgãos, sendo incompatível com a vida, até potros com tamanho próximo ao normal e com mínimas alterações (BAIN, 2004). Muitos potros podem, ainda, parecer normais ao nascimento e desenvolver sinais de comprometimento dentro das primeiras 72 horas de vida (MCAULIFFE, 2008). O desenvolvimento de sepse neonatal é uma consequência comum da placentite (SANCHEZ, 2005), sendo considerada a maior causa de morte em potros com menos de sete dias de vida (COHEN, 1994), assumindo importância clínica e econômica em todo mundo.

O reconhecimento precoce da sepse em potros é desejável por diversas razões. Primeiro, o atraso no estabelecimento do tratamento é um fator de risco para a baixa taxa de sobrevivência destes potros (GAYLE, 1998). Em segundo lugar, o custo associado ao tratamento de potros sépticos é elevado em relação ao de potros debilitados na ausência de sepse (CORLEY & FURR, 2003). Finalmente, a decisão de instituir terapia antimicrobiana, ou a escolha do agente antimicrobiano é claramente influenciada pelo fato dos potros serem sépticos ou não (CORLEY & FURR, 2003). Entretanto, a detecção precoce do processo séptico é, ainda hoje,

desafiadora, pois os sinais clínicos iniciais podem ser sutis e inespecíficos (BARTON, 2008). Além disso, potros em estado crítico podem apresentar sinais como febre, taquicardia, leucocitose e hiperventilação mesmo na ausência de processo infeccioso. A hemocultura, embora seja considerada como padrão ouro para o diagnóstico de sepse, apresenta algumas limitações (CORLEY & FURR, 2003). Resultados negativos não excluem em definitivo a presença de sepse e, muitas vezes, várias amostras sequenciais são necessárias para que ocorra o crescimento bacteriano, o que torna o diagnóstico demorado (BARTON, 2008).

A sepse neonatal tem sido associada a várias desordens metabólicas incluindo desregulação no metabolismo da glicose (HOLLIS et al., 2008; BARSNICK et al., 2011), do cálcio (HURCOMBE et al., 2009) e do ciclo do lactato (CORLEY et al., 2005). Em adição, com a resposta inflamatória sistêmica desencadeada pelo processo séptico, ocorre a liberação de diversos mediadores, como as proteínas de fase aguda (PFA), que tem sido amplamente utilizadas na medicina humana, no diagnóstico precoce de sepse em bebês neonatos (ENGUIX, et al., 2001).

As proteínas de fase aguda compreendem um grande grupo de proteínas que se reduzem (PFA negativas) ou se elevam (PFAs positivas) rapidamente em resposta às infecções ou lesões teciduais, e os níveis geralmente refletem o grau e extensão do processo inflamatório e infeccioso (CHAVATTE et al., 1992), sugerindo que alguns destes mediadores podem ser usados como marcadores precoces do processo séptico. Embora outros processos infecciosos ou lesões teciduais estejam associados com a resposta de PFAs, a resposta induzida por infecções bacterianas é geralmente muito acentuada, com grande e rápida variação nos níveis plasmáticos (CHAVATTE et al., 1992). Em neonatos equinos, entretanto, a quantificação das PFAs e sua utilidade como marcadores precoces da presença de sepse não está completamente esclarecida. A utilização de marcadores bioquímicos mais específicos, que avaliem as funções metabólica e energética, e o grau de resposta inflamatória através das PFAs em neonatos durante as primeiras horas de vida podem ser ferramentas úteis para estabelecimento de um diagnóstico precoce de sepse, e podem auxiliar na determinação de um prognóstico em potros em estado crítico.

Desta maneira ressalta-se a importância do reconhecimento precoce de potros que apresentam septicemia, permitindo o estabelecimento de tratamento adequado, e a necessidade do monitoramento clínico e laboratorial destes potros,

possibilitando maiores chances de sobrevivência e recuperação, com melhor prognóstico de vida atlética futura.

2 Hipótese

Potros sépticos apresentam alterações relacionadas ao metabolismo energético, lipídico, hepático e renal, com presença de hipoglicemia, hipertriglicidemia, hipercolesterolemia e hiperlactatemia e intensa resposta inflamatória; e que a magnitude dessas alterações está associada com a severidade da doença e com a sobrevivência desses potros.

3 Objetivos

3.1 Objetivos gerais

Avaliar os marcadores bioquímicos e inflamatórios em potros nascidos de éguas com placentite ascendente induzida experimentalmente, na presença de sepse, através da mensuração de marcadores bioquímicos e proteínas de fase aguda, avaliando sua utilidade como marcadores precoces do processo séptico e correlacionar esses marcadores com a sobrevivência destes potros.

3.2 Objetivos específicos

- Mensurar os níveis séricos das proteínas de fase aguda durante as primeiras horas de vida em potros controle e potros nascidos de éguas com placentite ascendente;
- Comparar a resposta clínica, hematológica, bioquímica e inflamatória de potros nascidos de éguas com placentite ascendente na presença e ausência de sepse, e potros controle, nascidos de éguas sem alterações placentárias;
- Avaliar a utilidade dos marcadores bioquímicos e das proteínas de fase aguda como marcadores precoces de sepse em potros e se existe correlação com a sobrevivência desses potros.

3 Artigo

3.1 Artigo 1

Peripheral blood markers of sepsis in foals born from mares with experimentally ascending placentitis

Luciana de Araujo Borba; Carlos Eduardo Wayne Nogueira; Fábio Rafael Pascotti Bruhn; Gabriela Castro da Silva; Lorena Soares Feijó; Igor Frederico Canisso; Bruna da Rosa Curcio

Artigo aceito para publicação na revista *Veterinary Records*

Peripheral blood markers of sepsis in foals born from mares with experimentally ascending placentitis

Luciana A. Borba [a], Carlos E.W. Nogueira [a], Fábio R.P. Bruhn [b], Gabriela C. Silva [a], Lorena S. Feijó [a], Igor F. Canisso [c*], Bruna R. Curcio [a,c*]

[a] *Departamento de Clínicas Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil.*
[b] *Departamento de Medicina Veterinária Preventiva, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil.*
[c] *Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana IL, USA.*

* Corresponding authors:
curciobruna@hotmail.com (Bruna da Rosa Curcio)
Campus Universitário, s/n, PoBox 354,
Pelotas, RS, Brazil 96160-000.
Tel: +55 53 3275-7295

canisso@illinois.edu (Igor Frederico Canisso)
1008 W Hazelwood Drive
Urbana IL 61802, USA
Tel: +1217 244-9040

ABSTRACT

This study aimed to compare blood markers in foals born from mares treated for experimentally induced ascending placentitis according to their sepsis scores. Thirty-five foals were allocated into healthy control foals (n=7) and placentitis foals: septic (n=9) and non-septic (n=19). Blood samples were obtained immediately after foaling and at 12, 24 and 48 hours. All samples were assessed for glucose, lactate, triglycerides, total cholesterol, urea, creatinine, total solids, fibrinogen, gamma-glutamyl transferase (GGT), serum amyloid A (SAA) and alpha-fetoprotein (AFP) concentrations. At foaling, glucose and GGT concentrations were lower in septic foals ($P<0.001$). Of interest SAA, AFP, creatinine and total cholesterol were higher in septic foals at parturition ($P<0.05$). Starting 12 hours, lactate, triglycerides and total cholesterol concentrations were higher in septic foals. At 24 and 48 hours, concentrations of SAA and AFP were higher in placentitis foals than control group. Total cholesterol and lactate appear suitable markers for sepsis during the first 24 hours postpartum. Septic foals displayed altered energy metabolism as determined by increased triglycerides and cholesterol concentrations, hypoglycaemia at birth and reduced activity of the GGT and increased lactate and urea concentrations. Sepsis was associated with high concentrations of SAA and AFP.

Keywords: serum amyloid A, alpha-fetoprotein**,** blood chemistry, energy metabolism.

INTRODUCTION

Abnormal conditions leading to placental insufficiency can result in anomalous fetal growth and depletion of energy reserves.[1] Normal fetal development and growth rely on a healthy uterine environment and a functional placenta to effectively supply needed nutrients to the foetus and enable adequate metabolic and endocrine exchanges between the foetus and dam.[2]

Placentitis is an important cause of placental insufficiency, abortion, premature delivery and neonatal death within the first 24 hours of life.[3-6] Neonatal sepsis during the first-week post-foaling is thought to be a common sequelae of placentitis.[3,5,7-8] Sepsis is associated with disturbances in the metabolism of glucose,[9-11] calcium,[12] and lactate.[13] Despite recent advancements in the diagnosis and treatment of sepsis in the newborn foal, the non-specific clinical signs and subtle nature of this disease may result in delayed diagnosis until severe progression of the disease, thus, early detection of sepsis remains critical for a favourable outcome.[14]

Recently, peripheral maternal markers, such as serum amyloid A (SAA) and alpha-fetoprotein (AFP) have been reported to be useful diagnostic tools for experimentally induced ascending placentitis in mares.[15-18] Serum amyloid A, a major acute phase protein increased in maternal plasma of mares with experimentally induced ascending placentitis shortly after induction.[16] Field studies demonstrated that SAA appears to be a sensitive diagnostic and prognostic marker for sepsis in foals.[19-20] Concentration of AFP was increased in maternal plasma of mares with experimental placentitis,[17] mares with spontaneous placentitis,[21] and in plasma of newborn foals that developed sepsis during the first week of life.[22] It remains to be determined if both SAA and AFP are useful markers for sepsis for foals born from mares with experimental placentitis.

While there have been numerous epidemiological studies assessing diagnostic and prognostic means for septic newborn foals,[23-25,14] the literature is scarce concerning foals born from mares with spontaneous[5] and experimentally induced ascending placentitis.[26] Experimental models offer the advantage of allowing systematic evaluations of a clinical condition under controlled and standardized conditions.

In a parallel study, we described the mares' and respective foals' clinical parameters (birth weight, survival and risk rates) in response to maternal treatment for experimentally induced ascending placentitis. [27]. In the present study using a subset these foals, we aimed to compare blood markers of sepsis for healthy foals and foals born from mares treated for experimentally induced ascending placentitis presenting high and low scores for sepsis. We hypothesized that septic foals present marked changes in the energy metabolism and blood markers response.

MATERIALS AND METHODS

Animals

All procedures carried out were approved by the Ethical Committee on Animal Experimentation of the Universidade Federal de Pelotas (UFPel) (#4750, 10/05/2010). Animal procedures carried out herein followed the guidelines of the European Union Directive (2010/63/EU) for animal experimentation. This study was conducted during three consecutive foaling seasons. All the animals were kept at Palma Farm of UFPel, Capão do Leão, Rio Grande do Sul, Brazil.

Forty-six Criollo-type mares carrying normal pregnancies were randomly assigned to serve as healthy controls (n=8) or to receive experimental induction of placentitis (n=38) as described in our parallel publication.[27] Ascending placentitis was induced via intracervical

inoculation of $1x10^7$ colony forming units of *Streptococcus equi* subspecies z*ooepidemicus* by 300 days of gestation (mean 301.7 ± 2.7, range 295-303 days) as previously described.[27]

All mares assigned to receive experimental placentitis showed clinical signs consistent with ascending placentitis (e.g., vulvar discharge, premature mammary gland development, placental separation, and increased combined thickness of uterus and placenta) by 48 hours post-induction.[27] Therapeutic combinations used for treatment of placentitis mares consisted of various combinations of estradiol cypionate (ECP, a long-acting estrogen) and altrenogest (ALT, a long-acting progestin) in addition to basic treatment for placentitis with trimethoprim-sulfamethoxazole (TMS) and flunixin meglumine (FM). The reader is referred to a previous publication for further details about the therapeutic regimen.[27] Mares with experimentally induced placentitis (n=38) were randomly assigned to different groups as follows: (1) TMS+FM (n=8); (2) TMS+FM+ALT (n=8); (3) TMS+FM+ALT+ECP (n=6); (4) TMS+FM+ECP (n=6); and (5) mares receiving no treatment (INOC, n=10). Treatment was started at 48 hours post experimental induction of ascending placentitis and carried out for ten consecutive days, as described in our parallel study.[27] All mares were closely monitored during foaling. Foetal membranes from all mares had a complete pathological, and microbiological evaluation,[4] and results are described in a parallel study.[27]. Two foals from the TMS+FM, one foal from control group and another foal from the TMS+FM+ALT had insufficient plasma samples to be analyzed in the present study. Seven stillborn foals delivered from mares receiving no treatment were not enrolled in the present study.

Clinical evaluation and sepsis score system

All foals had a full physical examination performed immediately after delivery, at 12 hours, and then repeated daily for the first seven days post-partum. Complete red and white blood counts were carried out in all of the foals at 12, 24 and 48 hours. Immunoglobulin (Ig)

levels were evaluated at 12 hours of life by the zinc sulphate turbidity test (TSZ, Embryolab, Santa Maria, Brazil). The results of IgG levels were used to attain the sepsis scores (S-Figure 1). Placentitis foals with leucopenia (<5000 cells/µL) or leucocytosis (>14000 cells/µL) and clinical signs of sepsis, received 20 mg/kg of ampicillin intravenously every 8 hours (Ampicilina Veterinária, Vetnil, Louveira, Brazil) and 0.5 mg/kg of flunixin meglumine (Desflan, Ouro Fino Saúde Animal, Cravinhos, Brazil) intravenous every 12 hours during seven days, starting at 48 hours post birth. Intravenous fluid therapy with crystalloid solution (Ringer's Lactate Solution, Eurofarma, Itapevi, Brazil) was administered when clinical findings and blood work indicated dehydration.[28] Enteral feeding with mare's milk (5-10% of body weight at 2-hour intervals) was administered to foals displaying inadequate sucking reflex.

Sepsis scoring (SS) was performed from delivery to 12 hours post-foaling (S-Figure 1).[29] Foals born from mares with experimental placentitis were classified as septic (n=9, i.e. SS $\geq$ to 11) and non-septic (n = 19 i.e., SS <11). Foals born from mares carrying and delivering normal pregnancies served as controls (n=7, i.e., SS <4)

Complete blood count and chemistry

Blood was obtained from foals by jugular venipuncture at 0 hour, (i.e., before 15 min after delivery), and then repeated at 12, 24 and 48 hours of post-foaling. Blood samples were collected in: (a) into tubes containing EDTA for complete blood count and fibrinogen; (b) plain tubes for determination of serum triglycerides, total cholesterol, urea, creatinine, total solids (TS) and gamma-glutamyl transferase (GGT); (c) tubes containing sodium fluoride for glucose and lactate assessments; and (d) into heparinized tubes for SAA and AFP measurements. Immediately after collection, the blood was centrifuged at 1500 *g* for 10 min,

and serum and plasma aliquots were separated, placed in cryovials, and frozen at -20°C until further analysis.

Red and white cells counts were determined using a commercial cell analyser (Sysmex Poch-1000VTM Hematology-Analyzer, Sysmex Corporation, Kobe, Japan). Smears were standardly prepared for leucocyte differential evaluation (Panótico Rápido, Laborclin, Pinhais, Brazil). Fibrinogen was determined by heat precipitation. Serum concentrations of triglycerides, total cholesterol, urea, creatinine, TS, GGT, glucose, and lactate were evaluated with spectrophotometric diagnostic kits using an automated analyser (Labmax Plenno, Labtest, Belo Horizonte, Brazil). Plasma concentrations of AFP were determined using a heterologous commercial immunoassay (AFP Siemens Healthcare Diagnostics Tarrytown) on a chemiluminescence platform (Immulite 1000 platform; Siemens Healthcare Diagnostics Tarrytown) at the University of Illinois. Concentrations of SAA were determined using a turbidometric immunoassay (Eiken Chemical Co, Ltd, Tokyo, Japan) at the University of Miami.

Statistical Analysis

Data were described using median (25th and 75th percentiles) to depict non-normally distributed data (biochemical markers) and mean and standard error (SE) or ranges for normally distributed data (gestational length and blood count). Normality was assessed by Shapiro-Wilk test. Non-normally data were log-transformed and analysed by one-way ANOVA between neonatal groups (control, septic, and non-septic). When significant, post hoc comparisons were made by the LSD test. For those statistically different markers in the LSD test, the cut-off values of risk factors for sepsis were extracted from literature, for glucose[30] and lactate,[31] or based on means $\pm$ 1SE of the control group (SAA, AFP, triglycerides, total cholesterol, urea, creatinine, TS, fibrinogen and GGT). For non-normally

distributed data, the means ± 1SE were calculated by inverse logarithm of transformed values. The independent association between categorized markers with sepsis was evaluated using the Fisher's exact test, followed by the odds ratio (OR) determination with 95 percent confidence interval. For those markers related to sepsis, their usefulness as prognostic predictors of sepsis was evaluated by estimations of sensitivity and specificity, positive predictive value (PPV), negative predictive value (NPV) and positive likelihood ratio (LR+). The statistical analysis was conducted using commercial software (SPSS 20, IBM Corporation, Armonk, United States). Statistical significance was set as $P<0.05$.

