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

Tese

# Perfil energético de éguas gestantes e seus neonatos

**Hortencia Campos Mazzo**

Pelotas, 2021

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# Perfil energético de éguas gestantes e seus neonatos

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 Doutora em Ciências (área de concentração: Sanidade Animal).

Orientadora: Bruna da Rosa Curcio

Pelotas, 2021

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

M477p Mazzo, Hortencia Campos

Perfil energético de éguas gestantes e seus neonatos / Hortencia Campos Mazzo ; Bruna da Rosa Curcio, orientadora. — Pelotas, 2021.

48 f.

Tese (Doutorado) — Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, 2021.

1. Colesterol. 2. Égua gestante. 3. Periparto. 4. Potro. I. Curcio, Bruna da Rosa, orient. II. Título.

CDD : 636.108982

Elaborada por Gabriela Machado Lopes CRB: 10/1842

Hortencia Campos Mazzo

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

Data da Defesa: 13/08/2021

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***Á minha família,***

***pelo primeiro de muitos doutores.***

## Agradecimentos

Não teria como iniciar nenhum tipo de agradecimento sem pensar em como Deus esteve presente em minha vida durante todo esse tempo. Minha formação acadêmica foi um tanto quanto conturbada, mas eu nunca me senti sozinha. Sou grata a Deus e a minha mãe Maria por terem me dado forças para seguir em frente sempre.

Não existe nada em minha vida conquistado sem a ajuda dos meus pais. Eles sempre foram meus maiores e melhores incentivadores. Eu nunca poderei ser grata suficiente por tudo que já fizeram e fazem por mim. Pai e mãe, amo vocês e tudo isso não teria sentido algum sem poder comemorarmos juntos.

Aos meus irmãos, Luana e Luiz Carlos, meus sobrinhos Heloisa, Laura e Luiz Miguel e a todos os meus familiares que sempre acreditaram em meu potencial e vibravam comigo a cada vitória, amo vocês demais, muito obrigada.

A minha Nega, eu nunca saberei se foi eu que te adotei ou você que me adotou, obrigada.

Aos meus amigos “de longe” em especial a Camila Bianconi, Saulo Baracat, Raissa Gracie e família, Nicolas Duarte e família (Danone), Mina Padovan, Mariana Fragoso Rentas (Mel), Mauricio Xavier, minha turma da graduação e a tantos outros que levo comigo em meu coração, agradeço os telefonemas, mensagens de WhatsApp, incentivo e ouvido emprestado para as minhas lamentações. Essa vitória é nossa!

Aos meus amigos de Pelotas: da VTreino (Luísa, Marcos e Carol, Milena, Pedro e Luíza, Luana, Carmela, Matheus, Vitor, Darlan e Liene, Lourenço) por muito tempo foi o local onde eu melhor me sentia em Pelotas; do meu grupo de oração EFATÁ, EMAUS-Pelotas, grupo São João Paulo II, Pe. Wilson, por me acolherem e me deixarem mais perto de Deus; meus *roommates*: Hélio, Cadu, Gerson, Natalie, Lucas e Eduardo, aprendi muito com cada um de vocês e agradeço a convivência e paciência; meus amigos da Haus; minha amada amiga Isabel, que impulsionou minha ida pra Pelotas; meus amigos que levarei pra vida inteira Márcio Peixoto e Marina Barrios e toda sua família vocês foram por muitas vezes meu ponto de apoio, meu ombro amigo sem julgamentos, sem cobranças; meu grande amigo Fernando Rutz,

obrigada por me incentivar e ajudar tanto. Eu sou grata a Deus por ter colocado vocês em meu caminho!

Á Universidade Federal de Pelotas, ao Programa de Pós-graduação em Veterinária por me oportunizarem o doutorado. Aos meus colegas da pós-graduação. À Coordenação e Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão da bolsa de estudo.

Ao meu grupo de pesquisa ClinEq, em especial aos “melhorzinhos”, Gabriela Castro, Taís, Guida, Neto e Roberta, as minhas estagiárias amadas Gabriela Marocco, Rafa e Eliza, a Morgana, Aníbal e aos funcionários do HCV. Não existe trabalho sem equipe, não existe pesquisa sem equipe. Vocês foram essenciais para a construção de cada etapa desse título. Muito obrigada por serem tão vocês.

Aos meus amigos que levarei pra vida toda Henrique, Ruth (Rutilene) e José Victor (Zé). Eu preciso deixar registrado meu amor e minha admiração por vocês. Muito obrigada por tanto!

Aos meus orientadores Bruna e Nogueira, com vocês aprendi muito mais que pesquisar, ou coisas sobre equinos. Aprendi a olhar pro outro, respeitar, admirar. Aprendi que ter um pouco ou muito mais conhecimento que uma outra pessoa sobre algum assunto não te dá o direito em ser arrogante ou prepotente e que não sendo assim as pessoas irão te respeitar ainda mais. Vocês são meus maiores exemplos. Obrigada pela oportunidade. Por abrir as portas pra mim mesmo sem me conhecer. Por terem sido pais, conselheiros, amigos e grandes mestres!

Por fim e por serem o motivo de todo esse trabalho aos cavalos. Meu amor pela profissão que escolhi cresce a cada dia pelo amor a eles.

**ELE NÃO!**

***“It is our choices, Harry, that show what we truly are,***
***far more than our abilities.”***
J. K. Rowling, Harry Potter and the Chamber of Secrets

## Resumo

MAZZO, Hortencia Campos. **Perfil energético de éguas gestantes e seus neonatos.** 2021. 48f. Tese (Doutorado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2021.

A gestação e o período neonatal marcam importantes fases na vida dos seres. O estudo das alterações metabólicas ocorrentes nesses períodos é importante para se compreender, prever e controlar possíveis patologias. Nessa tese objetivou-se avaliar o perfil energético de éguas gestantes e seus neonatos visando compreender a relação égua-potro. No primeiro estudo foram coletados sangue de éguas crioulas nos períodos que antecederam o parto, além de feitas mensurações quanto ao seu ganho de peso seja pelo acompanhamento das diferentes formas de mensuração de peso ou pelas medidas de gordura corporal e metabólica. Nesse estudo pode-se constatar que essas variáveis estavam correlacionadas ($p < 0,0001$) e foi observado um aumento nos níveis de colesterol total sanguíneo das éguas no dia do parto ($p = 0,0016$). Assim, em continuidade foi desenvolvido o segundo estudo onde éguas crioulas no periparto e seus neonatos tiveram seu sangue coletado e avaliado quanto a quantidade de colesterol total, triglicerídeos, LDL (lipoproteína de baixa densidade) e HDL (lipoproteína de alta densidade). Ainda, nos potros foram mensurados os níveis sanguíneos de lactato e glicose. Foi observado o real aumento do colesterol no dia do parto das éguas com retorno dos valores no pós-parto imediato ($p = 0,02$). Houve correlação entre os níveis de colesterol total das éguas e dos potros no momento do parto ($r = 0,457$, $p = 0,011$). Além de correlação entre triglicerídeos na égua e colesterol total no potro, e colesterol total na égua e LDL no potro. Observou-se também, um comportamento inversamente proporcional dos níveis de HDL e LDL sanguíneo do potro. Nossos resultados demonstram como o perfil lipídico dos neonatos flutua e pode ser interferido de várias maneiras. A égua prenhe apresentou aumento nos níveis de colesterol no dia do parto. Além disso, nosso estudo demonstrou a relação entre colesterol total e triglicerídeos na égua e colesterol total do neonato, e colesterol total na égua e LDL do neonato. Houve uma correlação inversamente proporcional entre os níveis de HDL e LDL no sangue do potro neonato, o que influenciou na manutenção dos níveis de outros componentes, como a glicose, que se correlaciona com o lactato no dia do parto, o que demonstra que devemos estar atentos à avaliação do momento desses componentes.



