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# Serum levels of mitochondrial uncoupling protein 1, leptin, and lipids during late pregnancy and the early postpartum period in mares

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ABSTRACT

The aim of this study was to investigate the changes of serum mitochondrial uncoupling protein 1 (UCP1) and leptin levels as well as of lipid and lipoprotein profiles in mares during the peripartum period. Ten pregnant mares (group A) were monitored from 15 ± 3 days of pregnancy until 15 days after foaling, and 10 nonpregnant nonlactating mares constituted the control (group B). In group A, blood sampling was performed on Days 15 ± 3 and 7 ± 3 before foaling, on the day of foaling, and on Days 7 and 15 after foaling. In group B, blood sampling was performed on the same days as in group A. Serum levels were determined for UCP1, leptin, total lipids, phospholipids, triglycerides, total cholesterol (Total-Chol), high-density lipoproteins, low-density lipoproteins (LDLs), and very low–density lipoproteins (VLDLs). Two-way repeated-measures ANOVA was applied to evaluate the effects of peripartum period and group membership. All studied parameters except phospholipid levels ($P > 0.05$) showed significant changes in group A over the peripartum period ($P < 0.0001$). A significant effect of pregnancy was found on all studied parameters ($P < 0.001$), which showed lower levels in group A than in group B for most of the time points considered. Significant negative correlations were found between UCP1 and total lipids, triglycerides, VLDLs, Total-Chol, and LDL values. Positive correlations were found between leptin and total lipids, triglycerides, VLDLs, Total-Chol, and LDLs. These changes observed in mares during the peripartum period could represent a response to hormonal and metabolic adaptations occurring during specific physiological conditions such as late pregnancy and early postpartum. These changes should compensate for the energy loss occurring during these particular life phases and ensure a good body condition to protect mares against negative energy balance.



## 1. Introduction

During pregnancy, the maternal body system is adapted to ensure fetus growth and development [1]. Despite the action of homeostatic mechanisms to maintain blood parameters within physiological levels, the biochemical changes that occur throughout the peripartum period [2] make mares physiologically unstable [3] and more susceptible to a number of metabolic diseases at this stage than during other life periods [3]. In the recent years, hyperlipidemia has become a more frequent pathological condition during the peripartum period, and variation in leptin levels might be related to disturbances in lipid metabolism [4]. Leptin, a tissue hormone involved in the regulation of energy balance, is secreted mainly by adipocytes [5]. In the horse, leptin concentration in peripheral blood is notably related to fat mass and body condition score (BCS) [6,7]. The main effect of this hormone is reduction in food intake through appetite suppression [8,9]. In recent years, leptin has been shown to act on various organs and to be produced not only by adipose tissue but by other peripheral tissues as well [10].

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The finding that leptin is synthesized in the placenta [11,12] raises the interesting hypothesis that it is involved in the complex biology of pregnancy. It has been suggested that high serum leptin levels correspond positively with reproduction ability in mares [13,14]. Despite the numerous studies in this field, leptin blood levels do not appear to be related to implantation, gestation, and parturition [15]; rather, leptin could be a key player in the regulation of maternal nutrition, more specifically during pregnancy [15]. Basically, higher levels of leptin may lead to a decrease in food intake and to an increase of energy expenditure by stimulating brown adipose tissue through the sympathetic nervous system and directly influencing the expression of the mitochondrial uncoupling protein 1 (UCP1) and increasing lipolysis and fatty acid oxidation [16–18]. The UCP1, enriched in brown adipose tissue, is a regulated proton carrier that dissipates the mitochondrial membrane potential generated by the respiratory chain, uncoupling ATP synthesis from respiration and releasing heat from oxidation of substrates [19]. The UCP1 plays important roles in metabolic and energy balance and regulation, in cold- and diet-induced thermogenesis and in decreasing oxidative stress associated with the pathogenesis of obesity [19]; its activity is regulated by purine nucleotides (inhibitors) and by fatty acids (activators) [19]. Although many research bodies dealt with several aspects of a mare's physiology during the peripartum period [20–24], there is a lack of information concerning the changes of serum leptin and UCP1 values as well as serum lipids and lipoproteins values of mares in response to the dynamic adaptation processes that characterize the peripartum period. In view of this, the aim of this study was to investigate the changes of serum UCP1 and leptin values and of lipid and lipoprotein profiles in mares during the peripartum period. We also aimed to assess whether there is a correlation between serum UCP1 and leptin levels as well as between serum UCP1 or leptin levels and the levels of various lipid components throughout the peripartum period.