RESULTS

There were no significant differences in complete red, or white blood cell counts among groups during the first 48 hours (see supplemental material for this article, S-Table 1). The gestational length (days) of the septic group (313.40 ± 2.58, range: 295-318) was shorter than non-septic (336.14 ± 4.59, range: 312-365) and control group (339.40 ± 4.22, range: 331-350) ($P<0.01$). Although, according treatment for placentitis the TMS+FM+ECP group had the longest time from inoculation to delivery. Gestation length of TMS+FM+ECP (346 ± 5.2, range: 336-365) and TMS+FM+ALT+ECP (330 ± 11.2, range: 317-352) groups were not significantly different than that of healthy CONT group [27]. The results of our parallel study [27] showed that foal survival at parturition (range 87.5 -. 100%) and seven days post-delivery were similar across all treated groups (range 66.7 - 100%). The inclusion of ECP in the treatments resulted in foals with body weight similar to control group ($P>0.05$). [27]. Despite the numerical difference, there were no significant differences across groups for foals being classified as septic (TMS+FM 33.3% n=2/6; TMS+FM+ALT 28.5%, n=2/7; TMS+FM+ALT+ECP 33.3%, n=2/6; TMS+FM+ECP 0%, n=0/6; Control 0% n=0/6; and no treatment 100%, n=3/3). Thus, group receiving ECP and control group not experience septic

foals after foaling. There were no significant differences in blood markers for foals from the different groups of treatment (S-Table 2).

The SAA concentration was higher in septic foals compared with control and non-septic foals during the first 48 hours of life (Table 1). At birth, AFP was higher in the septic group compared with non-septic and healthy foals. After 24 hours, the non-septic group presented a reduction of AFP concentrations, not differing from the control group, while remained increased in the septic group (Table 1).

Table 1

Concentrations of serum amyloid A (SAA) and alpha-fetoprotein (AFP) in neonatal foals at birth (0), 24 and, 48 hours according to the clinical diagnosis (healthy control, non-septic, and septic).

		Groups according to diagnosis		
Time	Variables	Control (n=7)	Non-septic (n=19)	Septic (n=9)
0 hour	SAA (mg/L)	0 (0-1.5)[b]	0 (0-0.8)[b]	0.5 (0-588.9)[a]
	AFP (µg/dL)	248.5 (242.2 -273.7)[c]	1660 (289-2210)[b]	2470 (1990-2845)[a]
24 hours	SAA (mg/L)	11.2 (0-113)[b]	12.1 (0.3-70.5)[b]	66.3 (3.6-630.8)[a]
	AFP (µg/dL)	225 (219.7-235.7)[b]	290 (249.5-2062.5)[b]	2360 (730.5-2467)[a]
48 hours	SAA (mg/L)	4.5 (1.4-95.7)[b]	13.4 (0.8-144.5)[b]	329.7 (20.1-962.8)[a]
	AFP (µg/dL)	215.5 (212.7)[b]	284 (262-1580)[ab]	1275 (268.7-2350)[a]

[a/b/c] Different superscript letters in rows indicate a statistical difference between groups by the LSD test (P<0.05). Data presented as median (25th and 75th percentiles).
SAA, serum amyloid A; AFP, alpha-feto protein;

At birth, septic foals presented hypoglycaemia [37 mg/dL (16.5 - 47.5 md/dL)], with higher concentrations of total cholesterol (4-fold increase) and creatinine, and lower activity of GGT compared with control and non-septic foals (P<0.001). After 12 hours, lactate, triglycerides, total cholesterol, urea, and creatinine concentrations were higher in septic foals (Fig. 1). In addition, these foals showed lower values of TS and GGT compared with control and non-septic groups (Fig. 2). Result of univariate analysis to predict sepsis based on peripheral blood markers are described in Table 2.

Table 2

Description of categorized blood chemistry markers related to sepsis in 28 foals born from mares with experimentally ascending placentitis at birth (0), 12, 24, 48 hours and, results of the univariate analysis (P <0.05).

Time	Parameter	Septic	Non-septic	Total	% Positivity	Fisher's P Value	OR	95% CI
Birth (0 hour)	**SAA**							
	> 1.37mg/L	4	3	7	57.1	0.165	–	–
	≤ 1.37mg/L	5	16	21	23.8			
	AFP							
	> 271ug/dL	9	16	25	36	0.530	–	–
	≤ 271ug/dL	0	3	3	0			
	Glucose							
	< 42mg/dL	6	0	6	100	0.000	33,3	(3,2 - 350,9)
	≥ 42mg/dL	3	18	21	14.3			
	Cholesterol							
	> 185 mg/dL	8	6	14	57.1	0.013	17.3	(1.7 – 171.6)
	≤ 185 mg/dL	1	13	14	7.1			
	Creatinine							
	> 3.3 mg/dL	4	2	6	66.7	0.06	–	–
	≤ 3.3mg/dL	5	17	22	22.7			
	GGT							
	< 26 UI/L	7	7	14	50	0.103	–	–
	≥ 26 UI/L	2	12	14	14.3			
12 hours	**Lactate**							
	> 2.8 mmol/L	9	11	20	45	0.020	7,5	(0.81 – 69.75)
	≤ 2.8mmol/L	0	8	8	0			
	Triglycerides							
	> 64mg/dL	7	0	7	100	0.00	53,3	(4.8 – 592.13)
	≤ 64mg/dL	2	19	21	9.5			
	Cholesterol							
	> 206 mg/dL	8	6	14	57.1	0.01	17.33	(1.7 – 171.6)
	≤ 206 mg/dL	1	13	14	7.1			
	Urea							
	> 43 mg/dL	7	6	13	53.8	0.04	7.58	(1.2 – 48)
	≤ 43 mg/dL	2	13	15	13.3			
	Creatinine							
	> 1.6 mg/dL	6	7	13	46.2	0.228	-	-
	≤ 1.6 mg/dL	3	12	15	20			
	TS							
	< 6.3 mg/dL	7	7	14	50	0.033	12.00	(1.21 – 118.89)
	≥ 6.3 mg/dL	1	12	13	7.7			
	GGT							
	< 33 UI/L	6	3	9	66.7	0.013	10.67	(1.67 – 68.18)
	≥ 33 UI/L	3	16	19	15.8			
	AFP							
	> 238 ug/dL	8	15	23	34.8	0.529	-	-
	≤ 238 ug/dL	0	3	3	0			
	Lactate							

	> 2.1 mmol/L	8	10	18	44.4	0.02	8.18	(0.87 – 76.60)
	≤ 2.1 mmol/L	0	9	9	0			
	Triglycerides							
	>66 mg/dL	7	6	13	53.8	0.05	-	
	≤ 66 mg/dL	0	13	13	0			
	Cholesterol							
	> 226 mg/dL	7	6	13	53.8	0.013	15.2	(1.51-152.5)
24 hours	≤ 226 mg/dL	1	13	14	7.1			
	Urea							
	> 40mg/dL	7	6	13	53.8	0.013	15.2	(1.51-152.46)
	≤ 40mg/dL	1	13	14	7.1			
	Creatinine							
	> 1.2 mg/dL	6	9	15	40	0.236	-	-
	≤ 1.2 mg/dL	2	10	12	16.7			
	GGT							
	< 39 UI/L	7	15	22	31.8	1.000	–	–
	≥ 39 UI/L	1	4	7	20			
	AFP							
	>220 ug/dL	6	17	23	26.1	1.000	–	–
	≤ 220 ug/dL	0	2	2	0			
	Lactate							
	> 1.7 mmol/L	7	13	20	35	0.283	–	–
	≤ 1.7 mmol/L	0	4	4	0			
	Cholesterol							
	> 212 mg/dL	6	9	15	40	0.051	–	–
48 hours	≤ 212 mg/dL	0	10	10	0			
	Urea							
	> 27 mg/dL	5	9	14	35.7	0.180	–	–
	≤ 27 mg/dL	1	10	11	9.1			
	TS							
	< 5.4 mg/dL	5	4	9	55.6	0.028	9.37	(1.30 – 67.65)
	≥ 5.4 mg/dL	2	15	17	11.8			
	GGT							
	< 32 UI/L	4	11	15	26.7	1.000	–	–
	≥ 32 UI/L	2	8	10	20.0			

OR, odds ratio; SAA, serum amyloid A; AFP, alpha-fetoprotein; GGT, gamma-glutamyl transferase; TS, total solids.

At birth, foals with hypoglycaemia (<42 mg/dL) or total cholesterol concentrations above 185 mg/dL were 33.3 and 17.3 times more likely to develop sepsis, respectively. At 12 hours, foals with high concentrations of lactate (>2.8 mmol/L), triglycerides (>64 mg/dL) and total cholesterol (>206 mg/dL) were more likely to have sepsis. Values of LR+, sensitivity, specificity, PPV, and NPV for sepsis for each parameter at birth, 12 and 24 hours are depicted in Table 3.

Table 3

LR+, Sensitivity, Specificity, PPV, and NPV of those variables useful as prognostic predictors of sepsis.

Time	Parameter	LR+	Sensitivity	Specificity	PPV	NPV
0 hour	Glucose < 42 mg/dL	-*	66.6	100	100	85.7
	Cholesterol > 185 mg/dL	2.8	88.8	68.4	47.1	92.8
12 hours	Lactate > 2.74 mmol/L	1.7	100	42.1	45	100
	Triglycerides > 64 mg/dL	-*	77.7	100	100	90.4
	Cholesterol > 208 mg/dL	2.8	88.8	68.4	47.1	92.8
	Urea > 43 UI/L	2.4	77.7	68.4	53.8	86.6
	TS < 6.3 mg/dL	2.7	87.5	63.1	50.0	92.3
	GGT < 33 UI/L	6.3	66.6	84.2	66.6	84.2
24 hours	Lactate > 2.1 mmol/L	1.9	100	47.3	44.4	100
	Cholesterol > 226 mg/dL	2.7	87.5	68.4	53.8	92.8
	Urea > 40 UI/L	2.7	87.5	68.4	53.8	92.8
48 hours	TS < 5.4 mg/dL	3.3	71.4	78.9	55.5	88.2

* Not possible to calculate because specificity = 100 per cent.
LR+, positive likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; GGT, γ-glutamyl transferase; TS, total solids.

Total cholesterol was the more sensitive parameter to predict sepsis at birth and 12 hours (sensitivity 88.8 percent; NPV 92.8 percent) and at 24 hours (sensitivity 87.5 percent; NPV 92.8 per cent). With 12 and 24 h, lactate concentrations (>2.7 mmol/L and 2.1mmol/L, respectively) being the most suitable predictor for sepsis (sensitivity 100 percent, NPV 100 percent).

DISCUSSION

This is apparently the first study to assess peripheral blood markers and sepsis scores in foals born from mares with experimentally induced placentitis. While experimental placentitis does not necessary reflect spontaneous clinical cases, controlled studies have the advantage of minimizing confounding variables commonly seen in clinical practice, for instance, foals in the present study were not treated for 48 hours thus allowing us to assess

peripheral markers in untreated animals. Therefore, only 33% of foals were septic from mares treated after experimental placentites, while mares receiving no treatment had 100% of septic foals. Being that septic foals showed special findings in the blood evaluation.

While plasma SAA increased in all groups in the first 24 hours, septic foals presented significantly higher concentrations, and non-septic and healthy control had SAA concentrations within normal ranges. Concentrations of SAA may increase mildly in the first few days after parturition in the normal neonatal foal but should remain within normal reported ranges.[31,33] As observed in our study, SAA concentration rise significantly during infection and has been demonstrated as a useful marker for sepsis.[20,34,19] Of interest, septic foals had lower magnitude of SAA increase at birth, this finding concurs with a recent study that shown that aborted foetuses from mares with placentitis had high concentrations of SAA but with lower magnitude than observed in adults and older newborn foals observed herein.[35]

Our results concur with a recent study where foals becoming sick in the first week of life had increased plasma concentrations of AFP at newborn foal examined at 12-24 hours post-delivery.[22] It is worth noting that, AFP is a protein present in the early equine conceptus, foetal fluids, maternal plasma and newborn foal.[36,37,17,22] Recently, it has been shown that AFP concentrations increase in the plasma of mares diagnosed with ascending placentitis.[17] The metabolism of AFP in foals is not known, but in humans, its half-life in serum is estimated to be approximately five days after delivery.[37,38] In foals, the biological role of AFP has yet to be determined. The AFP is a protein produced by the fetus (and present in the fetal fluids), can be detected in the plasma of mares diagnosed with ascending placentitis, probable due increased permeability of the fetoplacental unit upon inflamation. [17]

At birth, fibrinogen concentrations did not differ among groups. The hyperfibrinogenaemia (>700 mg/dL) observed in septic foals at 12 and 24 hours can be considered an inflammatory indicator of intrauterine sepsis in late gestation, suggesting that the foetus was exposed to an inflammatory process in utero,[39] which is consistent with the experimental induction of placentitis. Higher concentrations of fibrinogen in foals less than 2 days of age is evidence of intrauterine sepsis and inflammatory process, since hyperfibrinogenaemia indicate an inflammatory response for at least 24 to 48 hours.[39] The progressive decline in fibrinogen concentration observed in septic foals during the first 48 hours of age can be used as an indication of favourable prognosis, as describe elsewhere.[40]

It is worth noting that, at birth, TS was not different across groups, but lower in septic foals at 12 and 48 hours. Septic foals may catabolize their immunoglobulins, resulting in hypoproteinaemia.[39,41] Therefore hypoproteinaemia in 24 hours of age should be a warning for conditions such as sepsis.[42]

Septic foals had marked hypoglycaemia, hypertriglyceridemia, and hypercholesterolemia during the early postpartum period. These findings are consistent with retrospective studies involving foals with developing sepsis in the first week of life.[9,11-12] The hypoglycaemia recorded here in septic foals [37 mg/dL (16.5 to 47.5 mg/dL)] at birth is consistent with the cut-off value for hypoglycaemia (i.e. 41.95 mg/dL) at birth reported by Pirrone and others,[30,43] possibly as a result of the low glycogen reserves and poor nursing behaviour in these animals, or secondary to increased catabolism[9] and increase in energy consumption by the proliferating microorganisms.[44]

At 12 and 24 hours, triglyceride levels in septic foals were three times higher than control and non-septic foals. This is consistent with previous studies,[10-11,45] and may be due to lipid mobilization during sepsis. Lipid mobilization is controlled mostly by epinephrine and glucagon, which are secreted in response to low blood glucose concentrations.[46] Thus,

as hypoglycaemia was recorded here, it is likely that low blood glucose levels triggered the lipid catabolism. Similarly, hypertriglyceridemia is the main feature of altered fat metabolism in critically ill humans.[47]

Little is known about peripheral cholesterol concentrations under physiological and pathological conditions in neonatal foals. In our study, high cholesterol concentrations were observed at birth in septic foals [433 mg/dL (225-532.5 mg/dL)], suggesting that high cholesterol concentrations during the first 48 hours of life in foals may be an indicator of sepsis. These may be associated with immaturity and hepatic insufficiency during sepsis process,[48] which can lead to a cholestasis and subsequent blood cholesterol increase.

The high lactate concentrations in septic foals were maintained from birth to 48 hours of life, which concurs with previous studies involving critically ill foals.[31,43] It has been suggested that lactate clearance occurs more slowly in sick foals.[31,43] In this study, as reported by Corley and others,[13] the sequential measurement of lactate in critically ill foals gives important prognostic information and is associated with the presence of bacteraemia and evidence of the systemic inflammatory response syndrome (SIRS).

The increased urea and creatinine concentrations observed here in septic foals at 12 hours postpartum are not specific marker for sepsis. Furthermore, we observed that the creatinine concentrations in septic foals, although lower than observed at birth, remained higher than the other groups during the first 48 hours postpartum, possibly as a result of tissue hypoperfusion and renal damage, as suggested elsewhere.[40] Increased concentrations can be found in critically ill foals due to the negative energy balance, as well as in situations where there is renal compromise,[41] or may also be a transient finding in asphyxiated foals or foals delivered from mares with placentitis.[40]

The lower activity of GGT in septic foals during the first 48 hours is similar to the results obtained by Armengou and others,[45] in septic foals. Sepsis-induced intrahepatic

cholestasis may be associated with both, low or high GGT.[49] Thus, we speculate that the association of low GGT with poor prognosis in sepsis foals as seen in the present study suggests that sepsis, but not liver disease, is more severe with a poor prognosis.

Maturation in the equine foetus starts 4-5 days prior to delivery and continues in the first days postpartum, which is considered delayed in comparison with other species [50, 51]. Mares suffering with placentitis have shorter gestation, which may lead to abortion, stillbirth or delivery of a premature foal. Premature foals display abnormal hepatic and renal metabolism in the postpartum adjustment period adaptation of post-natal life [52]. Therefore, changes in blood markers for liver and renal functions assessed in the present study could be confounded with prematurity. This is a limitation of the present study design. However, since we had clinical evidence of sepsis substantiated by changes in blood markers such fibrinogen, AFP and SAA, it is clear that sepsis played a role in the results obtained in the present study.

As previously shown in our parallel study, neonates delivered from estradiol-treated mares benefited from ECP therapy as characterized by normal foal birth weight, no deaths in the first seven days post-partum, and no foals classified as high-risk. [27] Although, the findings in the present study enhance that therapeutic estrogen supplementation may aid recovery from ascending placentitis, as evidenced by no septic foals in TMS+FM+ECP group.

Considering the importance of septicaemia in neonatal foals and the necessity for early detection of sepsis and intervention, it was paramount to investigate markers that presenting high sensitivity that can be easily assessed in clinical practice. Cholesterol and lactate appeared to fulfill these principals to predict sepsis in foals born from mares with placentitis during the first 24 hours of life.

CONCLUSION

Septic foals born from mares with placentitis showed changes in energy metabolism characterized by hypertriglyceridemia and hypercholesterolemia, with hypoglycaemia at birth and reduction in the activity of the GGT enzyme, and increased serum lactate and urea. In addition, septic foals demonstrated high concentrations of fibrinogen, AFP, and SAA. Peripheral blood markers such as cholesterol and lactate are suitable markers to detect sepsis in foals born from mares with placentitis and glucose, and triglycerides may be used as prognostic tools for critically ill foals.