**Palavras-chave:** colesterol; égua gestante; periparto; potro

## Abstract

MAZZO, Hortencia Campos. **Energetic profile of pregnant mares and their newborns.** 2021. 48f. Thesis (Doctor degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2021.

Pregnancy and the neonatal period mark important stages in the lives of beings. The study of metabolic changes that occur during these periods is important to understand, predict and control possible pathologies. This thesis aimed to determine and evaluate the energy profile of pregnant mares and their newborns, to understand the mare-foal relationship. In the first study, blood was collected from native mares in the periods prior to parturition, in addition to measurements made regarding weight gain, either by monitoring the different ways of measuring weight, or by measuring body and metabolic fat. In the present study, these variables correlated ($p < 0,0001$) and there was an increase in the total blood cholesterol levels of the mares on the day of delivery ($p = 0,0016$). Thus, continuing the study, the second article was developed where native peripartum mares and their newborns had their blood collected and evaluated for levels of total cholesterol, triglycerides, LDL (low-density lipoprotein) and HDL (high-density lipoprotein). In addition, in the foals, blood lactate and glucose levels were measured. A real increase in cholesterol was observed in mares on the day of parturition, with a return of values in the immediate postpartum period ($p=0.02$). Correlation was found between the total cholesterol levels of mares and foals at the time of parturition ($r = 0,457$, $P = 0,011$). In addition, there was a correlation between total cholesterol and triglycerides in the mare and total cholesterol in the foal, and total cholesterol in the mare and LDL in the foal. Our results demonstrate how the lipid profile of neonates fluctuates and can be interfered with in various ways. Pregnant mare showed an increase in cholesterol levels at day of foaling. Furthermore, our study demonstrated the relationship between total cholesterol and triglycerides in the mare and total cholesterol in the newborn foal, and total cholesterol in the mare and LDL in the newborn foal. There was an inversely proportional correlation between the levels of HDL and LDL levels in the blood of the newborn foal, which influenced the maintenance of the levels of other components, such as glucose, which correlates with lactate on the day of foaling, which demonstrates that we must be attentive to the evaluation of the moment of these components.



**Keywords**: cholesterol; pregnant mare; peripartum; foal

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## Lista de Abreviaturas e Siglas

| | |
|---|---|
| tCho | Total cholesterol |
| HDL | High density lipoprotein cholesterol |
| LDL | Low density lipoprotein cholesterol |

## Lista de Símbolos

| | |
|---|---|
| < | Menor |
| > | Maior |
| ≤ | Menor ou igual |
| © | Copyright |

**Sumário**

## 1 Introdução

O grupo de Ensino, Pesquisa e Extensão em Medicina de Equinos – UFPel (ClinEq) vem sendo pioneiro nos estudos referentes a raça Crioula, que possui caraterísticas próprias principalmente pela sua rusticidade, comportamento e morfofisiologia. O Crioulo é uma das principais raças de equinos do Brasil, contando com 419,43 mil exemplares registrados no ano de 2019 segundo a Associação dos Criadores de Cavalos Crioulos (ABCCC).

Na criação de equinos uma das fases mais importantes é a gestação, pois ela impacta diretamente na concepção de potros viáveis que darão continuidade ao processo. Diversos parâmetros são alterados nessa fase, principalmente quando se fala da composição corpórea das éguas. Esses parâmetros terão implicação direta no desenvolvimento dos potros, e conhecer essas alterações facilita a toma de decisão de uma possível interferência externa. Éguas no periparto e neonatos são instáveis fisiologicamente e possuem alterações hematológicas e bioquímicas ainda pouco estudas.

As principais alterações metabólicas ocorrem na gestação, lactação e na fase neonatal (ARFUSO *et al.*, 2016b; BARRETO *et al.*, 2020; SILVA *et al.*, 2019). A gestação e lactação requerem um aumento da demanda metabólica (KRAKOWSKI *et al.*, 2020). Todo sistema corporal sofre adaptação para garantir o desenvolvimento pleno do feto (HADDEN; MCLAUGHLIN, 2009). O último trimestre aumenta as necessidades nutricionais devido as necessidades do feto (NRC, 2007). São descritas alterações fisiológicas a nível cardiovascular e metabólico relacionados ao aumento do útero que consequentemente causa efeitos sobre o sistema renal, hepático e cardiovascular (HADDAD *et al.*, 2009).

Ainda, o terço final é marcado pelo incremento de peso sendo ainda mais expressivo na raça crioula. Em trabalho avaliando éguas gestantes dessa raça em condição de escore corporal descrita como normal, no terço final da gestação, foi observado grande aumento nas medidas de perímetro torácico e abdominal, além de acúmulo de gordura na base da cauda (MARCHIORI *et al.*, 2015). Todo esse incremento está correlacionado as reservas corporais de

gordura que serão utilizadas na fase de lactação e na manutenção da égua no periparto.

Demandas energéticas, condição de saúde, nível de estresse no periparto são algumas das principais causas de variações nos parâmetros bioquímicos (ARFUSO *et al.*, 2016b). O terço final é marcado por alterações no metabolismo lipídico com

aumento dos triglicerídeos no plasma afim de suprir a demanda de crescimento fetal, e um aumento fisiológico dos níveis de colesterol pela elevada síntese de hormônios esteroides gonadais (KASINGER *et al.*, 2020). Alguns trabalhos já relatam sobre esses parâmetros contudo sem acompanhar as fases anteriores ao parto (SILVA *et al.*, 2019). Existem algumas especulações sobre o comportamento dos principais componentes lipídicos no periparto de éguas gestantes. Contudo, sabemos muito pouco sobre como esses parâmetros bioquímicos interferem nessa fase.

