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Hepcidin, ferritin and iron homeostasis in pregnant Spanish Purebred mares

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ABSTRACT

During pregnancy, maternal erythropoietic expansion and fetal development require greater mobilization of available iron (Fe) stores. These adjustments in Fe metabolism in humans and rodents are largely mediated by the hormone hepcidin (Hepc), which controls the expression of ferroportin (Fpn), a transporter responsible for exporting Fe from stores to extracellular fluid and plasma. These mechanisms based on the regulation of Hepc on the availability of Fe during gestation in healthy mares remain unknown.

The objective of this study was to determine the existence of interrelationships among concentrations of Hepc, ferritin (Ferr), Fe, and estrone (E_1) and progesterone (P_4) in Spanish Purebred mares along the whole gestation. Blood samples were taken from 31 Spanish Purebred mares each month, during 11 months of pregnancy. Fe and Ferr significantly increased and Hepc decreased during pregnancy ($P < 0.05$). The secretion peak of estrone (E_1) was reached in the 5th month and progesterone (P_4) between the 2nd and 3rd months of gestation ($P < 0.05$). Fe and Ferr were weakly positively correlated ($r = 0.57$; $P < 0.05$). Fe and Ferr were negatively correlated with Hepc ($r = -0.80$ and $r = -0.67$, respectively) ($P < 0.05$). P_4 was positively correlated with Hepc ($r = 0.53$; $P < 0.05$). Pregnancy in the Spanish Purebred mare was characterized by a progressive increase in Fe and Ferr and a reduction in Hepc concentrations. E_1 was partially responsible for the suppression of Hepc; on the other hand, P_4 induced its stimulation during pregnancy in the mare.

1. Introduction

Iron (Fe) is an essential micronutrient required by all tissues for metabolic and cellular processes [1]. Iron requirements substantially increase during pregnancy to ensure both the feto-placental unit development and the maternal erythropoietic expansion [2]. To meet these demands, the maternal and fetal iron homeostasis change [3–7], the absorption from the diet increases and its cellular stores, metabolism and regulation dynamically occur. The regulation of the availability of this micronutrient depends, at least in part, on maternal hepcidin (Hepc) [4,8].

Hepc is an iron-regulatory hormone produced by the liver that controls plasma Fe concentrations and tissue iron distribution [8,9]. Hepc inhibits the major Fe flow into the plasma, represented by the intestinal Fe absorption, the release by macrophages (that recycle Fe from aged red blood cells), and the mobilization of Fe stored in hepatocytes. Hepc exerts its effects through its receptor, the iron exporter ferroportin (Fpn). Fpn is expressed in all tissues that actively export Fe to plasma [1]. Once Hepc binds to Fpn, its degradation is triggered, resulting in Fe sequestration in target cells and reduced Fe flux to plasma. Therefore, Fe delivery to consumptive tissues, such as bone marrow, placenta and fetus, is inversely correlated with Hepc concentrations.

The finding on serum Hepc concentrations and their dynamic changes during pregnancy are limited. Hepc concentrations gradually decline along the pregnancy, reaching the lowest and even undetectable levels in the third trimester in humans [10–12] or during the third week of gestation in rats [13].

The reduction of maternal Hepc increases intestinal absorption and cell mobilization of Fe, therefore maximizing Fe disponibility for the pregnant mother and transplacental transfer to the fetus [12,14,15]. In pregnant women maternal Hepc is positively

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associated with placental transfer of iron derived from dietary heme and nonheme sources [3]. On the other hand, during the gestational period, Hepc is positively correlated with Ferr saturation, transferrin, and hemoglobin concentrations [10,16–18], suggesting the regulation of Hepc by Fe status and preservation of maternal erythropoiesis during pregnancy [19]. Fetal Fe requirements, which increase over pregnancy and become the highest in the 3rd trimester, were proposed as a possible factor for the progressive decrease in maternal Hepc levels [14,20]. What is more, experimental data indicate that Hepc may be directly transcriptionally regulated by estrogens and that steroid hormones may directly upregulate Hepc expression [21–23]. In fact, Fe and Ferr concentrations are inversely correlated with placental hormones during the first period of gestation in woman [24].