ACKNOWLEDGMENTS

The funding agencies CAPES and CNPq are acknowledged for providing scholarships to graduate students (L.A.B., G.C.S., and L.S.F.), and post-doctoral fellowship (B.R.C. Estágio Sênior no Exterior Processo CAPES#99999.005570/2015-08). Funds provided by the Universidade Federal de Pelotas and by Department of Veterinary Clinical Medicine of the University of Illinois Urbana-Champaign. We would like to thank Dr. Robyn Ellerbrock for her providential assistance editing this manuscript. Different excerpts of this manuscript were orally presented as an abstract at the Annual Meeting of the Society for Theriogenology, Fort Collins, 2-5 August 2017.

COMPETING INTERESTS

The authors declare no conflict of interest or personal relationship with any third party that could bias the publication of this manuscript.

REFERENCES

1 Koterba AM. Neonatal asphyxia. In: Koterba AM, Drummond WH, Kosch PC, eds. Equine Clinical Neonatology. Philadelphia, TN: Lea & Febiger 1990:124–135.

2 Rossdale PD, Ousey JC, Chavatte P. Readiness for birth: an endocrinological duet between fetal foal and mare. *Equine Vet J* 1997;24:96–99.

3 Giles RC, Donahue JM, Hong CB, et al. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). *J Am Vet Med Assoc* 1993;203:1170–1175.

4 Hong CB, Donahue JM, Giles RC, et al. Etiology and pathology of equine placentitis. *J Vet Diagn Invest* 1993;5:55–63.

5 Barr BS. The outcome of foals born to mares treated for placentitis. *Havemeyer Found Monogr Ser* 2005;19:49.

6 Laugier C, Foucher N, Sevin C, et al. 24-year retrospective study of equine abortion in Normandy (France). *Journal of Equine Veterinary Science* 2011;31:116–123.

7 Cohen ND. Causes of and farm management factors associated with disease and death in foals. *J Am Vet Med Assoc* 1994;204:1644–1651.

8 Sanchez LC. Equine neonatal sepsis. *Vet Clin North Am Equine Pract* 2005;21:273–293.

9 Hollis AR, Furr MO, Magdesian KG, et al. Blood glucose concentrations in critically ill neonatal foals. *J Vet Intern Med* 2008;22:1223–1227.

10 Barsnick RJ, Toribio RE. Endocrinology of the equine neonate energy metabolism in health and critical illness. *Vet Clin North Am Equine Pract* 2011;27:49–58.

11 Barsnick RJ, Hurcombe SD, Smith PA, et al. Insulin, glucagon, and leptin in critically ill foals. *J Vet Intern Med* 2011;25:123–131.

12 Hurcombe SD, Toribio RE, Slovis NM, et al. Calcium-regulating hormones and serum calcium and magnesium concentrations in septic and critically ill foals and their association with survival. *J Vet Intern Med* 2009;23:335–343.

13 Corley KTT, Donaldson LL, Furr MO. Arterial lactate concentration, hospital survival, sepsis and SIRS in critically ill neonatal foals. *Equine Vet J* 2005;37:53–59.

14 Guiguère S, Weber EJ, Sanchez LC. Factors associated with outcome and gradual improvement in survival over time in 1065 equine neonates admitted to an intensive care unit. *Equine Vet J* 2017;49:45–50.

15 Coutinho da Silva MA, Canisso IF, Macpherson ML, et al. Serum amyloid A concentration in healthy periparturient mares and mares with experimentally ascending placentitis. *Equine Vet J* 2013;45:619–24.

16 Canisso IF, Ball BA, Cray C, et al. Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. *Am J Reprod Immunol* 2014;72:376–385.

17 Canisso IF, Ball BA, Scoggin KE, et al. Alpha-fetoprotein is present in the fetal fluids and is increased in plasma of mares with experimentally induced ascending placentitis. *Anim Reprod Sci* 2015;154:48–55

18 Canisso IF, Ball BA, Cray C, et al. Use of a qualitative horse-side test to measure serum amyloid A in mares with experimentally induced ascending placentitis. *J Equine Vet Sci* 2015;35:54–59.

19 Paltrinieri S, Giordano A, Villani M, et al. Influence of age and foaling on plasma protein electrophoresis and serum amyloid A and their possible role as markers of equine neonatal septicaemia. *Vet J* 2008;176:393–396.

20 Chavatte PM, Pepys MB, Roberts B, et al. Measurement of serum amyloid A protein (SAA) as an aid to differential diagnosis of infection in newborn foals. Equine Infectious Diseases VI1992, 33–38.

21 Rothrock L. Estradiol-17β and alfa-fetoprotein as diagnostic markers for ascending placentitis in a Quarter Horse broodmare [abstract]. Proceedings of the Theriogenology Annual Conference, Asheville, July 27-30, 2016.

22 Prell M. Alpha-fetoprotein as a marker for equine neonatal disease [abstract]. In: Proceedings of the Theriogenology Annual Conference, Asheville, July 27-30, 2016.

23 Gayle JM, Cohen ND, Chaffin MK.. Factors associated with survival in septicemic foals: 65 cases (1988–1995). *J Vet Intern Med* 1998;12, 140–146.

24 Sanchez LC, Giguère S, Lester GD.. Factors associated with survival of neonatal foals with bacteraemia and racing performance of surviving Thoroughbreds: 423 cases (1982–2007). *J Am Vet Med Assoc* 2008;233:1446–1452.

25 Dunkel B, Corley KTT. Pathophysiology, diagnosis and treatment of neonatal sepsis. *Equine Vet Educ* 2015;27:92–98.

26 Bailey CS, Macpherson ML, Pozor MA, et al. Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. *Theriogenology* 2010;74:402–412.

27 Curcio BR, Canisso IF, Pazinato FM, et al. Estradiol cypionate aided treatment for experimentally induced ascending placentitis in mares. *Theriogenology* 2017;102:98–107.

28 Koterba AM. Physical examination. In: Koterba AM, Drummond WH, Kosch PC, eds. Equine Clinical Neonatology. Philadelphia, TN: Lea & Febiger 1990:71–85.

29 Brewer BD, Koterba AM,. Development of a scoring system for the early diagnosis of equine neonatal sepsis. *Equine Vet J* 1988;20:18–22.

30 Pirrone A, Antonelli C, Mariella J, et al. Gross placental morphology and foal serum biochemistry as predictors of foal health. *Theriogenology* 2014;81:1293–1299.

31 Castagnetti C, Pirrone A, Mariella J, et al. Venous blood lactate evaluation in equine neonatal intensive care. *Theriogenology* 2010;73:343–357.

32 Nunokawa Y, Fujinaga T, Taira T, et al. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci* 1993;55:1011–1016.

33 Duggan V, Holyoak GR, MacAllister CG, et al. Influence of induction of parturition on the neonatal acute phase response in foals. *Theriogenology* 2007;67:372–381.

34 Stoneham SJ, Palmer L, Cash R, et al. Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. *Equine Vet J* 2001;33:599–603.

35 Erol E, Jackson C, Horohov D, et al. Elevated serum amyloid A levels in cases of aborted equine fetuses due to fetal and placental infections. *Theriogenology* 2016;86:971–975.

36 Simpson, K.S., Adams, M.H., Behrendt-Adam, C.Y., Baker, C.B., McDowell, K.J. 2000. Differential gene expression in day 12 and day 15 equine conceptuses *J Reprod Fertil Suppl* 56, 539-547.

37 Bader D, Riskin A, Vafsi O, et al. Alpha-fetoprotein in the early neonatal period - a large study and review of the literature. *Clin Chim Acta* 2004;349:15–23.

38 Caballero C, Vekemans M, Lopez del Campo JG, et al. Serum alpha-fetoprotein in adults, in women during pregnancy, in children at birth, and during the first week of life: a sex difference. *Am J Clin Exp Obstet Gynecol* 1977;127:384–389.

39 Brewer BD. Neonatal infection. In: Koterba AM, Drummond WH, Kosch PC, eds. Equine Clinical Neonatology. Philadelphia, TN: Lea & Febiger 1990:295–316.

40 Schott HC. Review of azotemia in foals. Proceedings of the Annual Convention of the American Association of Equine Practitioners, San Antonio, November 18-22, 2011. p 328-324.

41 Axon JE, Palmer JE. Clinical pathology of the foal. *Vet Clin North Am Equine Pract* 2008;24:375–385.

42 Morresey PR. Prenatal and perinatal indicators of neonatal viability. Clinical Techniques in Equine Practice 2005;4:238–249.

43 Pirrone A, Mariella J, Gentilini F, et al. Amniotic fluid and blood lactate concentrations in mares and foals in the early postpartum period. *Theriogenology* 2012;78:1182–1189.

44 Barton MH. Early recognition of the septicemic foal. In: Proceedings of the Annual convention of the American Association of Equine Practitioners, Austin, July 19-21, 2008. p 101-109.

45 Armengou L, Cunilleras EJ, Ríos J, et al. Metabolic and endocrine profiles in sick neonatal foals are related to survival. *J Vet Intern Med* 2013;27:567–575.

46 Gonzáles FHD, Silva SC. Introdução à bioquímica veterinária. Porto Alegre, RS:Editora da UFGRS 2006:364.

47 Das S, Misra B, Roul L, et al. Insulin resistance and beta cell function as prognostic indicator in multi-organ dysfunction syndrome. *Metab Syndr Relat Disord* 2009;7:47–51.

48 Kulisa M, Dlugosz B, Luszczynski J, et al. Cholesterol level in serum in a thoroughbred foals bred in two different studs. *J Anim Sci Biotechnol* 2005;21:77–80.

49 Oswari H, Widjaja RK, Rohsiswatmo R, et al. Prognostic value of biochemical liver parameters in neonatal sepsis-associated cholestasis. *Paediatr Child Health* 2013;9:6–11.

50. Rossdale PD & Ousey J. Studies on equine prematurity 6: guidelines for assessment of foal maturity. *Equine Vet. J*. 1984;16:300-302.

51. Ousey J, Rossdale PD, Fowden A, et al. Effects of manipulating intrauterine growth on post natal adrenocortical development and other parameters of maturity in neonatal foals. *Equine Vet. J*. 2004;36:616-621.

52. Axon JE Critical care – assessment. In: McKinnon, AO, Squires, EL, Vaala, WE, Varner, D.D., eds. Equine Reproduction, Second Ed. Oxford, UK: Wiley- Blackwell 2011:167–176.

Figure Captions

Fig. 1. Median (25th and 75th percentiles) obtained for glucose, lactate, triglycerides, total cholesterol, urea, and creatinine for time (hours) at 0, 12, 24 and 48 hours in foals born mares with experimentally induced ascending placentitis according to diagnosis (i.e., control, non-septic and septic).

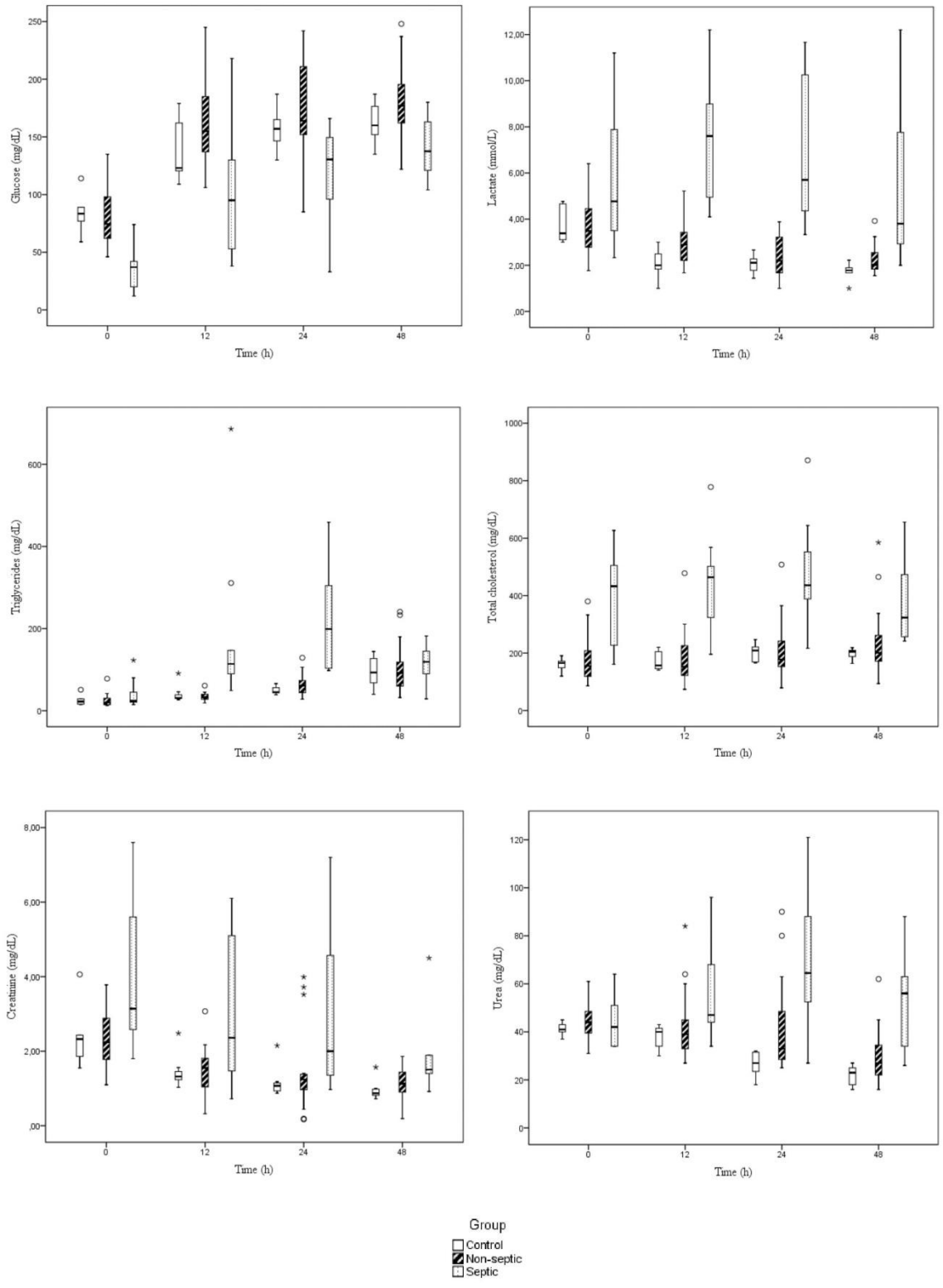
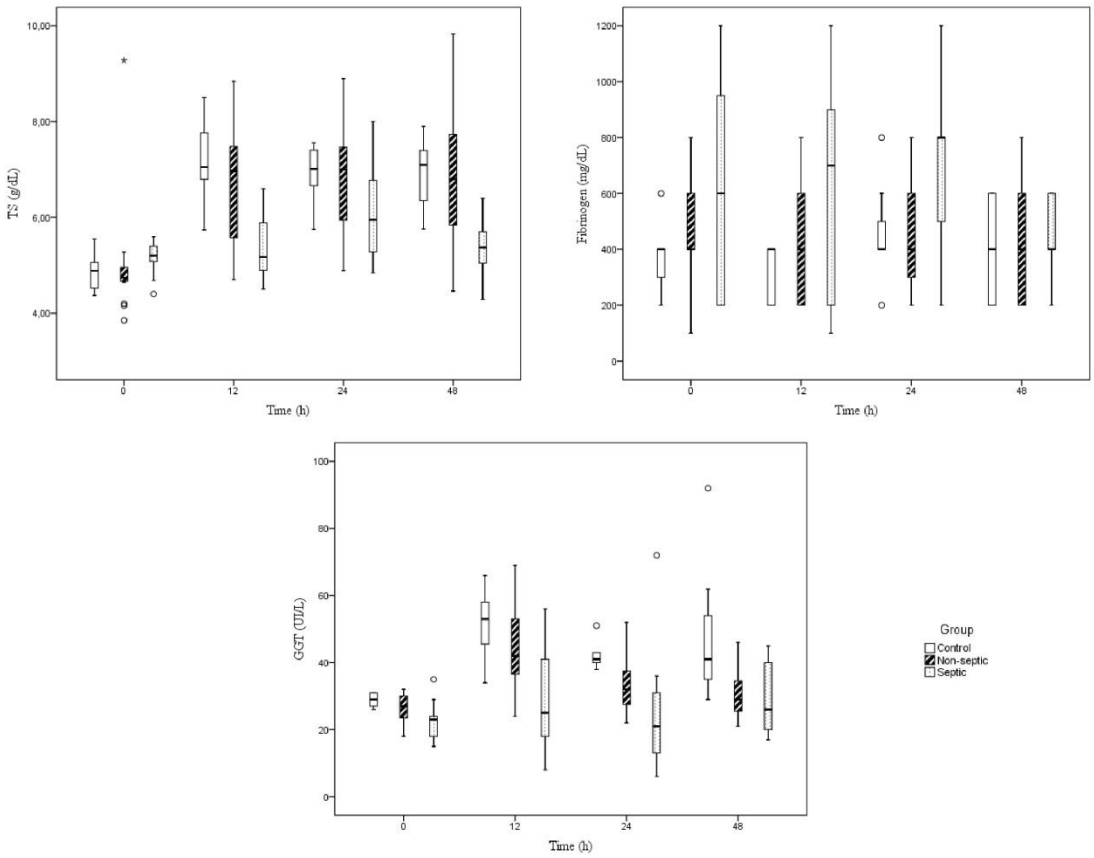


Fig.2. Median (25th and 75th percentiles) obtained for total solids (TS), fibrinogen and gamma-glutamyl transferase (GGT) for time (hours) at 0, 12, 24 and 48 hours in foals born mares with experimentally induced ascending placentitis according to diagnosis (i.e., control, non-septic and septic).



S-Figure 1. Modified sepsis score adapted from Brewer & Koterba (1988) used to classified foals in septic and non-septic.