Já o período neonatal é uma fase crítica de adaptação e ajustes a vida extrauterina com a transição da respiração, circulação, digestão, metabolismo e eliminação fetal para neonatal e crescimento (CHIBA *et al.*, 2017; ORSINI, 2011). O perfil energético no periparto e seu reflexo no potro neonato são parâmetros importantes para avaliar o estado fisiológico e/ou patológico dos envolvidos.

As primeiras semanas de vida são importantes para o amadurecimento e adaptação dos potros (CHIBA *et al.*, 2017). Nessa fase há uma instabilidade metabólica visto que a condição do neonato se altera a cada instante por se tratar de uma transição ativa (ORSINI, 2011). Além disso, existe uma forte relação entre a saúde do potro e fatores pré-natais demonstrando assim o quanto a égua pode vir a causar danos à saúde do neonato ou comprometer seu desenvolvimento pleno (FEIJÓ *et al.*, 2014).

A principal fonte lipídica dos potros neonatos está relacionada a nutrição. O colostro e leite de éguas possuem níveis altos de gordura que alteram os níveis de colesterol total, LDL, HDL e triglicerídeos. Esses níveis estão relacionados a raça e idade das éguas (BARRETO *et al.*, 2020). Éguas crioulas

demonstram ter níveis mais baixos de gordura no leite que outras raças (COSTA *et al.*, 2019).

Potros neonatos apresentam valores bioquímicos diversos a depender da fase de vida e da sua condição de saúde. Neonatos apresentam concentrações de glicose ao nascimento correspondentes a aproximadamente 50% dos valores maternos com seus valores mais baixos duas horas após o nascimento, aumentando nos próximos 1 a 2 dias (KANEKO; HARVEY; BRUSS, 2008). Já as concentrações de lactato são maiores no nascimento e diminuem nas 24h seguintes (CHIBA *et al.*, 2017). Esses animais possuem ainda hipertrigliceridemia transitória leve na primeira semana que está associada a imaturidade hepática ao nascimento e esses valores altos também são acompanhados pelo colesterol (AXON; PALMER, 2008). Estudo avaliando potros sépticos demonstrou que estes apresentam alterações nos níveis sanguíneos com aumento do lactato, triglicerídeos e colesterol e hipoglicemia demonstrando assim que esses marcadores poderiam ser utilizados como detectores de sepse (BORBA *et al.*, 2020).

Estudos relacionados ao acompanhamento no periparto de éguas e neonatal dos respectivos potros neonatos tem como principal objetivo prevenir que alterações que causem danos a esses animais. Muitos trabalhos tem enfoque em estudos com animais debilitados, descrevendo valores séricos sempre com alterações devido a sua condição, mas conhecer os níveis em animais normais são importantes para auxiliar nos tratamentos (ARFUSO *et al.*, 2016b; CHIBA *et al.*, 2017; CONLEY, 2016).

Entender a fase do periparto de éguas é um desafio. Saber que essa fase interfere tanto e de forma tão abrupta na vida do potro nos instiga a querer mensurar as variáveis envolvidas, sendo importante conhecer essa relação, potro e égua, e suas variações a fim de desenvolver ferramentas para prevenir possíveis problemas.

O objetivo desse estudo foi demonstrar e avaliar as possíveis alterações no ganho de peso, de medidas morfométricas e no perfil lipídico de éguas no periparto e de potros neonatos da raça Crioula. A hipótese do presente estudo é que existe uma correlação entre o peso e as medidas de gordura com os níveis de colesterol e triglicerídeos no terço final da gestação, com marcado incremento

dos níveis de colesterol total no dia do parto. Além disso, existe uma correlação entre os valores de colesterol total e frações e triglicerídeos e os níveis dos mesmos componentes lipídicos nos potros neonatos.

Paralelo a esses estudos foram desenvolvidos projetos de pesquisa, ensino e extensão que possuem uma grande importância na formação social dos envolvidos. Dentre os projetos de pesquisa, foi realizado experimento afim de desenvolver uma nova metodologia para aferição do peso de éguas gestantes crioulas. Estando este em fase de depósito de patente na instituição.

# 2 Artigos

## 2.1 Artigo 1

### Changes In Cholesterol, Triglycerides And Body Composition In Pregnant Mares

Hortencia Campos Mazzo, Carlos Eduardo Wayne Nogueira, Ruth Patten, Henrique dos Reis Noronha, Gabriela Castro da Silva, Bruna da Rosa Curcio

Submetido à revista Acta Scientiae Veterinariae
Aceito para publicação na revista 7 de Março de 2021

**RESARCH ARTICLE**

# Changes In Cholesterol, Triglycerides And Body Composition In Pregnant Mares

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## ABSTRACT

***Background*:** Mares are very different from other species during pregnancy, and studies on the physiological changes of this period are important. During late pregnancy, the distribution of weight and body fat are often used as indicators of adequate nutrition. This is a physiological period that results in an increase in metabolic demand. There is a tendency for the Criollo breed to have a higher body condition score that becomes more evident during pregnancy, a period when mares tend to gain more weight. The current study monitored serum cholesterol and triglyceride levels in pregnant mares during late gestation to determine a possible correlation with the distribution of fat or body weight.

***Materials, Methods & Results*:** Thirty-four Criollo-type mares were during late gestation and the following parameters were measured: body weight measured with a weight scale, body weight using a commercial weight tape, total body fat and fat thickness and the serum levels of total cholesterol and triglycerides. The fat thickness was measured in an ultrasound device and the prediction of total body fat was calculated using an equation. According to the days prior foaling, biometric monitoring and blood collection were carried out in five periods: F-90 (± 90 days prior to foaling) n = 33; F-60 (± 60 days prior to foaling) n = 33; F-30 (± 30 days prior to foaling) n = 31; F-15 (± 15 days prior to foaling) n = 29 and Foaling (at day of foaling) n = 14. Mares were monitored daily and accompanied foaling was also performed, ensuring collection at the right time. Comparisons of means were performed between variables in addition to the Pearson correlation test. Statistical significance was established at $P < 0.05$. There was no difference in relation to the period in body composition measurements ($P > 0.05$). A strong positive correlation was observed between the average weights ($P < 0.001$). The fat thickness showed a correlation between the weights ($P < 0.01$). There was no correlation with body

composition ($P > 0.068$). There was a strong positive correlation between weights ($P < 0.001$). In addition, differences in total cholesterol levels ($P = 0.0016$) were observed, with higher levels found in the Foaling period. The same was not observed for triglyceride levels ($P = 0.443$). There was no correlation between blood variables in this period ($P > 0.191$). There was also no correlation with the body composition ($P > 0.068$).