In equines, research on Hepc is limited, being related only to hypoferremia in horses treated with lipopolysaccharides [25], with Freund's adjuvant [26] and with pentosan polysulfate [27]. In these cases, hypoferremia was associated with upregulated expression of hepatic mRNA Hepc, which causes Fe accumulation in macrophages and reduces intestinal iron absorption without evidence of anemia or inflammation [24]. The cloning and sequencing of mRNA Hepc in equids were reported by Oliveira-Filho et al. [28].

At present, the knowledge related to circulating Hepc during healthy whole pregnancy in mares is unknown. Given that Hepc regulates the maternal systemic bioavailability of Fe and that this hormone is regulated by placental hormones, it is important to evaluate the interrelationships among the conventional parameters of iron status indicators (Fe, Ferr, Hepc), and concentrations of estrone (E_1), and progesterone (P_4) in normal pregnancies in order to improve knowledge about the physiology of Fe homeostasis during the whole pregnancy in the equine species.

2. Materials and methods

2.1. Mares

All methods and procedures used in this study were in compliance with the guidelines of the Spanish law (RD 37/2014) that regulated the protection of animals used for scientific purposes. The Animal Ethics Committee for the Care and Use of Animals of the CEU-Cardenal Herrera University (Spain) concluded that the proposed study did not need ethical approval, since this experiment was part of the clinical evaluation of the animals at this period of the cycle.

A total of 31 reproductive Spanish Purebred mares, aged between 4 and 17 years old, were studied during the whole pregnancy. The criteria for inclusion of mares in this research were: absence of infections or diseases that required hospitalization from at least 1 month prior to sampling, absence of antibiotic or glucocorticoid treatments within the sampling period, absence of supplement with vitamins or iron for 3 months prior to the examinations, and to be submitted to an appropriate vaccination and deworming program. All mares were healthy and were bred under the same handling conditions, receiving the same diet and reproductive management. During the sampling period, the mares' diet was a combination of fiber and compounded food, divided into two meals a day. The fiber consisted of 2–3 kg of alfalfa hay and straw, with a natural iron content of 30 to 1.200 mg/kg and 30–300 mg/kg, respectively (Table 1). Mares based their diet on hay ad libitum (equivalent to approximately 7.5 kg) and 4 kg of concentrated feed based on barley, oats, corn and wheat during the first 8 months of gestation. From this moment until the moment of delivery, they were supplemented using a special feed (Pavo®) at a dose of 0.42 kg per 100 kg of live weight per day. In addition, mares generally eat everything but that 100 g can remain in one of the feeders. Hay is usually eaten whole. Water was given ad libitum.

Table 1
Type of diet, content, quantities, distribution, and supplementation.

Composition/kg of food	Administered (/100 kg BW)
Alfalfa pellets	**1.25 kg**
Vitamin E 20 mg	25.00 mg
Biotin (Vit.H) 0.2 mg	0.25 mg
Vitamin D3 910 IE	1137.50 IE
Calcium 15 g	18.75 g
Phosphorus 3.5 g	4.38 g
Magnesium 1.8 g	2.25 g
Sodium 0.6 g	0.75 g
Potassium 25 g	31.25 g
Iron 273 mg	341.25 mg
Copper 7 mg	8.75 mg
Manganese 26 mg	32.50 mg
Zinc 17 mg	21.25 mg
Selenium 0.2 mg	0.25 mg
Concentrated feed	**0.67 kg**
Calcium 9.00 g	6.00 g
Phosphorus 4.00 g	2.67 g
Sodium 5.00 g	3.33 g
Potassium 1.20 g	0.80 g
Magnesium 5.00 g	3.33 g
Copper* 40 mg	26.67 mg
Iron* 130 mg	86.67 mg
Zinc* 150 mg	100.00 mg
Manganese* 110 mg	73.33 mg
Selenium 0.4 mg	0.27 mg
Iodine 0.9 mg	0.60 mg
Vitamin A 15000 IE	10000.00 IE
Vitamin E 250 mg	166.67 mg
Vitamin B1 15 mg	10.00 mg
Vitamin B2 15 mg	10.00 mg
Vitamin B6 10 mg	6.67 mg
Vitamin B12 150 ug	100.00 ug
Vitamin K3 4 mg	2.67 mg
Pantothenic acid 20 mg	13.33 mg
Choline 500 mg	333.33 mg
Folic acid 10 mg	6.67 mg
Niacin 36 mg	24.00 mg
D-Biotin 250 ug	166.67 ug
Lysine 6.5 g	4.33 g
Methionine 2.4 g	1.60 g
PAVO*from 8th month of gestation	**0.42 kg**
Vitamin A 16.2 IU	6.80 IU
Vitamin D3 2.7 IU	1.13 IU
Vitamin E 383 mg	160.86 mg
Vitamin K3 4 mg	1.68 mg
Vitamin B1 41 mg	17.22 mg
Vitamin B2 18 mg	7.56 mg
Niacinamide 32 mg	13.44 mg
Pantothenic acid 23 mg	9.66 mg
Vitamin B6 11 mg	4.62 mg
D-Biotin 576 mg	241.92 mg
Folic acid 9 mg	3.78 mg
Copper 71 mg	29.82 mg
Iron 129 mg	54.18 mg
Zinc 281 mg	118.02 mg
Manganese 178 mg	74.76 mg
Selenium 0.8 mg	0.34 mg
Iodine 1.1 mg	0.46 mg