Parameter	Score					
	4	**3**	**2**	**1**	**0**	**Score**
I. History:						
1. Placentitis, vulvar discharge prior to delivery, dystocia, long transport of mare, mare sick, prolonged gestation (> 365 days), foal induced		Yes			No	
2. Prematurity (days of gestation)		< 300	300-310	311-330	> 330	
II. Clinical examination:						
1. Petechiae, scleral injection not secondary to eye disease or trauma		Marked	Moderate	Mild	None	
2. Fever			>38.9º C	< 37.9ºC		
3. Hypotonia, coma, depression, convulsions			Marked	Mild	Normal	
4. Anterior uveitis, diarrhea, respiratory distress, swollen joints, open wounds		Yes			None	
III. Laboratory Data:						
1. PMN count (cells/µl)		< 2000	2000 - 4000 or > 12000	8000 - 12000	Normal	
2. Band Neutrofil count (cells/µl)		> 200	50-200		< 50	
3. Any toxic change in PMN	Marked	Moderate	Slight		None	
4. Fibrinogen (mg/dl)			> 600	400-600	≤ 400	
5. Blood glucose (mg/dl)			< 50	50-80	> 80	
6. Zinc sulphate turbidity test (mg/dl)	< 200	200-400	400-800		> 800	

Total points for this case: _____

In this system, the values of each parameter are converted into a score, on a scale ranging from 0 to 4. If the sum of all 12 parameters assessed reaches a value greater than or equal to 11, the foal is deemed as septic.

S -Table 1. Mean ± Standard Error (SE) for hematology at birth (0), 12, 24, 48 hours according to the clinical diagnosis (healthy control, non-septic, and septic).

Time	Parameter	Groups according to diagnosis		
		Control (*n* = 7)	Non-septic (*n* = 19)	Septic (*n* = 9)
0h	Red blood cells (10^6/mL)	10.9 ± 0.7	11.5 ± 0.3	10.3 ± 0.8
	Hemoglobin (g/dL)	13.4 ± 0.8	14.0 ± 0.3	13.9 ±0.5
	Packed cell volume (%)	44.2 ± 2.9	46.5 ± 1.0	44.8 ± 1.7
	Mean cell volume (fL)	40.2 ± 0.6	40.6 ± 0.6	46.5 ± 5.5
	Mean cell hemoglobin concentration (%)	30.5 ± 0.4	30.1 ± 0.3	31.1 ± 0.5
	Platelet count (10^3/mL	348.9 ± 43.9	347.6 ± 23.1	254.7 ± 27.5
	White blood cells (µL)	8042.9 ± 937.3	7568.4 ± 552.8	5600± 1572.7
	Neutrophils (µL)	5836.4 ± 680.4	5543.7 ± 493.5	3373.7 ± 1381
	Bands (µL)	0^b	0^b	14.8 ± 9.8^a
	Lymphocytes (µL)	2081.9 ± 343.6	1886.2 ± 131.0	2098.6 ± 326.3
	Monocytes (µL)	118.4 ± 58.9	104.6 ± 24.1	97.7 ± 34.2
	Eosinophils (µL)	11.8 ± 11.8	33.9 ± 15.2	15.3 ± 15.3
12h	Red blood cells (10^6/mL)	9.8 ± 0.5	9.6 ± 0.3	8.4 ± 0.4
	Hemoglobin (g/dL)	12.0 ± 0.7	11.6 ± 0.3	11.2 ± 0.6
	Packed cell volume (%)	38.9 ± 2.3	38.1 ± 1.0	35.5 ± 1.7
	Mean cell volume (fL)	39.7 ± 0.6	39.7 ± 0.5	42.5 ± 1.6
	Mean cell hemoglobin concentration (%)	30.9 ± 0.2	30.4 ± 0.2	31.4 ± 0.5
	Platelet count (10^3/mL	296.4 ± 38.3	284.9 ± 18.553	225.3 ± 26.4
	White blood cells (µL)	11200 ± 1052.0	10189 ± 778.2	6855.6 ± 2590.1
	Neutrophils (µL)	8925 ± 1187.8	8397.5 ± 744.6	5275 ± 2414.2
	Bands (µL)	0^b	0^b	10.9 ± 6.0^a
	Lymphocytes (µL)	2180 ± 253.0	1684.1 ± 161.4	1449.1 ± 233.0
	Monocytes (µL)	77.714 ± 59.1	84.5 ± 20.5	120.3 ± 54.1
	Eosinophils (µL)	17.0 ± 17.0	21.3 ± 9.8	0
24h	Red blood cells (10^6/mL)	9.37±0.48	9.5 ± 0.3	8.4 ± 0.6
	Hemoglobin (g/dL)	11.9 ± 0.7	11.411 ± 0.3	10.9 ± 0.8
	Packed cell volume (%)	37.6 ± 2.2	36.921 ± 0.9	34.7 ± 2.5
	Mean cell volume (fL)	40.4 ± 1.3^{ab}	38.926 ± 0.5^b	41.7 ± 1.7^a
	Mean cell hemoglobin concentration (%)	31.5 ± 0.2	30.958 ± 0.2	31.5 ± 0.4
	Platelet count (10^3/mL	243.0 ± 31.2	260.68 ± 16.3	257.7 ± 46.6
	White blood cells (µL)	7785.7 ± 546.2	8973.7 ± 1028.6	9200 ± 3462.3
	Neutrophils (µL)	5588.7 ± 473.1	7050.3 ±1006.7	6951.7 ± 3491.
	Bands (µL)	0	0	60.4 ± 43.1
	Lymphocytes (µL)	1977.4 ± 153.7	1694.7 ± 236.9	2049.0 ±397.3
	Monocytes (µL)	56.7 ± 35.4	154.5 ± 54.3	53.0 ± 35.9
	Eosinophils (µL)	162.9 ± 126.6	44.2 ± 13.4	85.8 ± 60.9
48h	Red blood cells (10^6/mL)	9.0 ± 0.4	9.3 ± 0.3	8.2 ± 0.4
	Hemoglobin (g/dL)	11.1 ± 0.6^a	11.3 ± 0.3^a	9.9 ± 0.3^b
	Packed cell volume (%)	34.6 ± 2.0	35.8 ± 1.2	32.1 ± 1.1
	Mean cell volume (fL)	38.4 ± 0.6	38.3 ± 0.5	39.4 ± 1.4
	Mean cell hemoglobin concentration (%)	32.3 ± 0.4	31.7 ± 0.3	31.1 ± 0.4

Platelet count (10^3/mL	351.3 ± 88.9	279.3 ± 15.5	317.7 ± 44.6
White blood cells (μL)	7114.3 ± 921.8	7960.0 ± 935.6	9666.7 ± 2748
Neutrophils (μL)	5574.1 ± 992.1	6460.6 ± 892.9	7768.7 ± 2442
Bands (μL)	8.3 ± 8.3	0	13.3 ± 13.3
Lymphocytes (μL)	1459.3 ± 169.4	1173.5 175.0	1716.5 ± 344.7
Monocytes (μL)	42.8 ± 32.9	90.7 ± 25.1	161.17 ± 94.5
Eosinophils (μL)	23.4 ± 11.2	9.3 ± 6.6	0

[a/b] Different superscript letters in rows indicate a statistical difference between groups by the LSD test ($P < 0.05$).

S -Table 2. Mean ± Standard Deviation (SD) for blood chemistry profiles at birth (0). 12. 24. 48 hours according to the mare's treatment groups for experimentally induced ascending placentitis

Parameter	Time (h)	Treatment of mares with experimentally induced placentitis					
		Control (n=7)	Untreated (n=3)	TMS+FM (n=6)	TMS+FM+ECP (n=6)	TMS+FM+ALT (n=7)	TMS+FM+ALT+ECP (n=6)
Glucose (mg/dL)	0	84.3 ± 17.9^{a}	17.7 ± 8.9^{b}	64.4 ± 34.4^{a}	77.0 ± 15.1^{a}	69.6 ± 30.6^{a}	73.0 ± 33.6^{a}
	12	139.4 ± 27.5	146.0 ± 91.5	141.7 ± 55.1	172.0 ± 50.6	138.1 ± 49.1	129.7 ± 45.8
	24	156.7 ± 19.3	94.5 ± 86.9	157.3 ± 47.8	193.5 ± 41.4	160.0 ± 43.9	149.5 ± 48.8
	48	162.7 ± 18.4	366.5 ± 263.7	159.8 ± 41.9	194.5 ± 38.3	186.0 ± 25.4	144.2 ± 26.6
Lactate (mmol/L)	0	3.7 ± 0.8	6.5 ± 4.1	4.3 ± 1.3	2.9 ± 0.8	3.8 ± 1.2	5.3 ± 3.0
	12	2.1 ± 0.6^{c}	7.9 ± 4.1^{a}	5.4 ± 3.8^{ab}	2.9 ± 0.6^{bc}	3.9 ± 2.5^{ab}	4.2 ± 2.2^{ab}
	24	2.0 ± 0.4	5.7 ± 1.1	4.5 ± 3.5	2.0 ± 0.7	4.3 ± 3.8	3.8 ± 2.8
	48	1.7 ± 0.4	4.0 ± 0.3	4.2 ± 4.0	2.3 ± 0.9	2.1 ± 0.2	3.8 ± 3.7
Triglycerides (mg/dL)	0	26.0 ± 13.4	28.3 ± 9.3	22.7 ± 10.6	21.0 ± 8.0	38.4 ± 38.2	42.0 ± 30.9
	12	40.8 ± 23.0	106.7 ± 45.1	81.0 ± 113.1	28.7 ± 8.2	139.0 ± 243.1	62.8 ± 31.3
	24	49.8 ± 11.2^{b}	209.0 ± 14.1^{a}	140.5 ± 156^{ab}	55.0 ± 28.9^{b}	76.8 ± 34.6^{b}	124.8 ± 132.9^{b}
	48	95.3 ± 40.5	87.0 ± 82.0	103.4 ± 57.4	110.0 ± 63.1	128.3 ± 71.3	99.5 ± 45.8
Cholesterol (mg/dL)	0	160.5 ± 24.4	462.7 ± 50.3	186.8 ± 105.9	186.3 ± 78.7	274.9 ± 192.6	228.3 ± 170.8
	12	174.3 ± 34.2	405.3 ± 89.9	256.7 ± 175.3	172.5 ± 71.7	303.7 ± 241.8	243.5 ± 173.6
	24	200.4 ± 32.5	401.0 ± 22.6	297.5 ± 175.1	204.8 ± 53.6	340.9 ± 263.3	263.3 ± 195.4
	48	197.6 ± 19.0	323.5 ± 54.4	232.8 ± 142.2	226.0 ± 52.1	299.3 ± 184.8	301.8 ± 188.7
Urea (mg/dL)	0	41.2 ± 2.8	56.3 ± 6.8	45.8 ± 7.4409	44.8 ± 9.6	40.7 ± 6.9	40.8 ± 5.4
	12	37.7 ± 5.3^{b}	69.3 ± 26.0^{a}	51.2 ± 13.3^{ab}	42.8 ± 20.4^{b}	38.6 ± 8.0^{b}	44.0 ± 10.8^{b}
	24	26.7 ± 5.4^{c}	98.0 ± 32.5^{a}	63.2 ± 28.1^{a}	39.2 ± 21.6^{c}	38.7 ± 16.5^{c}	42.7 ± 12.6^{b}
	48	21.7 ± 4.5	48.5 ± 20.5	44.6 ± 25.5	31.5 ± 16.0	25.0 ± 8.7	36.0 ± 16.6
Creatinine (mg/dL)	0	2.4 ± 0.9^{a}	6.6 ± 1.0^{b}	2.5 ± 0.5^{a}	2.3 ± 0.5^{a}	2.1 ± 0.8^{a}	3.0 ± 0.4^{a}
	12	1.5 ± 0.5	4.4 ± 1.6	2.3 ± 2.1	1.2 ± 0.3	1.5 ± 0.4	1.7 ± 0.5
	24	1.2 ± 0.4	3.1 ± 1.7	2.3 ± 2.5	0.9 ± 0.3	2.1 ± 1.5	1.2 ± 0.6
	48	0.9 ± 0.3	1.4 ± 0.1	2.1 ± 1.3	1.0 ± 0.5	1.2 ± 0.4	1.3 ± 0.4
GGT (UI/L)	0	28.8 ± 2.1	22.3 ± 11.0	25.5 ± 4.8	27.7 ± 2.6	25.6 ± 5.2	24.6 ± 4.5
	12	51.4 ± 11.1	29.0 ± 23.5	44.3 ± 20.6	40.3 ± 10.8	39.7 ± 11.0	37.3 ± 15.3

	24	42.3 ± 4.3	42.5 ± 41.7	29.7 ± 16.1	31.8 ± 4.2	29.3 ± 8.9	30.5 ± 9.4
	48	48.6 ± 21.9[a]	31.0 ± 19.8[b]	28.4 ± 7.2[b]	32.3 ± 7.2[b]	31.7 ± 6.6[b]	27.7 ± 5.9[b]
	0	4.8 ± 0.4	5.1 ± 0.6	4.9 ± 0.5	4.8 ± 0.46	5.5 ± 1.7	4.8 ± 0.3
Total solids	12	7.2 ± 0.9	5.0 ± 0.5	6.5 ± 1.5	7.1 ± 0.6	6.2 ± 0.9	6.1 ± 1.6
(mg/dL)	24	6.9 ± 0.6	7.3 ± 0.9	6.1 ± 1.1	6.9 ± 0.8	6.4 ± 0.9	6.5 ± 1.6
	48	6.9 ± 0.8	6.0 ± 0.6	6.1 ± 1.7	7.4 ± 1.4	6.5 ± 1.1	5.9 ± 1.8
	0	383.3 ± 132.9[b]	200.0 ± 0[b]	480.0 ± 179.0[ab]	383.3 ± 240.1[b]	742.9 ± 299.2[a]	450.0 ± 266.5[b]
Fibrinogen	12	314.3 ± 106.9	200.0 ± 0	460.0 ± 328.6	320.0 ± 268.3	571.4 ± 390.4	566.7± 233.8
(mg/dL)	24	457.1 ± 190.2	600.0 ± 282.8	360.0 ± 260.8	433.3 ± 150.5	657.1 ± 276.0	533.3 ± 273.2
	48	400.0 ± 178.9	400.0 ± 0	450.0 ± 191.5	333.3 ± 103.3	560.0 ± 219.1	360.0 ± 167.3
	0	0.6 ± 0.74[b]	617.5 ± 57.9[a]	0.8 ± 1.5[b]	1.2 ± 1.5[b]	6.8 ± 17.3[b]	0.1 ± 0.3[b]
SAA (mg/L)	24	62.6 ± 89.8	570.5 ± 471.3	26.0 ± 41.6	65.2 ± 59.4	33.1 ± 46.7	21.8 ± 38.6
	48	88.9 ± 173.1	605.0*	531.2 ± 583.7	107.5 ± 70.0	17.5 ± 20.8	37.3 ± 76.9
	0	253.8 ± 17.4	2123.3 ± 335.3	1768.8 ± 766.7	1458.5 ± 666.2	2504.0 ± 2447.3	1898.8 ± 1256.1
AFP (µg/dL)	24	227.7 ± 9.7	1791.3 ± 1317.1	1466.5 ± 943.6	275.0 ± 21.0	1385.8 ± 1279.3	1854.4 ± 927.5
	48	216.0 ± 4.6	250.0*	831.2 ± 884.0	797.3 ± 624.1	1016.2 ± 1212.3	1264.2 ± 1121.2

TMS: trimethoprim-sulfamethoxazole; FM: flunixin meglumine; ALT: altrenogest; ECP: estradiol cypionate.

[a/b/c] Different superscript letters in rows indicate a statistical difference between groups by the LSD test ($P < 0.05$).

3.2 Artigo 2

Could the early evaluation of peripheral blood markers in foals be useful to predict survival during the first 30 days of life?

Luciana de Araujo Borba; Carlos Eduardo Wayne Nogueira; Augusto Luiz Postal Dalcin; Bruna dos Santos Suñé Moraes; Fábio Rafael Pascotti Bruhn; Gabriela Castro da Silva; Lorena Soares Feijó; Bruna R. Curcio

Submetido à revista *Pesquisa Veterinária Brasileira*

Could the early evaluation of peripheral blood markers in foals be useful in predicting non-survival during the first 30 days of life?

Luciana A. Borba[2], Carlos E.W. Nogueira[2], Augusto L.P. Dalcin[2], Bruna S.S. Moraes[2], Fábio R.P. Bruhn[3], Gabriela C. Silva[2], Lorena S. Feijó[2] and Bruna R. Curcio[2*]

ABSTRACT.– Borba L.A., Nogueira C.E.W., Dalcin A.L.P., Moraes B.S.S., Bruhn F.R.P., Silva.G.C., Feijó L.S., Curcio B.R. 2018. **Could the early evaluation of peripheral blood markers in foals be useful in predicting non-survival during the first 30 days of life?** *Pesquisa Veterinária Brasileira 00(0):00-00*. Faculdade de Veterinária, Universidade Federal de Pelotas (UFPel), Cx. Postal 354, Campus Capão do Leão, Pelotas, RS 96010-900, Brazil. E-mail: curciobruna@hotmail.com

The aim of the present study was to identify biochemical and inflammatory markers after foaling associated with non-survival, and to examine their usefulness as death predictors in neonatal foals. Twenty-eight foals born from mares with experimentally induced ascending placentitis were assigned into two groups according to survival during the first 30 days of life, classified as either: survivors (n=19) or non-survivors (n=9). Glucose, lactate, triglycerides, total cholesterol, urea, creatinine, total plasmatic protein (TPP), fibrinogen, gamma-glutamyl transferase (GGT), serum amyloid A (SAA) and alpha-fetoprotein (AFP) were measured. At birth, AFP (P=0.04), total cholesterol (P=0.00) and triglycerides (P=0.03) were higher in non-surviving foals, whereas glucose (P=0.02) and GGT activity (P=0.02) were lower. Hypoglycemia was maintained during 24 and 48h of life in foals that died (P<0.05). Higher total cholesterol and lower GGT were observed in the non-surviving group during 12, 24 and 48h of life (P<0.001). Non-surviving foals presented marked hyperlactatemia at 12h and high plasma levels were maintained until 24h. At birth, glucose levels <42 mg/dL was the most specific parameter to predict death, with high PPV (83.3%). At 12h of life, the best parameter to predict non-survival was the triglycerides (>64 mg/dL). Also, total cholesterol (>185 mg/dL) and GGT (>26 UI/L) were the most sensitive (88.8%) serum parameters to predict death. Non-surviving foals presented several metabolic and inflammatory response characterized by hypoglycemia, hypercholesterolemia, lower GGT activity and elevated levels of AFP at birth. These blood markers can be used to assist in the prediction of foal survival in the clinical routine.