***Discussion*:** The absence of difference between the periods in relation to the weight measures and the correlations existing in these measures is related to the period in which they were collected, since the maximum relative weight of the foal is reached in ten months, causing the mare's weight stability. Interestingly, an unexplained increase in total cholesterol levels was found on the day of foaling. As the same change in triglycerides was not observed and there was no change in the diet or feeding behavior of the mares, the effects of the diet can be excluded in this case, which requires further studies to explain this result. Our hypothesis is that this increase is linked to hormones that tend to change in this pre-delivery period and that have their metabolism strongly linked to cholesterol levels. Levels of body fat and mare weight can therefore be correlated in the late gestation, allowing for their use as indicators of adequate nutritional and energy reserves.

**Keywords:** equine, pregnancy, triglyceride, weight.

## INTRODUCTION

Mare pregnancy differs greatly from the gestations of other animal species, reflecting a dynamic physiological condition in the endocrine profile [4]. Understanding species-specific physiological changes is necessary for the correct management of pregnancies [13]. Any alterations should therefore be well known to readily recognize any abnormalities that may impair the health of the mare, foal or both.

There is a tendency for Criollo horses to have a higher body condition score [7]. This is due to the breed's morphological competitiveness, whereby animals with greater fat accumulation and consequently more rounded aspects are better evaluated [7,9]. Additionally, its genetic kinship with the Andaluz breed gives further cause to characteristics such as rusticity, better feed conversion and easy fat accumulation [11]. Such characteristics become even more apparent during pregnancy, a period when mares tend to gain more weight [7].

Body fat distribution and weight are often used as indicators of adequate nutrition and energy reserves during late pregnancy [6]. These variables correlate with changes in circulating factors that manage the energy reserves of the body. Cholesterol is an important part of the cell

membrane, a direct precursor of steroid hormones important for pregnancy, including corticosteroids, androgens, estrogens, progesterone and vitamin D [2]. Furthermore, serum concentrations of lipids and lipoproteins may be influenced by the amount and distribution of body fat [14].

The current study monitored serum cholesterol and triglyceride levels in mares during late gestation to determine the correlation with the distribution of fat or body weight.

## MATERIALS AND METHODS

*Animals and study design*

Thirty-four pregnant Criollo-type mares aged 7-12 years old, with body condition scores of 5-7. All mares were housed at the Palma Farm of Federal University of Pelotas (UFPel), Capão do Leão, Brazil, under uniform sanitary conditions. The mares were maintained on native grass pastures and fed commercial concentrate for maintenance. The water was provided ad libitum.

During the study, four body composition evaluations were performed: body weight, prediction of total body fat and fat thickness. Additionally, serum total cholesterol and triglycerides levels were estimated.

For measurements of body weight, it was performed two different methods: the actual weight (weight scale), was measured using a mechanical livestock scale, and the estimated weight (weight tape) was measured using a commercial weight tape.

Fat thickness (retroperitoneal and rump) was measured according to described by Westervelt *et al.* [15], using an ultrasound device (Pie Medical® Linear Array Scanner 450)[1], with a linear probe of 7.5 MHz (UST-5512U-7.5, 38 mm). As well the prediction of body fat was calculated from the equation 8,64+4,70.rump described by the same author. The total body fat (metabolic weight) was calculated from the equation using heart-girth circumference (heartgirth) and body length (BL), which were applied to the formula: $heartgirth^2$+BL/11880 described by Hall [5].

Blood samples were collected from the external jugular vein in vacuum tubes, using a separation gel to produce serum. Blood was centrifuged at 1500 g for 15 min to separate the serum and frozen until processing. Serum triglycerides and total cholesterol was evaluated with spectrophotometric diagnostic kits using an automated analyser (Labmax Plenno®)[2].

Biometric monitoring and blood samples were taken over five periods, starting at seven months of gestation. In order to make a clearly descriptions, the periods were described according days prior foaling, as follows: F-90 (± 90 days prior to foaling) n = 33; F-60 (± 60

days prior to foaling) n = 33; F-30 (± 30 days prior to foaling) n = 31; F-15 (± 15 days prior to foaling) n = 29 and Foaling (at day of foaling) n = 14.

Mares were monitored daily by means of physical evaluations and pH measurement of mammary gland secretions to predict the day of foaling. In addition, accompanied foaling was also performed, ensuring collection at the right time. Mares that had abortion or stillborn foal were withdrawals of the study.

*Statistical analysis*

Statistical analyzes were performed using SPSS 20.0 software (Statistical Package for the Social Sciences®)[3]. Normality was assessed using the Shapiro-Wilk test. Considering that all variables have a normal distribution, continuous data were assessed using analysis of variance (ANOVA), using a general linear model for repeated measures. Pearson's correlation test was performed to verify the relationship between quantitative variables (metabolic weight, weight scale, weight tape, fat thickness, triglyceride and total cholesterol). In addition, among the triglyceride and total cholesterol variables, Pearson's correlation was performed in each of the five periods evaluated. Statistical significance was established at $P < 0.05$.

## RESULTS

There was no difference regarding the period on body composition measurements: weight scale, tape weight, metabolic weight and fat thickness ($P > 0.05$) (Tabela 1).

**Table 1.** Mean and standard deviation of weight measurements, weight scale, weight tape, metabolic weight and fat thickness by period ($P > 0.05$).

| Parameter | Period* | | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| | F-90 | F-60 | F-30 | F-15 | Foaling | | |
| Weight scale (kg) | 392.5 | 384.1 | 416.3 | 425.3 | 424.4 | 4.52 | 0.052 |
| Weight tape (kg) | 385.0 | 396.4 | 388.1 | 393.4 | 397.3 | 4.56 | 0.716 |
| Metabolic weight (kg) | 366.9 | 371.2 | 372.3 | 374.0 | 386.2 | 4.03 | 0.465 |
| Body fat (%) | 13.0 | 13.5 | 13.8 | 13.8 | 13.3 | 0.13 | 0.348 |
| Retroperitoneal (cm) | 0.786 | 0.846 | 0.786 | 0.836 | 0.889 | 0.03 | 0.494 |
| Rump (cm) | 0.932 | 1.028 | 1.091 | 1.095 | 0.993 | 0.03 | 0.348 |

*F-90 (± 90 days prior to foaling); F-60 (± 60 days prior to foaling); F-30 (± 30 days prior to foaling); F-15 (± 15 days prior to foaling); Foaling (at day of foaling).

A strong positive correlation was observed between metabolic weight and scale weight (r = 0.9289, *P* < 0.001), between metabolic weight and weight tape (r = 0.9175, *P* < 0.001) and also between scale weight and the weight tape (r = 0.8872, *P* < 0.001). However, the same was not observed in blood variables in relation to weight measurements (*P* > 0.107).

Rump and body fat had weak correlations with weight scale (r = 0.344, *P* = 0.000), weight tape (r = 0.294, *P* = 0.000) and metabolic weight (r = 0.216, *P* = 0.011), but it showed strong correlation with the measurement of retroperitoneal fat (r = 0.728, *P* = 0.000). Which in turn, presented a weak correlation with weight scale (r = 0.218, *P* = 0.011) and on weight tape (r = 0.254, *P* = 0.003).