*considering the mean values of BW paired to 600 kg.

2.2. Blood withdrawal

Venous blood samples were extracted from 31 reproductive Spanish Purebred mares each month, during the 11 months of gestation. All the samples were taken before meals. All mares became pregnant in late February, March and early April. Mean pregnancy length was of 330.1 ± 10.1 days (range: 290–348 days). The last blood samples were taken 7–15 days before parturition.

After foaling, all mares were in the lactation period and had foals on their sides.

Blood collections were always performed by jugular venipuncture between 8:00 and 11:00 a.m., using 20 mL disposable syringes, with luer cone (Becton Dickinson Discardit® II) attached to 40 mm. 18–20 G needles (Sterican®, Braun Melsungen AG). A total of 20 mL was collected and each blood sample was added to glass tubes with clot activators and PS granules to collect serum (Tapval®). Samples were refrigerated at 4 °C for transport, and then were centrifuged at 3500 rpm for 10 min (P Selecta® Centrifuge); the serum obtained was stored at −20 °C until analyzed.

Fe and Ferr (μg/dl) concentrations were analyzed using a Spin 200E spectrophotometer using commercial house reagents based on colorimetry for Fe (FerroZine) and turbidimetry for Ferr (Latex) (Spinreact®, Barcelona, Spain). The sample detection limits for Fe y Ferr were 0.850 μg/dL to linearity limit of 1000 μg/dL, and 5.04 μg/l, respectively. The intra- and interassay coefficients of variation (CVs) were of 0.79% and 3.17%, and of 5.1% and 6.3%, for Fe and Ferr, respectively.

Serum Hepc (ng/dl) concentration was measured according to the manufacture's guideline of horse hepcidin (HEPC) Enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource.com, San Diego, CA, USA). The detection range was of 15.6 ng/ml-500 ng/ml, the sensivity of 2.0 ng/ml and both intra- and interassay CVs rate was <15%.

Serum E_1 concentrations (ng/ml) were also measured by RIA (DSL-5400, Diagnostic Systems Laboratories, Texas, USA). The sensitivity of the assay was 0.03 nmol/L, the intra- and inter-assay CVs were 3.1 and 7.9%, respectively. Serum P_4 concentrations (ng/ml) were determined using a solid-phase I-125 radioimmunoassay (RIA) (Coat-a-Count Progesterone, Diagnostic Products Co., Los Angeles, LA, USA). The minimal assay sensitivity of P_4 was 0.1 ng/ml. The inter- and intraassay CVs were 16.1% and 4.3% at 3.5 ng/mL, 7.3% and 8.5% at 22.5 ng/ml, and 23.3% and 6.4% at 54.8 nmol/l, respectively.

2.3. Statistical analysis

The normality and homoscedasticity of the data were verified using the Kolmogorov-Smirnov and Levene test. Logarithmic transformations of the data were performed to achieve homogeneity of variance. Differences of Fe, Ferr and Hepc among months of pregnancy were performed using repeated-measures ANOVAs test. Tukey HDS post hoc comparison test was used to determine the difference between pairs of months. The interrelationships between Fe, Ferr, Hepc, E_1 and P_4 were examined by linear regression analysis and the correlation was expressed by Pearson's correlation coefficients. The level of significance was $P < 0.05$.

