

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

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: Four body parameters of 34 Criollo-type mares in late gestation were evaluated: 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 (\pm 90 days prior to foaling) $n = 33$; F-60 (\pm 60 days prior to foaling) $n = 33$; F-30 (\pm 30 days prior to foaling) $n = 31$; F-15 (\pm 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 10 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.

DOI: 10.22456/1679-9216.110128

Received: 6 December 2020

Accepted: 7 March 2021

Published: 3 April 2021

Programa de Pós-Graduação em Veterinária (PPGV) & Departamento de Clínicas Veterinária, Faculdade de Medicina Veterinária, Universidade Federal de Pelotas (UFPEL), Capão do Leão, RS, Brazil. CORRESPONDENCE: H.C. Mazzo [hcmvet@gmail.com] & B.R. Curcio [curciobruna@hotmail.com]. Departamento de Clínicas Veterinária, Faculdade de Medicina Veterinária - UFPEL. Campus Capão do Leão s/n. CEP 96010-900 Capão do Leão, RS, Brazil.

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 [12]. Any alterations should therefore be well known in order 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 [6]. This is due to the breed's morphological competitiveness, whereby animals with greater fat accumulation and consequently more rounded aspects are better evaluated [6,8]. Additionally, its genetic kinship with the Andalus breed gives further cause to characteristics such as rusticity, better feed conversion and easy fat accumulation [10]. Such characteristics become even more apparent during pregnancy, a period when mares tend to gain more weight [6].

Body fat distribution and weight are often used as indicators of adequate nutrition and energy reserves during late pregnancy [5]. 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, 4 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 in 2 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: $\text{heartgirth}^2 + \text{BL} / 11880$ as described elsewhere [13].

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 were evaluated with spectrophotometric diagnostic kits using an automated analyser (Labmax Plenno®)².

Biometric monitoring and blood samples were taken over 5 periods, starting at 7 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 analysis 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 5 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 ($P > 0.05$) [Table 1].

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 ($r = 0.254$; $P = 0.003$).

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 ($r = 0.228$; $P < 0.01$).

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

Parameter	Periods*					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 (\pm at day of foaling).

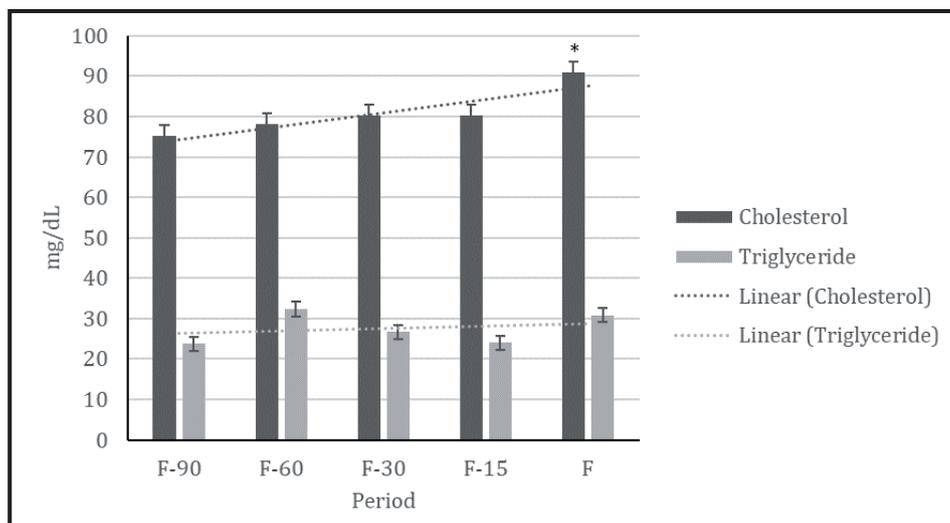


Figure 1. Means and standard deviation in triglyceride and cholesterol values in the periods: F-90 (± 90 days prior to foaling); F-60 (± 60 days prior to foaling); F-30 (± 30 days prior to foaling); F-15 (± 15 days prior to foaling); Foaling (\pm on the day of foaling). Asterisk shows difference in periods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

DISCUSSION

Mares in the late gestation showed an increase in total cholesterol between 15th 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.* [11] 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 [4,12]. However, it 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 [3,12]. 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 *et al.* [7] 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, 0.8 mm. The maximum value of our animals reached up to 0.889 mm of retroperitoneal fat and 1095 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 no observed differences in 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 [9]. 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.* [7], 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 an 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

¹Pie Medical Inc. Maastricht, Netherlands.

²Labtest Diagnóstica S.A. Vista Alegre, Lagoa Santa, MG, Brazil.

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

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.

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.

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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