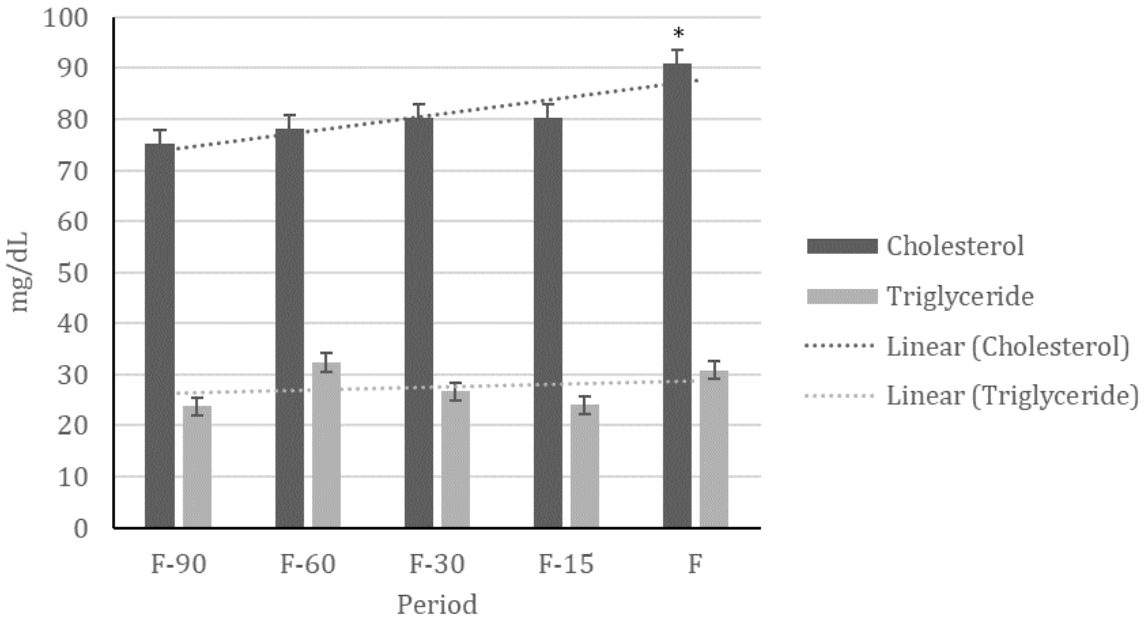


Figure 1. Means and standard deviation in triglyceride and cholesterol values in the periods: F-90 (± 90 days prior to foaling); F-60 (± 60 days prior to foaling); F-30 (± 30 days prior to foaling); F-15 (± 15 days prior to foaling); Foaling (± on the day of foaling). Asterisk shows difference in periods by Tukey's test (*P* < 0.05).

In regards to the biochemical variables, differences in total cholesterol levels were observed (*P* < 0.001), where higher levels were noted on the day of foaling (Figure 1). The same effect was not observed in triglyceride levels (*P* = 0.443). However, in both cases, the values did not exceed the reference levels for the species. There was no correlation of data in the F-90 period and in the Foaling period (*P* > 0.191), but there was a moderate correlation in the F-60 period (r = 0.425, *P* = 0.019), F-30 (r = 0.408, *P* = 0.02) and F-15 (r = 0.479, *P* = 0.007). There was also no correlation with the body composition (*P* > 0.068), except for a weak correlation between triglycerides and retroperitoneal fat (r = 0.228, *P* <0.01).

## DISCUSSION

Mares in the late gestation showed increase in total cholesterol between 15$^{th}$ days before parturition and day of foaling. Blood cholesterol levels are determined by factors related to diet and metabolism, such as intestinal absorption, hepatic synthase, body maintenance, bile and stool excretion and resorption [1]. Since there was no change in the diet or feeding behavior of the mares, the effects of diet can be excluded in this case.

The observed increase in total cholesterol levels on the day of foaling was similar to that found by Silva *et al.* [12] in Mangalarga Marchador mares, the hypotheses for this fact were related with synthesis of hormones or the increased of energy requirement in mares around parturition time [4,13]. However, still cannot be fully explained. We believe that the observed increase is related to the metabolic changes that occur during the pre-foaling period, specifically in regards to hormone levels which are tightly bound to cholesterol [4]. Two known mechanisms in the metabolism of pregnant mares existing at the end of gestation may be responsible for this increase. The decrease in progestogenic support that was the main responsible for the maintenance of pregnancy and the little or no steroid synthesis capacity performed by the adrenals of fetuses where they used cholesterol as a synthesizer [3,13]. We suspect that as progesterone levels decrease and fetal cortisol levels increase on the day of foaling, cholesterol levels increase due to a lack of synthesis.

It was to be expected that the fact that triglyceride is one of the components of adipose tissue correlated with fat measurements. Marchiori [8] evaluating non-obese pregnant mares noted that the average fat thickness in the rump and retroperitoneal was 1.22 mm and 1.919 mm, respectively, and differed from the pregnant obese mares that dissipated an average of 1.13 mm, 2, 08 mm. The maximum value of our animals reached up to 0.889 mm of retroperitoneal fat and 1,095 of fat on the rump. Therefore, this correlation cannot have been observed, as the values of body fat did not vary and were within normal limits. In addition, this work showed that the concentration of triglycerides in normal pregnant mares does not differ when compared with pregnant obese mares, which shows that this would not be a good indicator of energy reserves.

There were not observed difference of weight measurements between time before parturition, probable due to the period of late gestation that was evaluated in the present study. Well then, the foal's maximum relative weight is reached around the gestational age of ten months, causing the mare's weight to stabilize [10]. Given this stability, correlations between the different weight measurements and fat thickness during this period were expected. In addition, the correlations between measures of fat and metabolic weight corroborate the results

of Marchiori *et al.* [8], who demonstrate a difference between the values of fat increase in mares at the end of gestation when compared to other months. This is because the energy reserves for breastfeeding are acquired at this time of pregnancy, being an important tool to assess the differentiation of obese and healthy animals.

## CONCLUSION

Mares in late gestation showed increase in total cholesterol levels at day of foaling. Furthermore, body fat levels and mare body weight can be correlated in the late gestation, allowing for their use as indicators of adequate nutritional and energy reserves.

MANUFACTURERS

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[3] Statistical Package for the Social Sciences, IBM Corp, Armonk, NY, USA.

***Ethical approval.*** All procedures in the animals were approved by the Ethical Committee on Animal Experimentation of the College of Veterinary Medicine, UFPel under protocol number #1165-2016.

***Acknowledgements.*** Our thanks to the Brazilian Criollo Horse Breeder Association (ABCCC) and Brazilian funding agencies CAPES, FAPERGS and CNPQ for providing scholarships to the graduate and undergraduate students.