3. Results

Compared to the 1st month, Fe and Ferr concentrations increased from the 2nd and the 3rd month ($P < 0.05$), remaining constantly higher from the 4th to the 8th month ($P < 0.05$), increasing progressively and significantly from the 9th to the 11th month of gestation ($P < 0.05$); Hepc significantly decreased from the 2nd to the last month of pregnancy ($P < 0.05$) (Table 2).

Compared to the 1st month, E_1 (Table 2) concentrations significantly increased from the 2nd to the 6th month ($P < 0.05$), and significantly decreased from the 7th month until the end of gestation ($P < 0.05$), with the higher values than those of the 1st month of gestation ($P < 0.05$). The maximum P_4 concentrations were reached in the 3rd and 4th month compared to other periods ($P < 0.05$) (Table 2).

In order to have a synthetic vision of the results, in Figs. 1 and 2 data for Fe, Ferr and Hepc, and for E_1 and P_4 were respectively grouped into 3 trimesters (first, second and third trimester paired to 9 months) and into the last values of the 10–11 months of gestation.

Fig. 3 shows the correlation coefficients between all parameters considered in the study. Fe and Ferr were weakly positively correlated ($r = 0.57$; $P < 0.05$) (Fig. 3a). Fe and Ferr were negatively correlated with Hepc ($r = -0.80$ and $r = -0.67$, respectively) ($P < 0.05$) (Fig. 3b and c). P_4 was positively correlated with Hepc ($r = 0.53$; $P < 0.05$) (Fig. 3d).

4. Discussion

4.1. Effects of pregnancy on the Fe and Ferr homeostasis

As previously documented by Satué and Montesinos [29], serum Fe and Ferr concentrations significantly increased along pregnancy in Spanish Purebred mares. These results contrast with those observed in other animal species, as ewes [30], cows [31], sows [32], goats [33], and buffaloes [34]. In these later species significantly lower values of Fe and Ferr in the 2nd and 3rd period compared to the 1st period were found.

In addition, in both iron-supplemented and non-supplemented pregnant women, Ferr concentrations display a U-shaped curve, with decreasing concentrations in the 1st and 2nd trimester, a nadir at 35–38 weeks of gestation, and an increase in the postpartum period [35]. It has been hypothetized that the rapid fetal and placental growth, the increase in maternal erythrocyte mass and the hemodilution during pregnancy induce a decreased maternal Fe and Ferr concentrations [36,37]. The hemodilution produced by the expansion of plasma volume in woman is estimated at 46–50% and it is established in the 2nd trimester of gestation to facilitate uteroplacental arterial flow and adequate fetal growth [38]. It is believed that the fetal adrenal glands may initiate the increase in blood volume by delivering the estrogen precursor hormone dehydroepiandrosterone (DHEA) to the placenta. Estrogens in turn stimulate angiotensin production by the liver, which an increase of aldosterone production, and therefore, plasma retention [39]. Plasma volume expansion during pregnancy causes reductions in the concentrations of many analytes, including hemoglobin, being a potential mechanism for reduced Ferr levels in these species [40]. Then, physiological anemia due to dilution appears between weeks 30 and 34 of gestation, that is, at the end of the 2nd and beginning of the 3rd trimester. Then, to meet these demands, the absorption of Fe from the diet [3,4] and the efficient mobilization of reserve tissues, such as the liver and spleen [5–7], increase availability for transfer across the placenta and maternal metabolism [4,8].

The increase of free and stored Fe levels during the last two periods of pregnancy in Spanish Purebred mares, without exceeding the reference intervals established for healthy adult horses (range: 105–277 μg/dL) [41], could indicate that mares have adequate Fe stores to meet the demands imposed by the fetus developing and feto-placental unit. The pattern shown by serum Fe and Ferr seems to indicate that pregnant mare is quite efficient at regulating iron levels. This could explain that in mares receiving diets with around 80% of energy requirements, maternal malnutrition does not seem to affect intrauterine or early postnatal growth of the foal [42]. Placental adaptations, through increased vascularization and the transport and metabolism of amino acids, vitamins and minerals, seem to be sufficient to sustain fetal growth during the last stage of gestation [43].

Based on the coefficient of correlation between the Fe of the mother and the newborn paired to 0.827, Upadhyaya et al. [44] showed that maternal Fe status influences the Fe level of the newborn; therefore, a mother with adequate iron status tends to

Table 2
Concentrations of iron, ferritin, hepcidin, estrone, and progesterone (mean ± SD) in Spanish Purebred mares during 11 months of pregnancy.