INDEX TERMS: Septicemia, death predictors, placentitis, acute-phase proteins, biochemical markers, foals.

RESUMO.– [**A avaliação precoce de marcadores sanguíneos periféricos em potros pode ser útil na predição de não sobrevivência nos primeiros 30 dias de vida?**]

O objetivo deste estudo foi identificar, logo após o nascimento, marcadores bioquímicos e de resposta inflamatória associados à não sobrevivência, e sua utilidade como preditores de morte em potros neonatos. Vinte e oito potros nascidos de éguas submetidas à indução experimental de placentite foram divididos em dois grupos de acordo com a sobrevivência durante os primeiros 30 dias de vida em: sobreviventes (n=19) e não sobreviventes (n=9). Os níveis de glicose, lactato, triglicerídeos, colesterol total, ureia, creatinina, proteínas plasmáticas totais (PPT), fibrinogênio, gama-glutamil transferase (GGT), amiloide A sérica (SAA) e alfa-feto proteína (AFP) foram mensurados. Ao nascimento, potros não sobreviventes apresentaram elevadas concentrações de AFP (P=0.04), colesterol total (P=0.00), triglicerídeos (P=0.03) e menores concentrações de glicose (P=0.02) e GGT (P=0.02). Baixos níveis de glicose foram mantidos durante as 24 e 48h em potros não sobreviventes (P<0.05). Elevado colesterol total e baixa GGT foram observados durante as 12, 24 e 48h de vida (P<0.001). Potros não sobreviventes apresentaram acentuada hiperlactatemia nas 12h e os níveis permaneceram elevados até às 24h. Ao nascimento, glicose <42 mg/dL foi o parâmetro mais específico para predizer a não sobrevivência, com elevado valor preditivo positivo (83.3%). Com 12h, o melhor parâmetro para predizer a não sobrevivência foi avaliação de triglicerídeos (>64 mg/dL). Além disso, os níveis de colesterol total (>185 mg/dL) e GGT (>26 UI/L) foram os parâmetros mais sensíveis (88,83%) para prever morte. Potros não sobreviventes apresentaram desordens metabólicas e inflamatórias, caracterizadas por hipoglicemia, hipercolesterolemia, baixa atividade de GGT e elevados níveis de AFP no nascimento. Esses marcadores sanguíneos podem ser usados para auxiliar na predição de sobrevivência de potros na rotina clínica.

TERMOS DE INDEXAÇÃO: Septicemia, preditores de não-sobrevivência, placentite, proteínas de fase aguda, marcadores bioquímicos, potros.

[1] Departamento de Clínica Veterinária, Universidade Federal de Pelotas (UFPel), Campus Universitário, Capão do Leão, RS 96160-000, Brasil.
*Corresponding author: curciobruna@hotmail.com
[2] Departamento de Veterinária Preventiva, Universidade Federal de Pelotas (UFPel), Campus Universitário, Capão do Leão, RS 96160-000, Brasil.
[3] Veterinary Clinic Medicine, University of Illinois, 1008 W Hazelwood Drive, Urbana IL 61802, United States .
E-mails: luaraujo_sm@hotmail.com, cewn@terra.com, fabio_rpb@yahoo.com.br, gabicastrovini@gmail.com, augustopostal@gmail.com, canisso@illinois.edu, feijo.lorena.s@gmail.com, curciobruna@hotmail.com

INTRODUCTION

One third of deaths in foals during the neonatal period, including aborted fetuses, stillbirths and foals dying with < 1 day of age, occurs due to feto-placental infections (Barton 2006). Intensive care for critically ill foals is often very expensive and time consuming; therefore, the prognosis for survival is an important factor in the decision of the care given to these foals (Dembek et al. 2014). Clinical response to sepsis can be highly variable, depending on the duration and intensity of the septic insult. The initial response to infection should evoke non-specific signs of decreased activity, malaise, increased periods of recumbency, inability to track the mare, decreased frequency of nursing, and failure to gain weight. As the inflammatory response to infection intensifies, other signs of systemic disease appear including tachycardia, tachypnea, bilateral scleral injection, hyperemia of the coronary bands, unpigmented skin, and mucous membranes, petechial hemorrhages and edema. (Barton 2008). Thus, determining a diagnosis and establishing an accurate prognosis after birth or hospitalization is often difficult (Freeman & Paradis 1992).

Poor prognosis for survival, reduced performance at an expected level, and financial limitations are the main reasons why sick foals are euthanized (Dembek et al. 2014). Having access to more simple and cost-effective methods for field use to estimate the survival in newborn foals within the first few hours of life could be a valuable tool. Several studies have reported hematological and biochemical markers in septic foals (Gayle et al. 1998, Corley et al. 2005, Hollis et al. 2008, Wotman et al. 2009), but only a few have attempted to assess inflammatory and metabolic markers which may aid in predicting death in sick neonatal foals, if evaluated during the first few hours of life. The aim of the present study was to identify biochemical and inflammatory markers available shortly after foaling that were associated with non-survival and their usefulness as death predictors in a group of neonatal foals presenting high and low scores for sepsis. We hypothesized that non-surviving foals present marked changes in the lipid and energy metabolism and intense inflammatory response early after birth and that these markers can be used to predict death in foals during the first 48h of life.

MATERIALS AND METHODS

This experiment has been approved by the Ethical Committee on Animal Experimentation (CEEA) of the Universidade Federal de Pelotas (UFPel), Capão do Leão, RS, Brazil, (#4750;10/05/2010).

Experimental model. This study was conducted during three consecutive foaling seasons on animals kept at Palma Farm of UFPel, Capão do Leão, Rio Grande do Sul, Brazil. Foals born from mares with experimentally induced ascending placentitis and treated with various therapeutic combinations were used as the experimental model. Specific methodology and results about pregnancy outcomes of mares with induced placentitis were described in our parallel study (Curcio et al. 2017). In the present study we used a subset of twenty-eight foals, classified as septic or non-septic foals, according to the sepsis score system.

Foal evaluation. Foals were submitted to full physical examination immediately after birth, at 12h and then repeated daily for the first seven days post-delivery. Complete blood counts were performed at birth and 12, 24 and 48h. Foals that presented leukopenia (< 5000) or leukocytosis (> 14000) and clinical signs of sepsis, received Ampicillin (20 mg/kg, IV q 8h; Ampicilina Veterinaria, Vetnil), flunixin meglumine (0.5 mg/kg, IV q 12h; Desflan, Ouro Fino Saude Animal) during seven days, starting at 48h post birth. Intravenous fluid therapy with crystalloid solution (Lactated Ringer's Solution) was administered when clinical and laboratory findings indicated dehydration (Koterba 1990). Enteral feeding with mare's milk (5-10%/2h) was administered in foals that presented inadequate suckle reflex.

According to the sepsis score (Brewer & Koterba 1988), all foals were evaluated at 12h and classified as septic (n=9, i.e. foals with sepsis score $\geq$11) or non-septic (n=19 i.e. foals with a sepsis score <11). Foals were closely monitored during the first 30 days of life to determine the survival rate.

Blood sampling, hematological and biochemical evaluation. Jugular venipuncture was performed at 0h, (<15 min after foaling), and at 12, 24 and 48h of life. Blood samples were collected in: (a) tubes containing EDTA for complete blood count and fibrinogen; (b) plain tubes for serum levels measurement of triglycerides, total cholesterol, urea, creatinine, total plasma protein (TPP) and gamma-glutamiltransferase (GGT); (c) tubes containing sodium fluoride for glucose and lactate assessment; and (d) heparinized tubes for serum amyloid A (SSA) and alpha-fetoprotein (AFP) measurement. Immediately after collection, samples were centrifuged at 3000 g for 10 min, and serum and plasma aliquots were separated, placed in cryovials, and frozen at -20°C until further analysis.

Red and white blood cells counts were determined using a commercial cell analyzer (Sysmex pocH-1000VTM Hematology-Analyzer, Sysmex). Smears were prepared for differential leukocyte counts. Fibrinogen was determined by heat precipitation. Serum concentrations of triglyceride, cholesterol, urea, creatinine, total plasma protein, GGT, glucose and lactate were evaluated with spectrophotometric diagnostic kits (Labtest Diagnóstica SA, Lagoa Santa, Brazil) using an automatic biochemical system (Labmax Plenno, Labtest Diagnóstica SA, Lagoa Santa, Brazil). Plasma concentrations of AFP were determined using a heterologous commercial immunoassay (AFP Siemens Healthcare Diagnostics Tarrytown) on a chemiluminescence platform (Immulite 1000 platform; Siemens Healthcare Diagnostics Tarrytown) at the University of Illinois. Concentrations of SAA

were determined using a turbidometric immunoassay (Eiken Chemical Co, Ltd, Nagoya Naka Ward) at the University of Miami.

Statistical Analysis. Data were described using median and interquartile ranges (IR) to depict non-normally distributed data (biochemical and inflammatory markers) and, mean and standard error means and ranges for normally distributed data (gestational length and blood count), by Shapiro-Wilk normality test. Data without normal distribution were logarithmically transformed prior to analyses of independent samples T-test for groups according to survival (surviving and non-surviving). The interaction between the occurrence of sepsis and survival was evaluated by ANOVA two factors. For those statistically different markers, the cutoff values of risk factors for death were extracted from previous studies, as glucose (Pirrone et al. 2014) and lactate (Castagnetti et al. 2010), or based on means $\pm$ 1SD of the healthy control foals (SAA, AFP, triglycerides, total cholesterol, urea, creatinine, TPP, fibrinogen and GGT) of a parallel study (unpublished data of authors). The independent association between categorized markers with death was evaluated using the Fisher's exact test, followed by the odds ratio (OR) determination with 95% confidence interval. For those markers related (i.e. $P<0.05$) to death, their usefulness as prognostic predictors of death were evaluated by estimations of sensibility and specificity, positive predictive value (PPV), negative predictive value (NPV), and positive likelihood ratio (LR+). The statistical analysis was conducted using commercial software (SPSS 20, IBM Corporation). Statistical significance was set as $P<0.05$.

RESULTS

The SAA concentration was not different between survival groups during first 48h of life. At birth, AFP was higher in non-surviving foals compared with surviving group (Table 1). Moreover, non-surviving foals presented hypoglycemia (41 mg/dL, IR: 24–66 mg/dL) and hypercholesterolemia (400 mg/dL, IR: 225–518 mg/dL), higher concentrations of triglycerides (30 mg/dL, IR: 20–62.5 mg/dL) and lower levels of GGT (25 UI/L, IR: 15.5–39.5 UI/L) compared with surviving group at birth ($P<0.05$). Lower glucose concentrations were maintained during 24 and 48h of life in foals that did not survive ($P<0.05$). Higher total cholesterol concentrations and lower levels of GGT were observed in the non-surviving group at 12, 24 and 48h of life, differing from the surviving group ($P<0.001$). Non-surviving foals presented marked hyperlactatemia at 12h and greater plasma levels were maintained until 24h. At 48h of life, urea and creatinine concentrations were different between groups ($P<0.001$) (Fig 1 and 2). At birth, 12 and 48h fibrinogen concentrations did not differ between groups. At 24h, the fibrinogen concentration was higher in non-surviving foals. TPP was lower in non-surviving foals at 12 and 48 h. At birth, foals that presented glucose concentrations <42mg/dL were 21.25 times more likely to die. In addition, foals with total cholesterol concentrations >185 mg/dL and GGT activity <26 UI/L, were 17.33 times more likely to die. At 12h, foals with triglycerides >64 mg/dL and GGT activity <36 UI/L were 36 and 10.67 more likely to die, respectively (Table 2). Values of LR+, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for death for each parameter at birth, 12h and 24h after birth are showed in Table 3.

At birth, plasma glucose concentration (<42 mg/dL) was the most specific parameter to predict death with high PPV (83.3%). At 12h of life, the best parameter for detection of non-survival was the measurement of serum triglycerides (>64 mg/dL). Also, total cholesterol (>185 mg/dL) and GGT (>26 UI/L) were the most sensitive (88.83%) serum parameters to predict death at birth. After 24h, these markers did not show be good predictors of death.

Foal survival rate evaluated during the first 30 days showed that 19 (67.86%) foals survived and 9 (32.14%) did not survive. The non-surviving group included foals that died (n=4) and those that were euthanized due to extreme deteriorating conditions as a result of a lack of response to treatment (n=5). All of the markers in Table 3 showed an interaction between surviving and sepsis ($P<0.05$), except for creatinine and GGT. Rate of survival was lower in septic foals (n=2/9; 22.22%) than in non-septic foals (n=17/19; 89.47%) ($P<0.001$). Two non-surviving foals of non-septic group did not present clinical signs during the first week of life, but died due to diarrhea and arthritis at 29 and 25 days of life, respectively. The septic non-surviving group included foals that presented several clinical disorders during the first hours after birth such as weakness, inability to stand and nurse, reduced tolerance to oral feeding and increased susceptibility to hypothermia.

Results of hematological parameters are showed in Table 4. There were no differences in complete red blood counts among groups during the first 48h. In the WBC count, an increase in band neutrophils was observed at 12h in non-surviving foals. At 48h, white blood cells in the non-surviving group were higher than in the surviving group.

DISCUSSION

The present study showed that compromised non-surviving foals presented several metabolic and inflammatory changes characterized by marked hypoglycemia, hypertriglyceridemia, hypercholesterolemia and elevated levels of AFP after birth. However, as expected, these are related with sepsis and a variable clinical response.

At birth, non-surviving foals presented increased concentrations of AFP compared to surviving group. Foals which became septic in the first week of life had increased plasma concentrations of alpha-fetoprotein at 12-24h post-foaling (Prell et al. 2016), whereas to the author's knowledge this is the first study to compare values of AFP between surviving and non-surviving foals. In horses, Alpha-fetoprotein (AFP) is a protein produced by the fetal liver and that continues to be highly expressed during early pregnancy by the fetus (Simpson et al. 2000) and into the third trimester of gestation (Bader et al. 2004, Canisso et al. 2014), is present in the fetal fluids and is increased in plasma of mares with experimentally induced ascending placentitis (Canisso et al. 2015). Further, alpha-fetoprotein kinetics in foals is not completely known; in humans AFP half-life is estimated to be approximately 5 days after delivery (Caballero et al. 1977, Bader et al. 2004).

SAA did not present differences between surviving and non-surviving groups. SAA is a major acute phase protein in horses (Pepys et al.1989), and its concentration may increase mildly in the first few days after parturition in healthy neonatal foals (Nunokawa et al. 1993, Duggan et al. 2007). SAA and other proteins that act similarly increase rapidly in response to bacterial and other infections, and the levels reached generally reflect the extent and activity of disease (Chavatte et al. 1992), which demonstrate that SAA is a good marker for sepsis (Stoneham et al. 2001, Paltrinieri et al. 2008), but not for survival. SAA might raise values in several noninfectious conditions in neonate foals, although not as much in infections conditions (Chavatte et al. 1992). This justifies the lack of difference according to the survival evaluation in the present study, where some foals presented disorders such as prematurity, neonatal maladjustment syndrome and weakness resulting in mild increases of SAA concentrations.

Regarding to plasma glucose, non-surviving foals presented hypoglycemia at birth. Particularly, foals that presented glucose concentrations < 41mg/dL were 21.25 times more likely to die. In our study, hypoglycemia at birth was associated with poor prognosis of survival in neonate foals, corroborating with what is described in septic foals after hospital admission, despite nutritional and fluid support (Gayle et al. 1998, Hollis et al. 2008, Barsnick et al. 2011, Armengou et. al. 2013). In humans, hypoglycemia has been reported in critically neonates and is associated with non-survival as well (Wintergest et al. 2006). The mechanism of hypoglycemia, possibly, is a result of the low glycogen reserves and poor nursing behavior in these animals, or secondary to increased catabolism (Hollis et al. 2008) and increased energy consumption by the proliferating microorganisms (Barton 2008). Also, hypoglycemia is more common in neonatal foals than in adult horses due to foals born with very low fat and glycogen stores (Fowden et al. 1991).