**Declaration of interest.** The authors have no competing interests. The authors alone are responsible for the content and writing of the paper.

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## 2.2 Artigo 2

# Correlation between the lipid profile of peripartum Criollo mares and healthy newborn foals

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Será submetido à revista Journal of Equine Veterinary Science (JEVS)

**Correlation between the lipid profile of peripartum Criollo mares and healthy newborn foals**

***Abstract***

The neonatal phase is full of metabolic instabilities. There is still a prenatal relationship with the health of the foal. Monitoring the serum lipid profile in peripartum mares and newborn foals will be helpful for a more accurate diagnosis and may be applicable to the results of preventive medications. Thus, the objective was to evaluate the lipid profile during peripartum in Criollo mares and their respective neonatal foals. Blood samples were collected from pregnant mares in the two days prior to parturition. After parturition, the mares and their foals were collected at times: after parturition, 4h, 12h, 24h and in the 3rd week of life. Tests were performed for serum triglycerides, total cholesterol (tCho), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL). Still, at other times, serum analysis of lactate and glucose in foals was performed. Comparisons of means were performed between the variables in addition to the Pearson correlation test. Statistical significance was established at $P < 0.05$. There is a significant increase in the tCho of the mares on the day of foaling ($P < 0.02$). In addition to a correlation of this variable in the foal's tCho values over the same period ($r = 0.457$, $P = 0.011$). Furthermore, there was a strong negative correlation between HDL in mares and glucose and lactate in foals. These results demonstrate how the lipid profile of mares during peripartum is strongly related to changes that occur in neonates.

*Keywords*: cholesterol, lactate, glucose, newborn foal, pregnant mare.

## 1. Introduction

The neonatal period is a critical phase of adaptation and adjustments to extrauterine life with the transition from respiration, circulation, digestion, metabolism and fetal elimination to neonatal and growth [1,2]. Thus, due to these changes, one of the important metabolic compounds involved in foal maturation is lipids. In this phase, metabolic instability occurs, as the newborn's state changes all the time because it is an active transition [2]. In addition, the prenatal relationship with the health of the foal is already discussed and observed [3].

Nutrition from colostrum and mare's milk is the main source of lipids for newborn foals. Creole mares demonstrate lower levels of fat in milk than other breeds [4]. The energy profile in the peripartum and its reflection in the newborn foal are important parameters to assess the physiological and/or pathological status of those involved.

The biochemical values of newborn foals change depending on their stage of life and/or health condition. Glucose, for example, has levels at birth that correspond to approximately

50% of maternal values [5]. Mild transient hypertriglyceridemia is associated with liver immaturity and values are accompanied by cholesterol [6].

Therefore, monitoring the serum lipid profile in peripartum mares and newborn foals will be useful for a more accurate diagnosis and may be applicable to the results of preventive medications. However, the available literature still lacks information on the dynamic adaptation processes that characterize the peripartum and early postnatal period.

The overall aim of this study was to evaluate lipid profile during peripartum period in Criollo breed mares and their respective neonatal foals. Specific goals evaluated in this study were (i) to describe lipid profile of Criollo mares among two days before and 3 weeks after foaling; (ii) to assess lipid profile of neonatal foals in the first three weeks; and (iii) to evaluate the correlation of peripheral blood lipid markers: triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol between mares and their respective neonatal foals.

Our hypothesis is that there is a change in the lipid profile of mares in the peripartum period and a correlation between the values of total cholesterol and fractions and triglycerides and the levels of the same lipid components in neonatal foals.

## 2. Materials and methods

### 2.1 Animals and experimental design

All mares and foals were in uniform sanitary conditions. They were housed at the Palma Farm of Federal University of Pelotas (UFPel), Capão do Leão, Brazil. The mares were kept in native grass pastures and fed commercial concentrate for maintenance with water supplied ad libitum. Foals were fed only with colostrum and maternal milk.

All procedures in the animals were approved by the Ethical Committee on Animal Experimentation of the College of Veterinary Medicine, UFPel under protocol number #1165-2016. Animal procedure herein followed the guidelines of Council for the International Organizations of Medical Sciences.

Six pregnant Criollo mares aged 7-12 years old, with body condition scores of 5-7. The general condition and pregnancy course were fully monitored by routine clinical examinations, means of physical evaluations and ultrasound during the second and third trimesters of pregnancy (5th to 11th month of gestation). After 300 days, the mares were subjected to daily monitoring, which was carried out through physical assessments and measurement of the pH of the mammary gland secretions to predict the day of foaling, ensuring collection at the right time.

Six newborn foals (four colts and two fillies) from mares. All mares had eutocic parturition with healthy foals. All foalings were closely monitored. The foals had gestation time recorded

in days and were weighed and underwent a complete physical examination after birth. Foal attitude and demeanor was carefully assessed immediately after delivery [8].

Blood samples were collected from mares by jugular venipuncture two (-2) and one (-1) days before foaling, at foaling (F), and after foaling at times: 4h, 12h, 24h hours, and 3 weeks (3w). The concentrations of triglycerides, total cholesterol (tChol), HDL-cholesterol (HDL) and LDL-cholesterol (LDL) were measured in serum by enzymatic or colorimetric methods, by spectrophotometry, following the manufacturer's recommendation, in automated biochemical equipment (Labmax Plenno, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) using a specific commercial kit (Labtest Diagnóstica SA).

Blood collections by jugular vein puncture for glucose and lactate analysis were performed in all foals at birth, 12, 24, 48 and 120 hours. Serum glucose and serum lactate measurements were performed immediately after collection in a portable reagent tape measuring device (Lactimeter Portable-Accutrend Plus, Roche®, Germany) (On Call Plus® glucometer, Acon Laboratories®, San Diego, California, USA).

Blood samples were collected from newborn foals by jugular at birth (B) (before colostrum take), and after birth at times: 4h (Foal4), 12h (Foal12) and 24h (Foal24) and 3 weeks (Foal3w). The concentrations of triglycerides, total cholesterol (tChol), HDL-cholesterol (HDL) and LDL-cholesterol (LDL) were measured in serum by enzymatic or colorimetric methods, by spectrophotometry, following the manufacturer's recommendation, in automated biochemical equipment (Labmax Plenno, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) using a specific commercial kit (Labtest Diagnóstica SA).

The collection times were chosen due to the absence of changes in the biochemical values evaluated after 48h. The days of gestation and birth weight of the neonates were also measured.

2.4. Statistical analyzes

Statistical analyzes were performed using SPSS 20.0 software. Normality was assessed using the Shapiro-Wilk test. Considering that all variables have a normal distribution, continuous data were assessed using analysis of variance (ANOVA), using a general linear model for repeated measures over time (within-subjects). Pearson's correlation test was applied to verify the correlation between lipid profile values (triglycerides, total cholesterol (tChol), HDL-cholesterol (HDL) and LDL-cholesterol (LDL) of mares and foals, time of parturition of mares and the variables of lactate and serum glucose in foals and between the collection times of these same variables in foals. Statistical significance was established at $P \leq 0.05$.