Month	Fe (μg/dL)	Ferr (μg/dL)	Hepc (μg/dL)	E_1 (ng/mL)	P_4 (ng/mL)
1	136.4 ± 14.1	83.60 ± 6.37	158.4 ± 7.67	57.1 ± 19.4	34.9 ± 13.1
2	148.3 ± 13.9*	147.9 ± 3.42*	140.8 ± 7.69*	133.8 ± 50.9*	24.4 ± 10.2
3	142.9 ± 14.8*	163.2 ± 3.14*	136.6 ± 14.9*	326.4 ± 164.1*	38.1 ± 20.1**
4	137.8 ± 11.8*	148.8 ± 6.27*	126.6 ± 11.3*	854.1 ± 192.9*	39.1 ± 22.7**
5	138.5 ± 15.2*	149.1 ± 4.17*	116.7 ± 15.4*	1168.5 ± 261.7*	19.3 ± 3.82
6	151.4 ± 14.2*	155.7 ± 6.85*	97.4 ± 10.0*	843.6 ± 179.4*	13.9 ± 4.02
7	162.7 ± 9.70*	151.0 ± 9.86*	86.9 ± 9.28*	691.5 ± 156.5*	12.2 ± 3.71
8	167.6 ± 12.5*	153.5 ± 1.55*	79.7 ± 4.11*	593.6 ± 176.4*	12.1 ± 2.95
9	175.7 ± 18.9*	159.8 ± 10.0*	76.4 ± 15.4*	445.3 ± 286.2*	11.4 ± 3.13
10	182.1 ± 20.3*	169.1 ± 9.03*	74.3 ± 20.9*	232.0 ± 114.4*	11.5 ± 3.81
11	172.5 ± 18.1*	196.4 ± 11.6*	63.7 ± 26.2*	122.3 ± 69.7*	17.5 ± 8.19

Fe: iron; Ferr: ferritin; Hepc: hepcidin; E_1: estrone and P_4: progesterone.
Symbol indicates significant differences versus the 1st month: *($P < 0.05$).
Symbols indicate significant differences versus the other months: ** ($P < 0.05$).

produce newborns with correspondingly adequate iron levels. Indeed, it has been documented in the newborn that Ferr serves as a fetal Fe reserve and it is closely related to the mother's Ferr concentrations [45]. Taking into consideration these evidences, it could be suggested that the supplementation with this type of mineral is not necessary in these pregnant mares. Indeed, hay is known to be rich enough in Fe to meet the needs of the horse [46].

Although Fe and Ferr were correlated, the correlation was not very close between them, suggesting that plasma Fe levels do not depend entirely on body stores, so that other mechanisms, such as increased absorption in the diet could be present. Pregnant mares must consume sufficient nutrients both to maintain their own body weight throughout the pregnancy and also to create new tissues, those of the fetus and tissues that support the pregnancy such as the placenta. The Fe requirement is estimated to be 50 mg/kg of diet for pregnant mares [46]. It is known that the fetus indirectly receives Fe from the maternal circulation through a rapid and unidirectional process. Uteroferrin is the carrier in which the mare mediates maternal fetal transport of the Fe required for hemoglobin production and other enzyme cofactors in the fetus [47,48]. Uteroferrin remained detectable in almost every endometrial gland and taken up by areolas of the chorioallantoides from the time of endometrial cup development to day 309 of gestation, highlights the obvious need for iron transport across the placenta throughout gestation [49,50]. Iron deposition in fetal tissues is minimal during the first 4 months of pregnancy, but a need arises from the 5th month. Indeed, approximately 37, 38, and 92 mg of iron are deposited each day in the fetus and fetal membranes during months 9, 10, and 11 of pregnancy, respectively. In a 500-kg mare, this translates to 74, 76, and 184 μg/kg of mare weight. During the first 8 months of pregnancy, 80 mg/kg are necessary, increasing from this time until the last 2 months of pregnancy to 100 mg/kg