The hypertriglyceridemia observed in non-surviving foals during the first 12h of life can be a result of lipid mobilization produced during the sepsis process. Lipid mobilization is controlled mostly by epinephrine and glucagon, which are secreted in response to low blood glucose concentrations (Gonzáles & Silva2006). In critically ill foals, changes in energy metabolism are characterized by hypoglycemia and hypertriglyceridemia (Barsnick et al. 2011, Barsnick & Toribio 2011). Since carbohydrate stores in foals are limited, they are quickly depleted, leading to a mobilization of fat depots (Barsnick & Toribio 2011). Ultimately, when the liver is not able to maintain glucose production from fatty acids, blood triglycerides concentrations increases (Barsnick & Toribio, 2011). In our study, non-surviving foals demonstrated triglycerides concentrations > 100mg/dL at 12 and 24h of life, what is mean that elevated triglycerides concentrations can be associated with non-survival. Myers et al. (2009) reported similar results; in their study, triglyceride concentrations >200 mg/dL at admission were a negative prognostic indicator in ill foals receiving parenteral nutrition. Other studies have measured triglyceride levels in sick foals and concluded serum concentrations in non-surviving foals were higher than in survivors (Armengou et al. 2013, Berryhil et al. 2017).

In foals, the nature of changes in the lipid profile, particularly total cholesterol concentrations under physiological and pathological conditions, is controversial. According to Bauer (1990), total cholesterol concentrations may be remarkably elevated during the first 2 weeks of life in foals. In our study we observed high cholesterol concentrations in non-surviving foals at birth. These results may be associated with immaturity and hepatic insufficiency during the sepsis process (Kulisa et al. 2005) which can lead to a cholestasis and subsequent blood cholesterol increases. The present study has proved that serum total cholesterol is a good marker to predict death at birth.

A noteworthy aspect of this study was the assessment of lactate at birth and sequentially during the first 48 hours. At birth, both groups presented hyperlactatemia, with no difference between groups. Blood lactate concentrations are higher in normal neonatal foals than normal adult horses for the first 24 to 72h (Kitchen & Rossdale 1975, Magdesian 2003). Furthermore, lactate is an important carbohydrate substrate in the fetus and this might contribute to the normal neonatal hyperlactatemia (Tennent-Brown 2014). Elevated concentrations of plasma lactate measured at birth could be due to cortisol and catecholamine release or to the physiologic hypoxia during the birth process (Castagenetti et al. 2010). Time-dependent changes in venous blood lactate were different between surviving and non-surviving foals with a reduced lactate clearance in non-survivors. In healthy newborn foals, the lactate concentration is age-dependent and healthy foals should be able to reduce the blood levels during the first 24h to values of up to 2.1 mmol/L (Castagnetti et al. 2010). In our study, surviving

foals presented a reduction of plasma values to less than 2.1 mmol/L, while non-surviving foals maintained high plasma lactate concentrations.

No difference was observed in urea and creatinine between surviving and non-surviving groups until 24 hours. However, elevated creatinine concentrations in non-surviving foals at 48h of life recorded in our study was not a specific marker for survival. Critically ill foals can present increased concentrations of creatinine due to the negative energy balance, as well as in conditions of renal disorders (Axon & Palmer 2008). Also, elevated serum creatinine may be a transient finding in asphyxiated foals or foals delivered from mares with placentitis (Schott 2011).

During the first 24h of life, non-surviving foals showed lower GGT activity compared with surviving foals. The activity of GGT is not fully known in foals. Lower activity of GGT in the non-surviving group is similar to results obtained by Armengou et al. (2013) that described for the first time the activity of GGT in septic and non-surviving foals. When sepsis is present, intrahepatic cholestasis may be associated with both, low or high GGT activity (Oswari et al. 2013). Serum GGT activity was correlated with non-survival at birth and 12h and demonstrated to be a more sensitive marker of non-survival at birth. Thus, we encourage the use GGT levels to make an accurate prognosis in critically ill foals.

In neonatal equine practice, research about early markers that present high sensibility and that can be easily determined in clinical routine is crucial. The clinical importance of this study is in the early detection of foals that present a poor prognosis, and the viability of treatment for these foals. This study presented some limitations such as a small number of foals, and the maturity degree presented by some foals, which can interfere in the interpretation of results.

CONCLUSION

Non-surviving foals presented several metabolic and inflammatory responses characterized by hypoglycemia, hypercholesterolemia, lower GGT activity and elevated levels of AFP at birth. Measurement of glucose, triglycerides, and GGT activity at birth showed to be good markers to predict non-survival in a group of foals born from mares with placentitis, presenting high sensibility and specificity. These blood markers can be used to assist in making decisions about the chances of survival of foals in clinical routine.

Acknowledgments. - Our thanks to Ruth Patten for the English spelling check.

Conflict of interest statement. -The authors have no competing interests.

REFERENCES

Armengou L., Cunilleras E.J., Ríos J., Cesarini C., Viu J., Monreal L. 2013. Metabolic and endocrine profiles in sick neonatal foals are related to survival. J. Vet. Intern. Med. 27:567–575.

Axon J.E., Palmer J.E. 2008. Clinical pathology of the foal. Vet. Clin. North Am. Equine Pract. 24:375–385.

Bader D., Riskin A., Vafsi O., Tamir A., Peskin B., Israel N., Merksamer R., Dar H., David M. 2004. Alpha-fetoprotein in the early neonatal period - a large study and review of the literature. Clin. Chim. Acta. 349:15–23.

Barsnick R.J., Toribio R.E. 2011. Endocrinology of the equine neonate energy metabolism in health and critical illness. Vet. Clin. North Am. Equine Pract. 27:49–58.

Barsnick R.J., Hurcombe S.D., Smith P.A., Slovis N.M., Sprayberry K.A., Saville W.J.A., Toribio R.E. 2011. Insulin, glucagon, and leptin in critically ill foals. J. Vet. Intern. Med. 25:123–131.

Barton, M.H. 2006. Septicemia, p.75-97. In: Paradis, M.R. Equine Neonatal Medicine: A Case Based Approach, Elsevier, Philadelphia.

Barton M.H. 2008. Early recognition of the septicemic foal. Proceedings of the American Association of Equine Practitioners (AAEP) - Focus Meeting First Year of Life, Ausin, Texas, p 101-109.

Bauer, J.E. 1990. Normal blood chemistry, p.602-614. In: Koterba A.M., Drummond W.H. & Kosch P.C. (Eds), Equine Clinical Neonatology, Lea & Febiger, Philadelphia.

Berryhil E.H., Magdesian K.G., Kass P.H., Edman J.E. 2017. Triglyceride concentrations in neonatal foals: Serial measurement and effects of age and illness. Vet. J. 227:23–29.

Brewer B.D, Koterba A.M. 1988. Development of a scoring system for the early diagnosis of equine neonatal sepsis. Equine Vet J. 20:18–22.

Caballero C., Vekemans M., Lopez del Campo J.G., Robyn C. 1977. Serum alpha-fetoprotein in adults, in women during pregnancy, in children at birth, and during the first week of life: a sex difference. Am. J. Clin. Exp. Obstet. Gynecol. 127(4):384–389.

Canisso I.F., Ball B.A., Cray C., Williams N.M., Scogin K.E., Davolli G.M., Squires E.L., Troedsson M.H. 2014. Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. Am. J. Reprod. Immunol. 72:376–385.

Canisso I.F., Ball B.A., Scogin K.E., Squires E.L., Williams N.M., Troedsson M.H. 2015. Alpha-fetoprotein is present in the fetal fluids and is increased in plasma of mares with experimentally induced ascending placentitis. Anim. Reprod. Sci. 154:48–55.

Castagnetti C., Pirrone A., Mariella J., Mari G. 2010. Venous blood lactate evaluation in equine neonatal intensive care. Theriogenology. 73:343–357.

Chavatte P.M., Pepys M.B., Roberts B., Ousey J.C., McGladdery A.J., Rossdale P.D. 1992 Measurement of serum amyloid A protein (SAA) as an aid to differential diagnosis of infection in newborn foals. Eq. Infec. Dis. 33–38.

Corley K.T.T., Donaldson L.L., Furr M.O. 2005 Arterial lactate concentration, hospital survival, sepsis and SIRS in critically ill neonatal foals. Equine Vet. J. 37(1):53–59.

Curcio B.R., Canisso I.F., Pazinato F.M., Borba L.A., Feijo L.S., Muller V., Finger I.S., Toribio R.E., Nogueira C.E.W. 2017. Estradiol cypionate aided treatment for experimentally induced ascending placentitis in mares. Theriogenology. 102:98–107.

Dembek K.A., Hurcombe S.D., Frazer M.L., Morresey P.R., Toribio R.E. 2014. Development of a Likelihood of Survival Scoring System for Hospitalized Equine Neonates Using Generalized Boosted Regression Modeling. PLoS ONE 9(10): e109212.

Duggan V.E., Holyoak G.R., MacAllister C.G., Confer A.W. 2007. Influence of induction of parturition on the neonatal acute phase response in foals. Theriogenology. 67:372–381.

Fowden A.L., Mundy L., Ousey J.C., McGladdery A., Silver M. 1991. Tissue glycogen and glucose 6-phosphate levels in the fetal and newborn foals. J. Reprod. Fertil. Suppl. 44:537–42.

Freeman, L., Paradis, M.R., 1992. Evaluating the effectiveness of equine neonatal care. Vet. Med. 87, 921–926.

Gayle J.M., Cohen N.D., Chaffin M.K. 1998. Factors associated with survival in septicemic foals: 65 cases (1988–1995). J. Vet. Intern. Med. 12, 140–146.

Gonzáles F.H.D., Silva S.C. 2006. Introdução à bioquímica veterinária. 2ºed. Editora da UFGRS, Porto Alegre. 364p.

Hollis A.R., Furr M.O., Magdesian K.G., Axon J.E., Ludlow V., Boston R.C., Corley K.T.T. 2008. Blood glucose concentrations in critically ill neonatal foals. J. Vet. Intern. Med. 22:1223–1227.

Kitchen H., Rossdale P.D. 1975. Metabolic profiles of newborn foals. J. Reprod. Fert., Suppl. 23, 705-707.

Koterba A.M. 1990. Physical examination, 71–85. In: Koterba A.M., Drummond W.H. & Kosch P.C. (Eds), Equine Clinical Neonatology, Lea & Febiger, Philadelphia.

Kulisa M, Dlugosz B, Luszczynski J., Pieszka M., Sicinska R. 2005. Cholesterol level in serum in a thoroughbred foals bred in two different studs. J. Anim. Sci. Biotechnol. 21:77–80.

Magdesian G.K. 2003. Blood lactate levels in neonatal foals: normal values and temporal effects in the post-partum period. 9th International Veterinary Emergency and Critical Care Symposium, New Orleans, Louisiana p.174. (Abstract).

Myers C.J., Magdesian K.G., Kass P.H., Madigan J.E., Rhodes D.M., Marks S.L. 2009. Parenteral nutrition in neonatal foals: Clinical description, complications and outcome in 53 foals (1995–2005). Vet. J., 181:137–144.

Nunokawa Y., Fujinaga T., Taira T., Okumura M., Yamashita K., Tsunoda N., Hagio M. 1993. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. J. Vet. Med. Sci. 55(6):1011–1016.

Oswari H, Widjaja RK, Rohsiswatmo R, Cleghorn G. 2013. Prognostic value of biochemical liver parameters in neonatal sepsis-associated cholestasis. J. Paediatr. Child. Health. 49 (1):6–11.

Paltrinieri S., Giordano A., Villani M., Manfrin M., Panzani S., Veronesi M.C. 2008. Influence of age and foaling on plasma protein electrophoresis and serum amyloid A and their possible role as markers of equine neonatal septicaemia. Vet. J. 176:393–396.

Pepys M.B., Baltz M.L., Tennent G.A., Kent J., Ousey J., Rossdale P.D. 1989. Serum amyloid A (SAA) in horses: objective measurement of the acute phase response. Equine Vet. J. 21(2):106 –109.

Pirrone A., Antonelli C., Mariella J., Castagnetti C. 2014. Gross placental morphology and foal serum biochemistry as predictors of foal health. Theriogenology. 81:1293–1299.

Prell M., Canisso I.F., Schnobrich M., Riddle T., Ellerbrock R.E., Wilkins P. 2016. Alpha-fetoprotein as a marker for equine neonatal disease Proceedings of the Society for Theriogenology Annual Conference 2016, Asheville, North Carolina. (Abtract).

Schott H.C. 2011. Review of azotemia in foals. Proceedings of the 57th Annual Convention of the American Association of Equine Practitioners (AAEP), San Antonio, Texas, p. 328-324.

Simpson K.S., Adams M.H., Behrendt-Adam C.Y., Baker C.B., McDowell K.J. 2000. Differential gene expression in day 12 and day 15 equine conceptuses. J. Reprod. Fertil. Suppl. 56:539-547.

Stoneham S.J., Palmer L., Cash R., Rossdale P.D. 2001. Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. Equine Vet. J. 33:599–603.

Tennent-Brown B.S. 2014. Blood Lactate Measurement and Interpretation in Critically Ill Equine Adults and Neonates. Vet Clin. Equine. 30(2):399-413.

Wintergerst K.A., Buckingham B., Gandrud L., Wong B.J., Kache S., Wilson D.M. 2006. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care Unit. Pediatrics. 118 (1):173-179.

Wotman K., Wilkins P.A., Palmer J.E., Boston R.C. 2009. Association of blood lactate concentration and outcome in foals. J. Vet. Intern. Med. 23:598–605.

Figure legends.

Fig.1. Interquartile ranges of the medians obtained for glucose, lactate, triglycerides, total cholesterol, urea and creatinine over time (0 h, 12 h, 24 h, 48 h) in neonatal foals presenting high and low scores for sepsis according to survival (i.e. surviving and non-surviving).

Fig.2. Interquartile ranges of the medians obtained for total plasma protein (TPP), fibrinogen and gamma-glutamil-transferase (GGT) over time (0 h, 12 h, 24 h, 48 h) in neonatal foals presenting high and low scores for sepsis according to survival (i.e. surviving and non-surviving).

Table 1. Serum amyloid A (SAA) and alpha-fetoprotein (AFP) concentrations in neonatal foals at birth (0), 24h, 48h according to survival.

Time	Variables	Groups according to Survival	
		Surviving (n=19)	Non-surviving (n=9)
0h	SAA (mg/L)	0.2 (0-0.8)	0.5 (0-302)
	AFP (µg/dL)	1840 (293-2370)[b]	2470 (1650.5-2845)[a]
24h	SAA (mg/L)	22.4 (0.3-97.2)	14 (630.8- 879.5)
	AFP (µg/dL)	289 (249.5-2137.5)	2210 (288.5-2445)
48h	SAA (mg/L)	15.13 (0.6-163.6)	54 (3.1- 605.5)
	AFP (µg/dL)	283 (262.5-1625)	1080 (262-2290)

[a/b] Different superscript letters in rows indicate a statistical difference between groups (P<0.05). Data are presented as median and interquartile ranges.

Table 2. Description of categorized biochemical markers related to death within the first 30 days of life in 28 foals born from mares with experimentally induced ascending placentitis and results of the univariate analysis (P ≤ 0.05).

Time	Variable	Death	Survival	Total	Positivity	Fisher's P value	OR	95% CI
Birth (0h)	AFP							
	> 271µg/dL	9	16	25	36	0.530	-	-
	≤ 271µg/dL	0	3	3	0			
	Glucose							
	< 42mg/dL	5	1	6	83.3	0.008	21.2	(1.9 - 236)
	≥ 42mg/dL	4	17	21	19			
	Triglycerides							
	> 39mg/dL	4	3	7	57.1	0.165	-	-
	≤39mg/dL	5	16	21	23.8			
	Cholesterol							
	> 185 mg/dL	8	6	14	57.1	0.013	17.3	(1.7 - 171.6)
	≤ 185 mg/dL	1	13	14	7.1			
	GGT							
	< 26 UI/L	8	6	14	57.1	0.013	17.3	(1.7- 171.6)
	≥ 26 UI/L	1	13	14	7.1			
12 hours	Lactate							
	> 2.74mmol/L	8	12	20	40	0.214	-	-
	≤ 2.74mmol/L	1	7	8	12.5			
	Triglycerides							
	> 64mg/dL	6	1	7	85.7	0.001	36	(3.1 - 414.8)
	≤ 64mg/dL	3	18	21	14.3			
	Cholesterol							
	> 206 mg/dL	7	7	14	50	0.103	-	-
	≤ 206 mg/dL	2	12	14	14.3			
	PPT							
	< 6.3 mg/dL	6	8	14	42.9	0.209	-	-
	≥ 6.3 mg/dL	2	11	13	15.4			
	GGT							
	< 33 UI/L	6	3	9	66.7	0.013	10.7	(1.7 - 68.2)
	≥ 33 UI/L	3	16	19	15.8			
	Lactate							
	> 2.1 mmol/L	6	12	18	33.3	0.676	-	-
	≤ 2.1 mmol/L	2	7	9	22.2			
	Cholesterol							
	> 226 mg/dL	6	7	13	46.2	0.103	-	-
	≤ 226 mg/dL	2	12	14	14.3			

24 hours	Creatinine							
	> 1.2 mg/dL	6	9	15	40	0.236	-	-
	≤ 1.2 mg/dL	2	10	12	16.7			
	Fibrinogen							
	> 400 mg/dL	5	8	13	38.5	0.673	-	-
	≤400 mg/dL	3	10	13	23.1			
	GGT							
	< 39 UI/L	8	14	22	36.4	0.280	-	-
	≥ 39 UI/L	0	5	5	0			
48 hours	Cholesterol							
	> 212 mg/dL	6	9	15	40	0.179	-	-
	≤ 212 mg/dL	1	9	10	10			
	Urea							
	> 27 mg/dL	5	9	14	35.7	0.407	-	-
	≤ 27 mg/dL	2	9	11	18.2			
	Creatinine							
	> 1.24 mg/dL	5	7	12	41.7	0.202	-	-
	≤ 1.24mg/dL	2	11	13	15.4			
	TPP							
	< 5.4 mg/dL	5	4	9	55.6	0.028	9.4	(1.3 - 67.6)
	≥ 5.4 mg/dL	2	15	17	11.8			
	GGT							
	< 32 UI/L	6	9	15	40	0.179	-	-
	≥ 32 UI/L	1	9	10	10			

OR = Odds Ratio, AFP = Alpha-fetoprotein, GGT = Gamma-glutamyl transferase, TPP = Total Plasma Protein

Table 3. LR+, sensitivity, specificity, PPV and P-value of variables useful as prognostic predictors.