## 3. Results

In all analyses, both in mares and foals, the values have not exceed the reference levels for the species.

In the analysis carried out in mares (Figure 1), differences in total cholesterol levels were observed ($P < 0.02$), where higher levels were noted on the day of foaling (F). The same effect was observed in triglyceride levels ($P = 0.443$), but the values only differed when comparing the periods F, 4h and 3w.

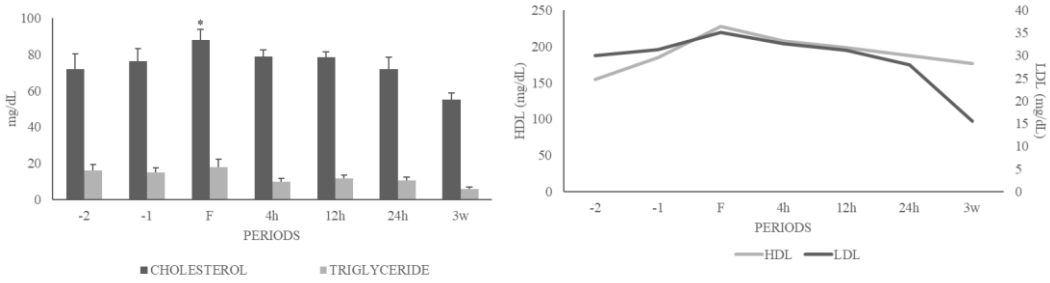


**Figure 1**. Changes in lipids profile (triglycerides, total cholesterol, HDL cholesterol (HDL), and LDL cholesterol (LDL)) of peripartum mares in the periods: -2 (2 days before foaling); -1 (1 day before foaling); F (day of foaling); 4h (4 hours after foaling); 12h (12 hours after foaling); 24h (24 hours after foaling); 3w (3 weeks postpartum), mean ± SEM, asterisk shows difference in periods by LSD test ($P < 0.05$).

The plasma LDL values highlight a sharp drop in 3w when compared to the other periods ($P < 0.02$). HDL and LDL levels were higher in the period F, but statistically the periods in which these differences were demonstrated, 24h ($P = 0.008$), as in the case of LDL, or in -1 ($P = 0.007$) and 4h ($P = 0.048$), as in the case of HDL, did not differ from the other periods (Figure 1).

Gestational length (days) averaged 354,80±13,66. The average weight of the foals was 37,950±6,327kg.

In the present study, there was a significant increase ($p < 0.01$) in blood glucose levels when comparing the time of birth (91.80 ± 22.98mg/dL) and 24 hours after (165±32.85mg/dL). On the other hand, blood lactate levels decreased significantly (4±1.48mmol/L, 2.4±0.76mmol/L). Both outcomes indicated that there was no additional significant change at other times. At birth time, there was a strong positive correlation between lactate and glucose (r=0,9968, $P = 0,000$), which does not occur at other times.

In foals (Fig. 2), plasma tChol did not differ between periods when compared to period F ($P > 0.05$). There was an increase in triglyceride plasma levels in period Foal24 followed by

a fall with a return to previous values in Foal3w ($P < 0.02$). The plasma levels of LDL and HDL had opposite courses in the analyzed periods, with an increase in HDL and a decrease in LDL in the Foal 3w ($P < 0.02$).

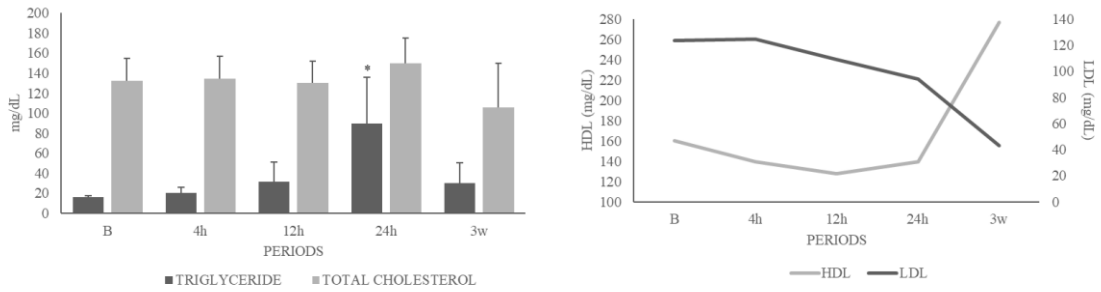


**Figure 2**. Changes in lipids profile (triglycerides, total cholesterol, HDL cholesterol (HDL), and LDL cholesterol (LDL)) of newborn foals in the periods: B (day of birth); 4h (4 hours after birth); 12h (12 hours after birth); 24h (24 hours after birth); 3w (3 weeks after birth), mean ± SEM, asterisk shows difference in periods by LSD test ($P < 0.05$).

In relation to mares and foals, a moderate correlation was found between the mare's tChol and the foal's tChol (r = 0,457, $P$ = 0,011) and LDL (r = 0,649, $P$ = 0,000) when the periods were not used. Likewise, a moderate correlation between the mare's triglycerides and the foal's tChol (r=0,433, $P$ = 0,017).

When considering the periods, the HDL of mares at parturition showed a strong negative correlation with glucose (r = -0,9964, $P \geq 0,05$) and lactate (r = -1, $P$ =0,0028) from foal to birth.

## 4. Discussion

Although within the physiological range, the serum concentration of tCho and triglycerides showed its highest values on the day of foaling and its lowest values in the 3w period in mares [5]. It is speculated that in the case of tCho this is related to an alteration that also occurs in lipoproteins linked to hepatic regulatory elements. In an endocrine way, cholesterol is used by organs such as the ovaries and the placenta for hormone synthesis during pregnancy [9,10]. After parturition, the need for these hormones is abruptly reduced and there is also an increase in the body's energy consumption causing cholesterol homeostasis, remaining within the ideal values for the mare and developing for the foal [11]. However, it is still required for synthesis of other important biological components, such as bile salts, 7-deydrocholesterol and cell membranes [12]. Triglycerides, on the other hand, are likely related to the transfer of lipids across the placenta to fetal energy requirements, which tend to be

greatest in the final third of pregnancy and have a cumulative effect [13]; fat from the mammary gland, where milk production would cause these levels to drop [10].