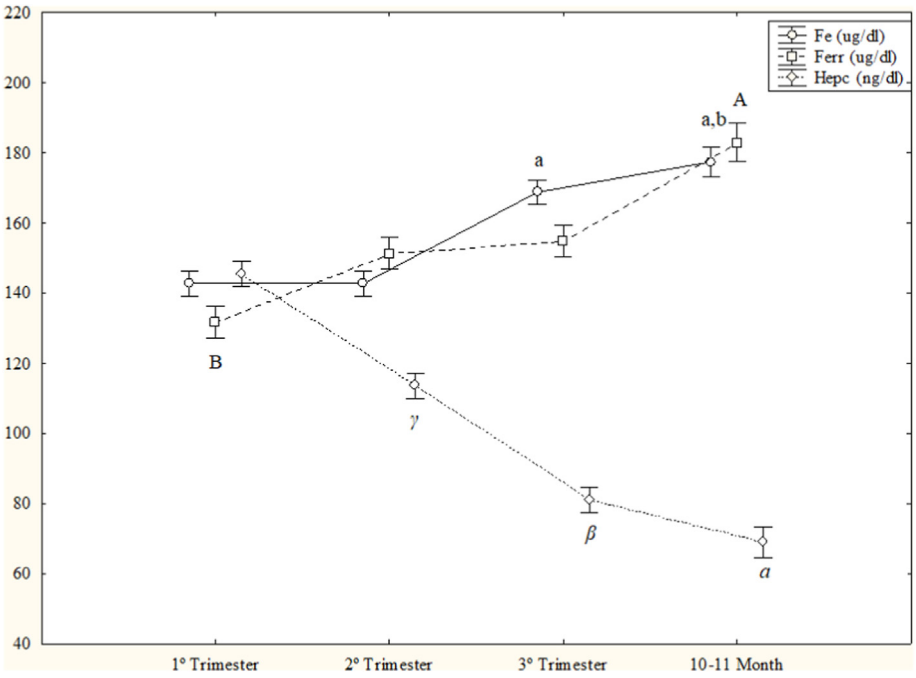

Fig. 1. Circulating iron, ferritin and hepcidin concentration (mean ± SD) in pregnant Spanish Purebred mares grouped into 3 trimesters (paired to 9 months) and the joint values of the 10–11 months of gestation.
Letters indicate significant differences paired to $P < 0.05$.
Fe: a = vs 1st and 2nd Trimester; b = vs 3rd Trimester.
Ferr: A = vs 1st - 3rd Trimester; B = vs 2nd and 3rd Trimester.
Hepc: α = vs 1st - 3rd Trimester; β = vs 1st and 2nd Trimester; γ = vs 1st Trimester.

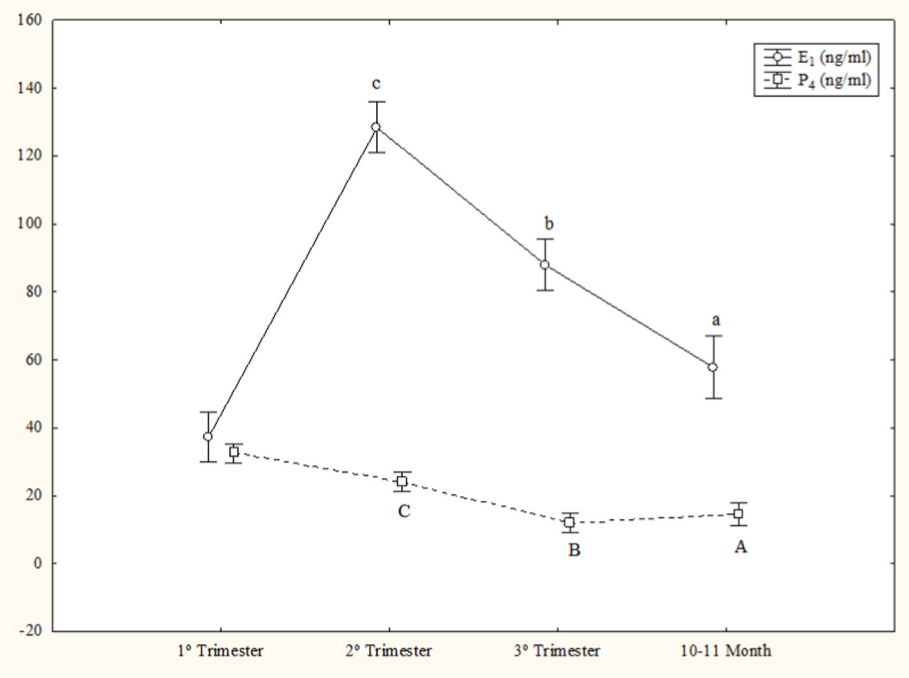

Fig. 2. Circulating oestrone sulfate and progesterone concentrations (mean ± SD) in pregnant Spanish purebred mares grouped into 3 trimesters (paired to 9 months) and the joint values of the 10–11 months of gestation.
Letters indicate significant differences paired to $P < 0.05$.
E_1: a = vs 2nd and 3rd Trimester; b = vs 1st and 2nd Trimester; c = vs 1st Trimester.
P_4: A = vs 1st Trimester; B = vs 3rdTrimester and 10–11 Months.