Time	Parameter	LR+	Sensitivity*	Specificity*	PPV*	NPV*	Fisher's P-value
0h	Glucose< 42 mg/dL	9.9	55.56	94.44	83.33	80.95	0.008
	Cholesterol> 185 mg/dL	2.8	88.83	68.42	57.14	92.86	0.013
	GGT < 26 UI/L	2.8	88.83	68.42	57.14	92.86	0.013
12 h	Triglycerides> 64 mg/dL	12.7	66.67	94.74	85.71	85.71	0.001
	GGT < 40 UI/L	6.3	66.67	84.21	66.67	84.21	0.013
48h	PPT < 5.4 mg/dL	3.4	71.43	78.95	55.56	88.24	0.028

LR = Likelihood ratio, PPV = Positive predictive value, NPV = Negative predictive value. * Data are expressed as %.

Table 4. Mean ± Standard Error (SE) of hematological parameters of foals at birth (0), 12, 24, 48 hours according to survival.

Time	Parameter	Surviving (*n* = 19)	Non-surviving (*n* = 9)
0h	Red blood cells (10^6/mL)	11.3 ± 0.3	10.7 ± 0.9
	Hemoglobin (g/dL)	13.8 ± 0.3	14.4 ±0.5
	Packed cell volume (%)	45.7 ± 0.9	46.5 ± 2.0
	Mean cell volume (fL)	40.5 ± 0.6	46.7 ± 5.5
	Mean cell hemoglobin concentration (%)	30.1 ± 0.3	31.1 ± 0.5
	Platelet count (10^3/mL)	342.5 ± 22.2	265.4 ± 34.2
	White blood cells (µL)	7157.9 ± 515	6466.7 ± 1718.5
	Neutrophils (µL)	5056.2 ± 482.5	4403 ± 1523.6
	Bands (µL)	3.7± 3.7	7 ± 7
	Lymphocytes (µL)	1966 ± 164.5	1930.1 ± 251.2
	Monocytes (µL)	98.2 ± 23.2	111.2 ± 35.8
	Eosinophils (µL)	33.9 ± 15.2	15.3 ± 15.3
12h	Red blood cells (10^6/mL)	9.5 ± 0.3	8.6 ± 0.6
	Hemoglobin (g/dL)	11.5 ± 0.3	11.42 ± 0.6
	Packed cell volume (%)	37.7 ± 1.0	36.3 ± 1.7
	Mean cell volume (fL)	39.8 ± 0.5	42.7 ± 1.8
	Mean cell hemoglobin concentration (%)	30.4 ± 0.2	31.5 ± 0.6
	Platelet count (10^3/mL)	273.8 ± 18.9	244.2 ± 29.6
	White blood cells (µL)	9415.8 ± 746.2	8275 ± 3097.3
	Neutrophils (µL)	7674.8 ± 744	6601.3 ± 2841
	Bands (µL)	0^b	12.25 ± 7.0^a
	Lymphocytes (µL)	1636.7 ± 177.1	1532.3 ± 319.8
	Monocytes (µL)	82.63 ± 19.5	129.2 ± 60.5
	Eosinophils (µL)	20.1 ± 9.3^a	0^b
24h	Red blood cells (10^6/mL)	9.5 ± 0.3	8.48 ± 0.6
	Hemoglobin (g/dL)	11.4 ± 0.3	10.9 ± 0.8
	Packed cell volume (%)	36.9 ± 1.0	34.7 ± 2.5
	Mean cell volume (fL)	39.1 ± 0.5	41.7 ± 1.7
	Mean cell hemoglobin concentration (%)	30.9 ± 0.3	31.5 ± 0.4
	Platelet count (10^3/mL)	263.8 ± 16.4	250.3 ± 46.2
	White blood cells (µL)	8010.5 ± 963.5	11814 ± 3359.5
	Neutrophils (µL)	5971.1 ± 947.3	9881.1 ± 3356.4
	Bands (µL)	6.7 ± 6.7	42.1 ± 42.1
	Lymphocytes (µL)	1808.2 ± 253.3	1741 ± 326.9
	Monocytes (µL)	146.5 ± 54.9	74.7 ± 36.6
	Eosinophils (µL)	48.1 ± 13.2	75.3 ± 62.1
48h	Red blood cells (10^6/mL)	9.1 ± 0.3	8.8 ± 0.5
	Hemoglobin (g/dL)	11.1 ± 0.3	10.7 ± 0.5^b
	Packed cell volume (%)	35.1 ± 1.2	34.1 ± 4.7
	Mean cell volume (fL)	38.5 ± 0.7	38.7 ± 0.6
	Mean cell hemoglobin concentration (%)	31.7 ± 0.3	31.3 ± 0.3
	Platelet count (10^3/mL)	287.6 ± 19.4	294.2 ± 30.8
	White blood cells (µL)	6942.4 ± 861.3^b	12550 ± 6700^a
	Neutrophils (µL)	5472.4 ± 824.9^b	10569 ± 4891^a
	Bands (µL)	4.7 ± 4.7^a	0^b
	Lymphocytes (µL)	1162.2 ± 173.8	1748.7 ± 339.6
	Monocytes (µL)	65.4± 20.4	232.8 ± 86.3
	Eosinophils (µL)	11.8 ± 6.8^a	0^b

$^{a/b}$ Different superscript letters in rows indicate a statistical difference between groups ($P < 0.05$).

Figures

Figure 1.

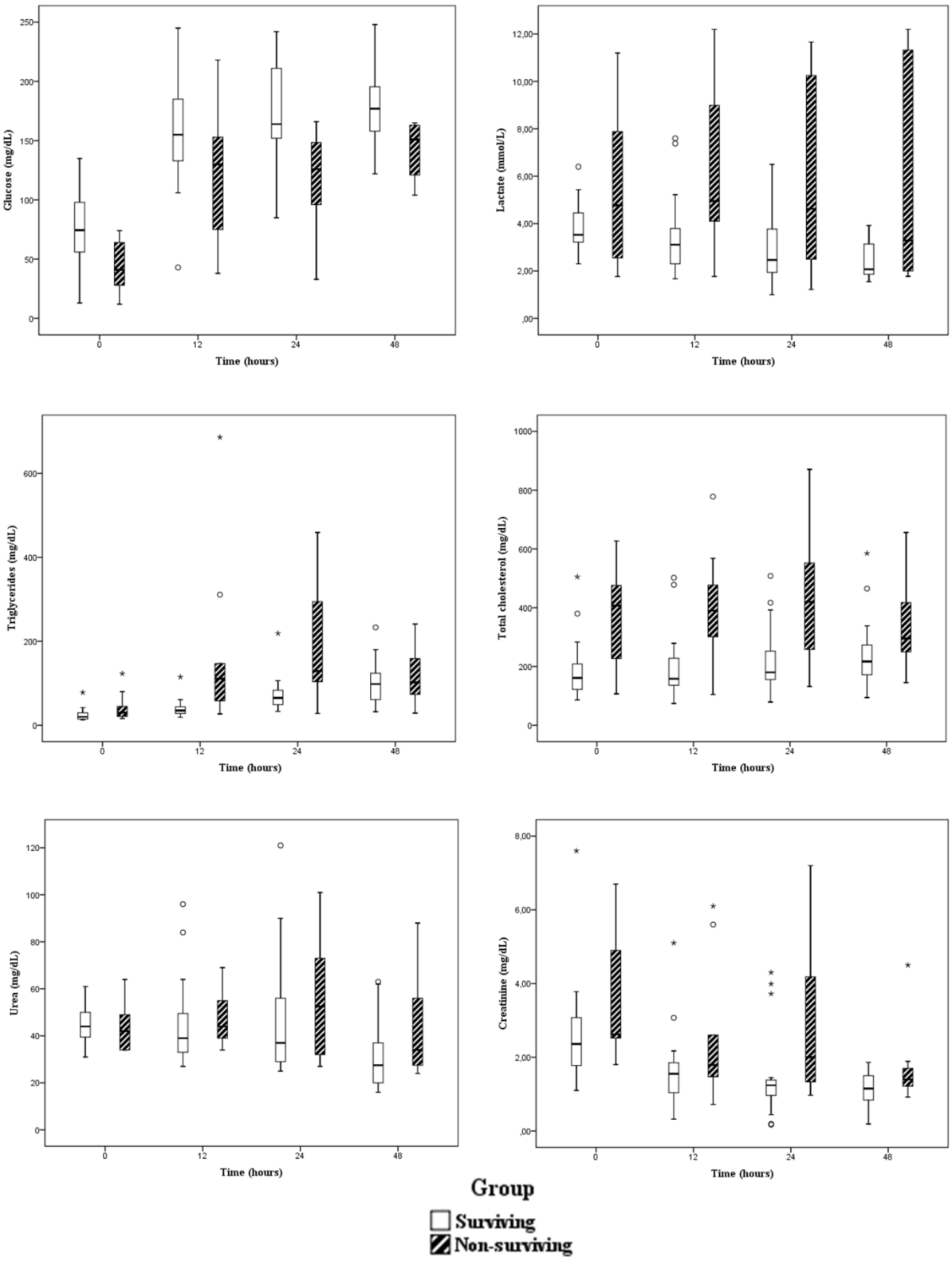
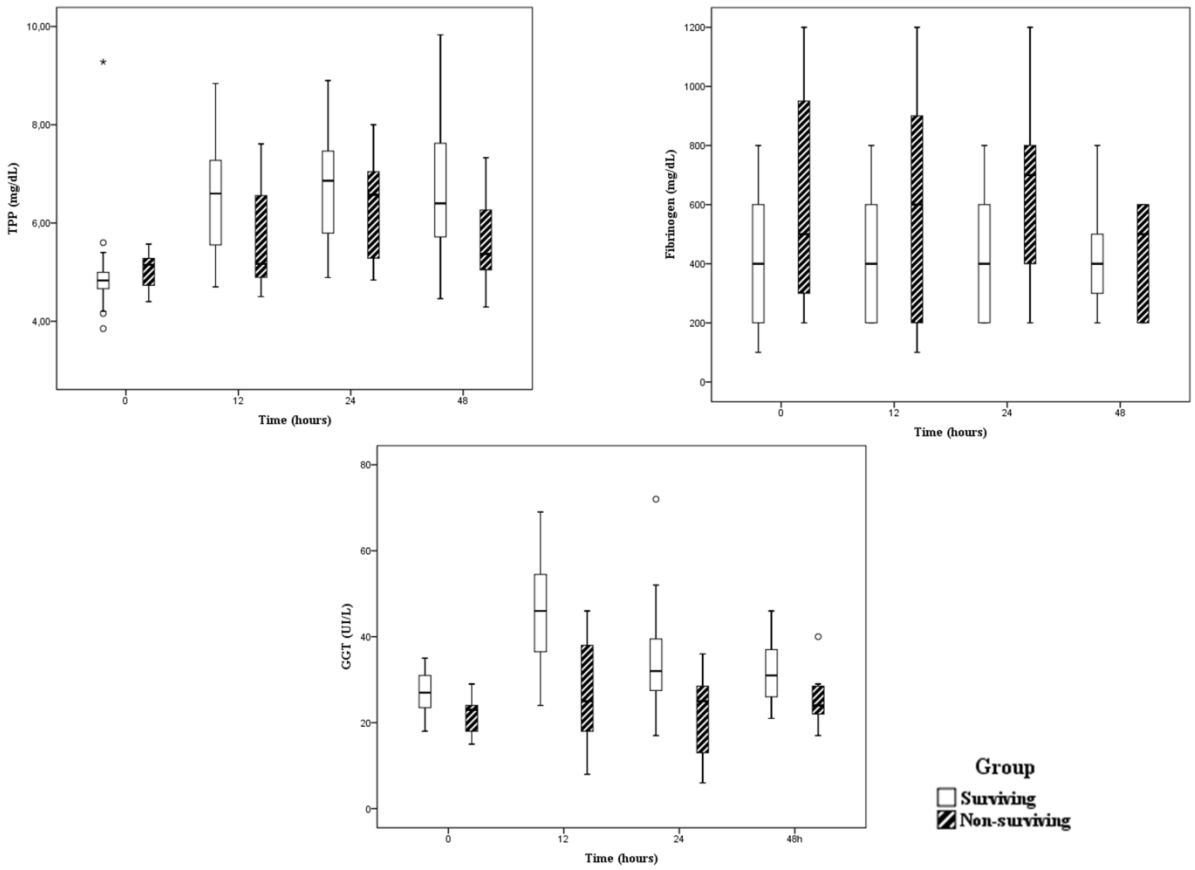


Figure 2.


TPP (mg/dL)
10,00
8,00
6,00
4,00
0
12
24
48
Time (hours)
Fibrinogen (mg/dL)
1200
1000
800
600
400
200
0
0
12
24
48
Time (hours)
GGT (UI/L)
80
60
40
20
0
0
12
24
48h
Time (hours)
Group
Surviving
Non-surviving

4 Considerações Finais

Após a realização deste estudo podemos observar que potros que sofrem algum tipo de infecção ainda na vida intrauterina e que apresentam sepse ao nascimento apresentam diversas desordens relacionadas ao metabolismo energético, lipídico e intensa resposta inflamatória, evidenciada pelas alterações observadas nos marcadores avaliados neste estudo, e que a magnitude destas alterações pode estar relacionada com a não sobrevivência destes potros. Alterações como hipoglicemia, hipercolesterolemia, hiperlactatemia, baixa atividade da enzima GGT e elevações nas proteínas de fase aguda SAA e AFP, observadas durante as primeiras 48h de vida, demostraram ser úteis na detecção de sepse e a mensuração sequencial destes marcadores permite quantificar o risco de desenvolvimento de sepse.

Da mesma maneira, potros não sobreviventes demonstraram marcadas alterações no metabolismo energético e lipídico, com manutenção de hipoglicemia, elevadas concentrações de colesterol e triglicerídeos e AFP. Foi possível observar também que, potros sépticos apresentam maior risco de não sobrevivência em relação a potros com outras alterações clínicas. Assim, ressalta-se a importância da avaliação bioquímica e do monitoramento durante as primeiras 48 horas de vida em potros nascidos de éguas com placentite, visando o reconhecimento precoce de animais que apresentam septicemia e que necessitam de tratamento e monitoramento intensivo. Como conclusão, temos que, a utilização de marcadores bioquímicos, como glicose, lactato, colesterol, triglicerídeos e AFP podem auxiliar na detecção precoce do processo séptico e no estabelecimento de terapêutica adequada, visando um incremento na taxa de sobrevivência, além de permitir o estabelecimento de um prognóstico e viabilidade de tratamento em potros em estado crítico.

Referências

ARMENGOU, L.; CUNILLERAS, J. E.; RÍOS, J.; CESARINI, C.; VIU, J.; MONREAL, L. Metabolic and endocrine profiles in sick neonatal foals are related to survival. **Journal of Veterinary Internal Medicine**, v.27, n.3, p.567-575, 2013.

AXON, J. E. Critical Care. In: McKINNON, A. O.; SQUIRES, E. L.; VAALA, W. E.; VARNER, D. D. **Equine Reproduction**. 2º ed. Oxford: Willey-Blackwell, 2011. p.167-176.

AXON, J. E.; PALMER, J. E. Clinical pathology of the foal. **Veterinary Clinics of North America: Equine Practice**, v.24, n.2, p.357-85, 2008.

BADER, D.; RISKIN, A.; VAFSI, O.; TAMIR, A.; PESKIN, B.; ISRAEL, N.; MERKSAMER, R.; DAR, H.; DAVID, M. Alpha-fetoprotein in the early neonatal period - a large study and review of the literature. **Clinica Chimica Acta**, v.349,p.15-23, 2004.

BAILEY, C. S.; MACPHERSON, M. L.; POZOR, M. A.; TROEDSSON, M. H. T.; BENSON, S.; GUIGUÈRE, S.; SANCHEZ, L. C.; LEBLANC, M. M.; VICKROY, T. W. Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. **Theriogenology**, v.74, n.3, p.402-412, 2010.

BAIN, F. T. Management of the foal from the mare with placentitis: a clinician's approach. **Proceedings of the Annual Convention of the American Association of Equine Practitioners (Denver, USA)**, 2004. p.1419-1204.

BARR, B. S. The outcome of foals born to mares treated for placentitis. **Proceedings of a Workshop on. Uterine infections in mares and women: a comparative study II (South Carolina, USA)**, 2005. p.49-50.

BARSNICK, R. J. I. M.; TORIBIO, R. E. Endocrinology of the equine neonate energy metabolism in health and critical illness. **Veterinary Clinics of North America: Equine Practice**, v.27, n.1, p.49-58, 2011.

BARSNICK, R. J. I. M.; HURCOMBE, S. D.; SMITH, P. A.; SLOVIS, N. M.; SPRAYBERRY, K. A.; SAVILLE, W. J. A.; TORIBIO, R. E. Insulin, glucagon and leptin in critically ill foals. **Journal of Veterinary Internal Medicine**, v.25, n.1, p.123-131, 2011.

BARTON, M. H. Septicemia. In: PARADIS, M. R. **Equine Neonatal Medicine: A Case Based Approach**. Philadelphia: Elsevier, 2006. p.75-97.

BARTON, M. H. Early recognition of the septicemic foal**. Proceedings of The Annual Convention of the American Association of Equine Practitioners (Austin, USA)**, 2008. p.101-109.