LDL values show similar behavior to tCho. This occurs through steroid synthesis and milk production, as the LDL pathway is the main way that cells in the ovary, placenta, and mammary gland acquire cholesterol. According to Arfuso et. al. [11], *de novo* synthesis of the mammary gland and LDL lipoproteins are responsible for the concentrations of cholesterol in milk, thus, LDL would be eliminated from the circulation in the lactation phase to compensate for the needs of the mammary glands. However, in that study, there was also a drop in LDL during pregnancy, which was not observed in the present study, which showed oscillation in values up to 24h postpartum, indicating that this need for the mammary glands may have been compensated by other factors, such as mares nutrition and good body condition score. This oscillation also occurred with HDL, which confirms the great influence of the physiological state on lipid homeostasis [14].

The final product of anaerobic glucose metabolism is lactate [15]. The placenta rapidly metabolizes glucose which consequently produces a large amount of lactate which is released into the fetal circulation causing a high concentration of lactate in the neonate [16]. Lactate is also an important carbohydrate substrate in the fetus, and this may contribute to normal periparturient hyperlactatemia [17]. Thus, it is normal for the concentration to decrease in the first 24 hours of life. This relationship also explains the strong positive correlation between lactate and glucose at the time of foaling and why there is no correlation at other times. This differs from studies that evaluated unhealthy newborn foals, where these values are negatively correlated [18].

Hypertriglyceridemia is common in neonatal foals, but this result was not observed in the present study [19]. The increase in triglycerides that occurred within 24 hours is within normal values and is believed to have occurred due to the composition of milk fat. Cholesterol levels were also within physiological values and varied as expected within the periods [19]. The hepatic pathways are not fully functional at birth and their function matures during the first two weeks of life [20]. The LDL has the function of distributing cholesterol throughout the body, where it forms plaque in the arteries due to the deposit in its inner area. Thus, HDL has the role of removing excess plaque and bringing cholesterol back to the liver [21]. Therefore, it is believed that the values of these lipoproteins have an inverse behavior over time.

Regarding the negative correlation between the HDL of the mare at birth and the glucose and lactate of the foal at birth, it is believed that it is due to the high levels of circulating cholesterol, which consequently implies a low amount of HDL at the time of birth[21]. HDL

reduces the amount of circulating glucose, which would imply a fall in the foal's values [22]. Even without the mare's glucose values, physiologically it is known that the values reflect on the foal via the placental route. Thus, low HDL values ensure a good amount of blood glucose in the mare. As stated before, lactate is the product of this metabolism, confirming this correlation.

There is no consensus on the correlation of maternal and fetal lipid values [23,24]. However, there is evidence that cholesterol can be transported from maternal to fetal circulation, one of which is the presence of plant sterols, from maternal nutrition, in the human newborn circulation [25]. Furthermore, in human studies, it was observed that even without a direct correlation between fetal cholesterol metabolism and maternal total cholesterol concentration, there was a direct correlation between fetal LDL metabolism and total cholesterol and cholesterol metabolism. LDL through the placenta [26]. Thus, our study reinforces that there is a correlation between these factors in horses.

Among the limitations of our study is the low number of animals. However, it should be noted that this herd is part of an experimental herd and, therefore, its homogeneity in handling and nutrition is guaranteed. In addition to greater control over collection times.

## 5. Conclusion

Studies in healthy neonates are extremely important to prevent possible pathologies. Our results demonstrate how the lipid profile of neonates fluctuates and can be interfered with in various ways. Pregnant mare showed an increase in cholesterol levels at day of foaling. Furthermore, our study demonstrated the relationship between total cholesterol and triglycerides in the mare and total cholesterol in the newborn foal, and total cholesterol in the mare and LDL in the newborn foal. There was an inversely proportional correlation between the levels of HDL and LDL levels in the blood of the newborn foal, which influenced the maintenance of the levels of other components, such as glucose, which correlates with lactate on the day of foaling, which demonstrates that we must be attentive to the evaluation of the moment of these components.

**Acknowledgements**

Our thanks to the Brazilian Criollo Horse Breeder Association (ABCCC) and Brazilian funding agencies CAPES, FAPERGS and CNPQ for providing scholarships to the graduate and undergraduate students.

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## 3 Considerações Finais

O período do periparto tem grande impacto na produção equina, principalmente no que diz respeito a neonatologia, uma vez que, éguas no terço final da gestação sofrem grandes mudanças corporais e metabólicas que impactam na saúde e desenvolvimento do potro.

Em nossos estudos foi possível observar que as medidas de peso e conformação da composição corpórea de éguas gestantes estão correlacionadas, e podem servir como indicador de reservas energéticas e da condição nutricional dessa categoria. Ainda, foi possível verificar que no dia do parto as éguas possuem um pico de colesterol com provável fonte relacionada as funções hormonais.

Além disso, nos potros neonatos foi observado um comportamento inversamente relacionado dos níveis de HDL e LDL sanguíneo, sendo esse comportamento importante para manutenção dos níveis de outros compostos como a glicose, por exemplo. Ainda, a correlação existente entre o lactato e a glicose observada no dia do parto demonstra que devemos nos atentar ao momento de avaliação desses componentes.

As correlações existentes entre alguns compostos lipídicos das éguas no periparto e de seus neonatos, demonstram o importante papel da saúde da égua no desenvolvimento pleno do potro. Assim, reafirma-se a importância de mais estudos para esclarecer as principais rotas fisiológicas de interação lipídica materno-fetal em éguas gestantes.

Esses estudos são o recorte de uma gama de outros experimentos realizados durante o doutorado. Houve participações em projetos de extensão, eventos sociais, participação em outros experimentos de mestrado, doutorado, iniciação científica, monitorias, trabalhos de conclusão de residência. Essas participações renderam publicações de artigos, apresentações de trabalhos, ministração de cursos, entre tantos outros aprendizados que não envolvem somente a parte acadêmica e que é extremamente importante para a formação não só de profissionais, mas de pessoas.

Ainda, foi desenvolvido um trabalho sobre estimação de peso de éguas gestantes por métodos alternativos, já em fase de patente, com colaboração de

profissionais de áreas distintas como a computação dando ênfase na importância da multidisciplinaridade para novas conquistas.

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# Anexo

## Anexo - Documento da Comissão de Ética e Experimentação Animal

UFPel
CEEA
Comissão de Ética em Experimentação Animal

Pelotas, 04 de abril de 2016

Certificado

Certificamos que a proposta intitulada "**Influência da obesidade materna em éguas gestantes sobre características metabólicas, morfométricas e comportamentais dos potros**", registrada com o nº23110.001165/2016-22, sob a responsabilidade de **Carlos Eduardo Wayne Nogueira** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de 21/03/2016.

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

Salientamos também a necessidade deste projeto ser cadastrado junto ao *COBALTO* para posterior registro no *COCEPE* (código para cadastro nº **CEEA 1165-2016**).

**M.V. Dra. Anelize de Oliveira Campello Felix**
***Presidente da CEEA***

Ciente em: ___/___/2016
Assinatura do Professor Responsável: _____