[46].

While increases of Fe and Ferr characterized the gestation in Spanish Purebred mares, in women, high Ferr levels in the 3rd trimester of pregnancy are indicative of a risk of preterm delivery associated with intrauterine infection and failure to expand the plasma volume during pregnancy. Ferr production is increased due to infection and inflammation as part of the mechanism of the acute phase response. In the presence of infection, macrophages produce inflammatory cytokines that generate reactive oxygen species, releasing free Fe from Ferr [51]. Since high Ferr concentration is a marker of both high iron and inflammation, it is unclear whether the adverse outcomes associated with high maternal Ferr are caused by high Fe, inflammation, or a combination of both, and which pathways they mediate fetal injury. Both a model of lipopolisacharides-induced sepsis and another model of diet-induced obesity developed embryotoxicity in pregnant woman.

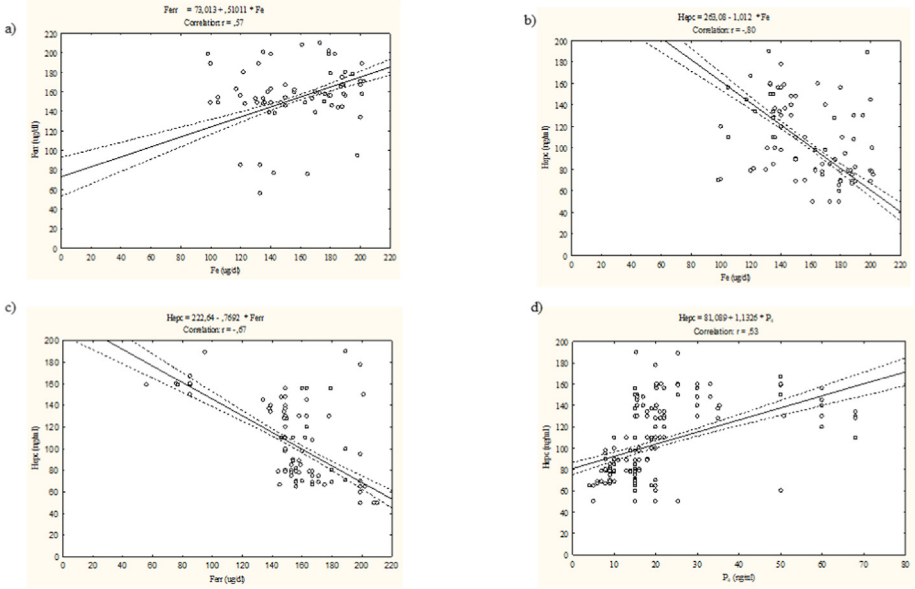

Fig. 3. Correlation coefficients between Fe and Ferr (a), Hepc and Fe (b), Hepc and Ferr (c), and Hepc and P_4 (d) in 31 pregnant Spanish Purebred mares.

The underlying mechanism has been linked to Fe-induced oxidative stress, which sensitizes the endothelium in the placenta and embryo to tumor necrosis factor, (TNFα)-mediated apoptosis [52]. These mechanisms remain unknown in the mare. However, these events could be similar to what happens in mares with placentitis, since concentrations of acute phase protein, such as amyloid type A serum (SAA), are increased after inoculation of the bacteria until the moment of abortion [53] compared to mares without placentitis in which SAA levels are undetectable [54].