## 2. Materials and methods

### 2.1. Animals

Twenty clinically healthy mares (seven Italian Saddle, seven Thoroughbred, and six Standardbred), aged between 8 and 12 years, were enrolled in the present study. Animals from the same breeding center were housed in individual straw-bedded boxes (latitude 37.46 N; longitude 14.93 E) under natural spring photoperiod (sunrise 5:15 AM and sunset 7 PM). Ten pregnant mares (group A) were monitored from 15 ± 3 days of pregnancy until 15 days after foaling, and 10 nonpregnant nonlactating mares constituted the control (group B). Table 1 summarizes the distribution of animals selected for experimental and control groups. Body condition score and body weight (BW) measurements were performed on both groups throughout the monitoring period. Body condition scoring was performed by the same operator using a 1-to-9 scale [25]. Body weight was measured by means of a weighting platform (PS3000HD Heavy Duty Floor Scale, Breckwell, UK). All the pregnant mares delivered between mid-April and mid-May, with the mean gestation length being 340 ± 9 days. After parturition, the mares were subjected to clinical examinations over three consecutive days, and ultrasound examinations (M-Turbo; FUJIFILM SonoSite, London, United Kingdom) were weekly performed to monitor the uterine involution and ovarian activity. Mares from groups A and B were fed the same diet but in a different dried grass hay and concentrate ratio. In particular, group A received 6 ± 1 kg/day dried grass hay (crude protein 9%, crude fiber 35%, Ca 0.4%, P 0.23%) and 5 ± 0.5 kg/day commercially available concentrates (crude protein 16%, crude fat 6%, crude fiber 7.35%, ash 10.09%, Ca/P 1.5:1, Na 0.46%, lysine 0.85%, methionine 0.35%, omega-3 0.65%). Group B received 5 ± 0.5 kg/day hay and 2 ± 0.5 kg/day concentrates. Water was available ad libitum. All animals were housed in individual boxes (4.0 × 4.0 m), and they were moved to paddocks from 10 AM to 4 PM daily.

All treatments, housing, and animal care were carried out in accordance with the standards recommended by European Union Directive 2010/63/EU for animal experiments.

**Table 1**
Breed and age (y) of pregnant (group A) and nonpregnant (group B) mares.

| Breed | Age (y) | | Parity | Gestation length (days) |
|---|---|---|---|---|
| | Group A | Group B | Group A | Group A |
| Standardbred | 9 | 10 | + | 337 |
| Standardbred | 10 | 12 | + | 345 |
| Standardbred | 8 | 8 | – | 344 |
| Italian Saddle | 12 | 10 | + | 346 |
| Italian Saddle | 12 | 12 | + | 333 |
| Italian Saddle | 10 | 8 | + | 358 |
| Italian Saddle | | 10 | | |
| Thoroughbred | 10 | 11 | + | 341 |
| Thoroughbred | 8 | 8 | – | 339 |
| Thoroughbred | 11 | 9 | + | 335 |
| Thoroughbred | | 9 | + | 325 |
| Mean ± SD | 10 ± 2 | 10 ± 1 | | 340 ± 9 |

Gestation length (days) and parity (+, multiparous; –, primiparous) have been indicated for group A.
Abbreviation: SD, standard deviation.

### 2.2. Data collection

In group A, blood sampling was collected by jugular venepuncture into two 10-mL tubes containing clot activators (Terumo Corporation, Tokyo, Japan), on Day 15 ± 3 and 7 ± 3 before foaling (−2, −1) in the morning (7 AM), at foaling day (P) and on Days 7 and 15 after foaling (+1, +2). In group B, blood sampling was performed at the same clock times as group A. Samples from the first tube were

**Table 2**
Mean values of body weight (BW) and body condition score (BCS) obtained from pregnant (group A) and nonpregnant (group B) mares during the monitoring period.

| Mares | Measurement | Weeks relative to parturition | | | | |
|---|---|---|---|---|---|---|
| | | −2 | −1 | P | +1 | +2 |
| Group A | BW (kg) | 643 | 657 | — | 579† | 578† |
| | BCS | 6.75 | 6.22 | — | 6.54 | 5.99 |
| Group B | BW (kg) | 575* | 576* | 575 | 575 | 575 |
| | BCS | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 |

Significances ($P < 0.0001$): (between group) * versus group A; (within group) † versus −2and −1.