BAUER, J.E. Normal blood chemistry. In: KOTERBA, A. M.; DRUMMOND, W. H.; KOSCH, P. C. **Equine Clinical Neonatology**. Philadelphia: Lea & Febiger, 1990. p.602-614.

BERRYHIL, E. H.; MAGDESIAN, K. G.; KASS, P. H.; EDMAN, J. E. Triglyceride concentrations in neonatal foals: Serial measurement and effects of age and illness. **The Veterinary Journal**, v.227, p.23-29, 2017.

BREWER, B. D.; KOTERBA, A. M. Development of a scoring system for the early diagnosis of equine neonatal sepsis. **Equine Veterinary Journal**, v.20, p.18-22, 1988.

BREWER, B. D. Neonatal infection, In: KOTERBA, A. M.; DRUMMOND, W. H.; KOSCH, P. C. **Equine Clinical Neonatology**. Philadelphia: Lea & Febiger, 1990. p.295-316.

BUCCA, S. Diagnosis of the compromised equine pregnancy. **Veterinary Clinics of North America: Equine Practice**, v.22, n.3, p.749-761, 2006.

CABALLERO, C.; VEKEMANS, M.; LOPEZ DEL CAMPO, J. G.; ROBYN, C. Serum alpha-fetoprotein in adults, in women during pregnancy, in children at birth, and during the first week of life: a sex difference. **American Journal of Obstetrics and Gynecology**., v.127, n.4, p.384-389, 1977.

CANISSO, I. F.; BALL, B. A.; CRAY, C.; WILLIAMS, N. M.; SCOGGIN, K. E.; DAVOLLI, G. M.; SQUIRES, E. L.; TROEDSSON, M. H. Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen

concentration. **American Journal of Reproductive Immunology**, v.72, p.376-385, 2014.

CANISSO, I. F.; BALL, B. A.; SCOGIN, K. E.; SQUIRES, E. L.; WILLIAMS, N. M.; TROEDSSON, M. H. Alpha-fetoprotein is present in the fetal fluids and is increased in plasma of mares with experimentally induced ascending placentitis. **Animal. Reproduction Science**, v.154, p.48-55, 2015.

CANISSO, I. F.; BALL, B. A.; CRAY, C.; SQUIRES, E. L.; TROEDSSON, M. H. Use of a qualitative horse-side test to measure serum amyloid A in mares with experimentally induced ascending placentitis. **Journal of Equine Veterinary Science**, v.35, n.1, p.54-59, 2015.

CASTAGNETTI, C.; PIRRONE, A.; MARIELLA, J. MARI, G. Venous blood lactate evaluation in equine neonatal intensive care. **Theriogenology**, v.73, p.343-57, 2010.

CHAVATTE, P. M.; PEPYS, M. B.; ROBERTS, B.; OUSEY, J. C.; MCGLADDERY, A. J.; ROSSDALE, P. D. Measurement of serum amyloid A protein (SAA) as an aid to differential diagnosis of infection in newborn foals. **Equine Infectious Diseases**, v.33-38, 1992.

COHEN, N. D. Causes of and farm management factors associated with disease and death in foals. **Journal of the American Veterinary Medical Association**, v.204, n.10, p.1644-1651, 1994.

CORLEY, K. T. T.; FURR, M. O. Evaluation of a score designed to predict sepsis in foals. **Journal of Veterinary Emergency and Critical Care**, v.13, n.3, p.149-155, 2003.

CORLEY, K. T. T.; DONALDSON, L. L.; FURR, M. O. Arterial lactate concentration, hospital survival, sepsis and SIRS in neonatal foals. **Equine Veterinary Journal**, v.37, n.1 p.53-59, 2005.

COUTINHO DA SILVA, M. A.; CANISSO, I. F., MACPHERSON, M. L.; JOHNSON, A. E. M.; DIVERS, T. J. Serum amyloid A concentration in healthy periparturient mares and mares with experimentally ascending placentitis. **Equine Veterinary Journal**, v.45, p.619-624, 2013.

CURCIO, B. R.; CANISSO, I. F.; PAZINATO, F. M.; BORBA, L. A.; FEIJO, L. S.; MULLER, V.; FINGER, I. S.; TORIBIO, R. E.; NOGUEIRA, C. E. W. Estradiol

cypionate aided treatment for experimentally induced ascending placentitis in mares. **Theriogenology**, v.102, p.98-107, 2017.

DAS, S.; MISRA, B.; ROUL, L.; MINS, N. T.; PATTNAIK, M.; BAIG, M. A. Insulin resistance and beta cell function as prognostic indicator in multi-organ dysfunction syndrome. **Metabolic Syndrome and Related Disorders**, v.7, n.1, p.47-51, 2009.

DEMBEK, K. A.; HURCOMBE, S. D.; FRAZER, M. L.; MORRESEY, P. R.; TORIBIO, R. E. Development of a Likelihood of Survival Scoring System for Hospitalized Equine Neonates Using Generalized Boosted Regression Modeling. **PLoS ONE**, v.9, n.10, p.e109212, 2014.

DUGGAN, V. E.; HOLYOAK, G. R.; MACALLISTER, C. G.; CONFER, A. W. Influence of induction of parturition on the neonatal acute phase response in foals. **Theriogenology**, v.67, p.372-381, 2007.

DUNKEL, B.; CORLEY, K. T. T. Pathophysiology, diagnosis and treatment of neonatal sepsis. **Equine Veterinary Education**, v.27, n, 2. p.92-98, 2015.

ENGUIX, A.; REY, C.; CONCHA, A.; MEDINA, A.; COTO, D.; DIÉGUEZ, M. A. Comparison of procalcitonin with C-reactive protein and serum amyloid for the early diagnosis of bacterial sepsis in critically ill neonates and children. **Intensive Care Medicine**, v.27, n.1, p.211-215, 2001.

EROL, E.; JACKSON, C.; HOROHOV, D.; LOCKE, S.; SMITH, J.; CARTER, C. Elevated serum amyloid A levels in cases of aborted equine fetuses due to fetal and placental infections. **Theriogenology**, v.86, p.971-975, 2016.

FOWDEN, A.L., MUNDY L., OUSEY J.C., MCGLADDERY A., SILVER M. Tissue glycogen and glucose 6-phosphate levels in the fetal and newborn foals. **Journal of Reproduction and Fertility. Supplement**, v.44, p.537-542, 1991.

FREEMAN, L.; PARADIS, M. R. Evaluating the effectiveness of equine neonatal care. **Veterinary Medicine**, v.87, p.921-926, 1992.

GAYLE, J. M.; COHEN, N. D.; CHAFFIN, M. K. Factors associated with survival in septicemic foals: 65 cases (1988–1995). **Journal of Veterinary Internal Medicine**, v.12, p.140-146, 1998.

GILES, R. C.; DONAHUE, J. M.; HONG, C. B.; TUTTLE, P. A.; PETRITES-MRPHY, M. B.; POONACHA, K. B.; ROBERTS, A. W.; TRAMONTIN, R. R.; SMITH, B.; SWERCZEK, T. W. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). **Journal of the American Veterinary Medical Association**, v.203, p.1170-1175, 1993.

GONZÁLEZ, F. H. D.; SILVA, S. C. Introdução à bioquímica veterinária. 2.ed. Porto Alegre: Editora da UFGRS, 2006. 364p.

GUIGUÈRE, S.; WEBER, E. J.; SANCHEZ, L. C. Factors associated with outcome and gradual improvement in survival over time in 1065 equine neonates admitted to an intensive care unit. **Equine Veterinary Journal**, v.49, p.45-50, 2017.

HOLLIS, A. R., FURR, M. O.; MAGDESIAN, K. G.; AXON, J. E.; LUDLOW, V.; BOSTON, R. C.; CORLEY, K. T. T. Blood glucose concentrations in critically ill neonatal foals. **Journal of Veterinary Internal Medicine**, v.22, n.5, p.1223-1227, 2008.

HONG, C. B.; DONAHUE, J. M.; GILES, R. C; PETRITES-MURPHY, M. B. JR.; POONACHA, K. B.; ROBERTS, A. W.; SMITH, B. J.; TRAMONTIN, R. R.; TUTTLE, P. A.; SWERCZEK, T. W. Etiology and pathology of equine placentitis. **Journal Veterinary Diagnostic and Investigation**, v.5, n.1, p.55-63, 1993.

HURCOMBE, S. D. A.; TORIBIO, R. E.; SLOVIS, N. M.; SAVILLE, W. J; MUDGE, M. C.; MACGILLIVRAY, K.; FRAZER, M. L. Calcium regulating hormones and serum calcium and magnesium concentrations in septic and critically ill foals and their association with survival. **Journal of Veterinary Internal Medicine**, v.23, n.2, p.335-343, 2009.

KITCHEN, H.; ROSSDALE, P. D. Metabolic profiles of newborn foals. **Journal of Reproduction and Fertility. Supplemment**, v.23, p.705-707, 1975.

KOTERBA, A. M. Physical Examination. In: KOTERBA, A. M.; DRUMMOND, W. H.; KOSCH, P. C. **Equine Clinical Neonatology**. Philadelphia: Lea & Febiger, 1990. p.71-85.

KOTERBA, A. M. Neonatal asfixia. In: KOTERBA, A. M.; DRUMMOND, W. H.; KOSCH, P. C. **Equine Clinical Neonatology**. Philadelphia: Lea & Febiger, 1990. p.124-135.

KULISA, M.; DLUGOSZ, B.; LUSZCZYNSKI, J.; PIESZKA, M.; SICINSKA. R. Cholesterol level in serum in a thoroughbred foals bred in two different studs. **Biotechnology in Animal Husbandry**, v.21, n.5-6, p.77-80, 2005.

LAUGIER, C.; FOUCHER, N.; SEVIN, C.; LEON, A.; TAPPREST, J. 24-year retrospective study of equine abortion in Normandy (France). **Journal of Equine Veterinary Science**, V.31, p.116-123, 2011.

MAGDESIAN, G. K. Blood lactate levels in neonatal foals: normal values and temporal effects in the post-partum period. **Journal of Veterinary Emergency and Critical Care,** v.13, n.13, p.174, 2003.

MARCONI, A. M.; PAOLINI, C. L.; STRAMARE, L.; CETIN, I.; FENNESSEY, P. V.; PARDI, G.; BATTAGLIA, F. C. Steady state maternal-fetal leucine enrichments in normal and intrauterine growth-restricted pregnancies. **Pediatric Research**, v.46, n.1, p.114-119, 1999.

MCAULIFFE, S. B. Neonatal examination, clinical procedures and nursing care. In: MCAULIFFE, S. B.; SLOVIS, N. M. **Color Atlas of Diseases and Disorders of the Foal**. Philadelphia: Saunders Elsevier, 2008. p.132-165.

MCKINNON, A. O. Maintenance of pregnancy. **Proceedings. of the Annual Resort Symposium of the American Association of Equine Practitioners (Gold Coast, Austrália)**, 2009. p.81-117.

MORRESEY, P. R. Prenatal and perinatal indicators of neonatal viability. **Clinical Techniques in Equine Practice**, v.4, n.3, p.238-249, 2005.

MYERS, C. J.; MAGDESIAN, K. G.; KASS, P. H.; MADIGAN, J. E.; RHODES, D. M.; MARKS, S. L. Parenteral nutrition in neonatal foals: clinical description, complications and outcome in 53 foals (1995–2005). **The Veterinary Journal**, v.181, p.137-144, 2009.

NUNOKAWA, Y.; FUJINAGA, T.; TAIRA, T.; OKUMURA, M.; YAMASHITA, K.; TSUNODA, N.; HAGIO, M. Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. **The Journal of Veterinary Medical Science**, v.55, n.6. p1011-1016, 1993.

OSWARI H, WIDJAJA RK, ROHSISWATMO R, CLEGHORN G. 2013. Prognostic value of biochemical liver parameters in neonatal sepsis-associated cholestasis. **Journal of Paediatrics and Child Health**, v.49, n.1, p.6-11, 2013.

OUSEY, J. C.; ROSSDALE, P. D.; FOWDEN, A. L.; PALMER, L.; TURNBULL, C.; ALLEN, W. R. Effects of manipulating intrauterine growth on post natal adrenocortical development and other parameters of maturity in neonatal foals. **Equine Veterinary Journal**, v.36, n.7, p.616-621, 2004.

PALTRINIERI, S.; GIORDANO, A.; VILLANI, M.; MANFRIN, M.; PANZANI, S.; VERONESI, M. C. Influence of age and foaling on plasma protein electrophoresis and serum amyloid A and their possible role as markers of equine neonatal septicaemia. **The Veterinary Journal**, v.176, p.393-396, 2008.

PEPYS, M. B.; BALTZ, M. L.; TENNENT, G. A.; KENT, J.; OUSEY, J.; ROSSDALE, P. D. Serum amyloid A (SAA) in horses: objective measurement of the acute phase response. **Equine Veterinary Journal**, v.21, n.2, p.106-109, 1989.

PIRRONE, A.; MARIELLA, J.; GENTILINI, F.; CASTAGNETTI, C. Amniotic fluid and blood lactate concentrations in mares and foals in the early postpartum period. **Theriogenology**, v.78, n.6, p.1182-1190, 2012.

PIRRONE, A.; ANTONELLI, C.; MARIELLA, J.; CASTAGNETTI, C. Gross placental morphology and foal serum biochemistry as predictors of foal health. **Theriogenology**, v.81, n.9, p.1293-1299, 2014.

PRELL, M.; CANISSO, I. F.; SCHNOBRICH, M.; RIDDLE, T.; ELLERBROCK, R. E.; WILKINS, P. Alpha-fetoprotein as a marker for equine neonatal disease. **Proceedings of The Theriogenology Annual Conference (Asheville, USA)**, 2016.

ROSSDALE, P. D.; OUSEY, J. C.; SILVER, M.; FOWDEN, A. Studies on equine prematurity 6: guidelines for assessment of foal maturity. **Equine Veterinary Journal**, v.16, n.4, p.300-302, 1984.

ROSSDALE, P. D.; OUSEY, J. C.; CHAVATTE, P. Readiness for birth: an endocrinological duet between fetal foal and mare. **Equine Veterinary Journal**, v.29, n.24, p.96-99, 1997.

ROTHROCK, L.; CANISSO, I. F. Estradiol-17β and alfa-fetoprotein as diagnostic markers for ascending placentitis in a Quarter Horse broodmare. **Proceedings of The Theriogenology Annual Conference (Asheville, USA)**, 2016.

SANCHEZ, L. C. Equine Neonatal Sepsis. **Veterinary Clinics of North America: Equine Practice**, v.21, n.1, p.273-293, 2005.

SANCHEZ, L. C.; GUIGUÈRE, S.; LESTER, G. D. Factors associated with survival of neonatal foals with bacteremia and racing performance of surviving Thoroughbreds: 423 cases (1982–2007). **Journal of the American Veterinary Medical Association**, v.233, n.9, p.1446-1452, 2008.

SCHOTT, H. C. Review of azotemia in foals. **Proceedings. of the Annual Convention of the American Association of Equine Practitioners (San Antonio, USA)**, 2011. p.328-334.

SIMPSON, K. S.; ADAMS, M. H.; BEHRENDT-ADAM, C. Y.; BAKER, C. B.; MCDOWELL, K. J.; Differential gene expression in day 12 and day 15 equine conceptuses. **Journal of Reproduction and Fertility. Supplement**, v.56, p.539-547, 2000.

STONEHAM, S. J.; PALMER, L.; CASH, R.; ROSSDALE, P. D. Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. **Equine Veterinary Journal**, v.33, n.6, p.599-603, 2001.

TENNENT-BROWN, B. S. Blood lactate measurement and interpretation in critically ill equine adults and neonates. **Veterinary Clinics: Equine Practice**, v.30, n.2, p.399-413, 2014.

WHITWELL, K. E. Investigations into fetal and neonatal losses in the horse. **Veterinary Clinics of North America: Equine Practice**, v.2, n.2, p.313-31, 1980.

WINTERGERST, K. A.; BUCKINGHAM, B.; GANDRUD, L.; WONG, B. J.; KACHE, S.; WILSON, D. M. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care Unit. **Pediatrics**, v.118, n.1, p.173-179, 2006.

WOTMAN, K.; WILKINS, P. A.; PALMER, J. E.; BOSTON, R. C. Association of blood lactate concentration and outcome in foals. **Journal of Veterinary Internal Medicine**, v.23, p.598-605, 2009.

Anexos

Anexo A

Parecer da Comissão de Ética em Experimentação Animal

Pelotas, 05 de julho de 2010.

De: Prof. Dr. Orlando Antonio Lucca Filho
Pres. da Comissão de Ética e Experimentação Animal (CEEA)

Para: Prof. Carlos Eduardo Wayne Nogueira
Departamento de Clínicas Veterinária
Faculdade de Veterinária

Senhor Professor:

A CEEA analisou o projeto intitulado: **"Alterações clínicas e metabólicas em potros neonatos e sua relação com os achados ginecológicos e obstétricos na égua".** Processo n° 23110.004750/2009-55 sendo de parecer **FAVORÁVEL** a sua execução considerando ser o assunto pertinente e a metodologia compatível com os princípios éticos em experimentação animal e com os objetivos propostos.

Solicitamos, após tomar ciência do parecer, reenviar o processo à CEEA.

Salientamos também a necessidade deste Projeto ser cadastrado junto ao Departamento de Pesquisa para posterior registro no COCEPE (Código para Cadastro nº CEEA 4750).

Sendo o que tínhamos para o momento, subscrevemo-nos.

Atenciosamente,

Prof. Dr. Orlando Antonio Lucca Filho
Presidente da CEEA