Several genes related to Fe-binding proteins in the fetal (chorioallantois) and maternal (endometrium) components including *uteroferrin*, *FTH1* (ferratin heavy chain 1), *HBA2* (hemoglobin alpha subunit 2), *LCN2* (lipocalin 2) and SERPINA14 (serpina family A member 14) have been identified by RNA-Seq in normal equine placentas [55]. Furthemore, non-coding RNAs (ncRNAs) play a regulatory role in placental function. In pregnant mares, most ncRNAs have been identified in the endometrium and chorioallantois, and include members of the microRNA (miRNA) group. RNA isolated from these tissues by RNA-Seq has succeeded in identifying more than 3.000 genes in Nocardioform placentitis, and almost 3.000 in the chorioallantois, and 1.000 in the endometrium in mares with ascending placentitis [56]. Given that these genes seem to be related to pregnancy disorders mediated by inflammation, apoptosis, and hypoxia, among others [56], it is possible that Fe-binding proteins may be altered, leading to alterations in Fe transfer to the fetus. However, future studies are necessary to verify these mechanisms in the mare.

4.2. Effects of pregnancy on the Hepc homeostasis

In pregnant Spanish Purebred mares Hepc progressively decreased during pregnancy in accordance with healthy human [2,10] and rodent species [13,57] in which maternal Hepc levels are profoundly decreased in the 2nd and 3rd trimester of pregnancy. In humans, a cross-sectional study compared the levels of Fe, Hepc, and other hematological parameters between non-pregnant and healthy pregnant women in the 1st and 2nd trimesters of gestation, finding that Hepc increases in the 1st trimester compared to non-pregnant women, even if it subsequently decreased in the 2nd quarter [12]. Although the mechanisms responsible of decreasing of Hepc are unknown, they are regulated by maternal Fe status [12,58]. Indeed, maternal availability of circulating Fe is essential for Fe transfer. Since the demand for Fe increases enormously with advancing gestation, additional Fe must be absorbed from the diet or mobilized from stores to meet the demands [2]. During pregnancy, maternal Hepc regulates iron uptake by the placenta from heme or non-heme iron in the maternal diet [2]. There is an increase in intestinal iron absorption in the duodenum 2 to 3 times higher than in the pre-pregnancy stage, which allows maintaining the higher iron requirements. Since the intake of iron into the body does not depend on the amount ingested through the diet, the increase in the absorption of Fe from the diet is strictly regulated through the Hepc [8,9].

Hepc production is predominantly regulated at the transcriptional level, mRNA Hepc and protein levels show high correlation [59] and controls the efflux of Fe into plasma by regulating Fpn. In addition to the placenta, Fpn is located on tissues that actively export iron including intestinal enterocytes, reticuloendothelial macrophages, and hepatocytes [9,60]. Hepc triggers Fpn degradation, reducing iron flux from these tissues thereby decreasing plasma Fe concentrations and systemic Fe bioavailability [61,62]. Hepc production by the liver is simultaneously regulated by circulating and stored iron, erythropoietic activity, and inflammation [63]. Then, Hepc expression is determined by the interplay of these pathways [64]. When body Fe levels are elevated or inflammation or infection is present, liver Hepc production is increased resulting in diminished Fpn expression. Conversely, when body Fe levels are depleted or anemia or hypoxia exists, Hepc expression is reduced, allowing for increased dietary iron absorption and mobilization from body stores via active Fpn [9].

Although maternal Hepc is low in normal pregnancies, inflammatory disorders could prevent appropriate suppression of Hepc, potentially compromising Fe availability during pregnancy. In addition, causing iron sequestration in the mother, elevated maternal Hepc could prevent iron absorption from supplements commonly prescribed to pregnant women. A study, utilizing stable iron isotope administration in healthy pregnancies, reported that higher maternal Hepc was associated with lower dietary Fe absorption and lower Fe transfer to the neonate [3,36,37]. Hepc binds to the receptor Fpn and the interactions between Hepc and Fpn block Fe release from storage sites and prevent Fe from being exported from the enterocyte into the body [65]. Then, the differences of Fe and Ferr among species could be explained by the fact that net Hepc concentrations can be determined by the relative strength of the opposing stimuli of maternal inflammation and Fe depletion. However, not inflammatory causes were related with decreased of Hepc in pregnant Spanish Purebred mares.

Then, the reduction in Hepc levels, with increased serum Fe and Ferr concentrations in the mare, suggests the active Hepc suppression. Possibly, decreased maternal Hepc allows even more for increased absorption of dietary iron and mobilization of iron from stores as spleen and liver, thus increasing the availability of Fe for transfer across the placenta, as it occurs in humans [12]. This evidence could also indicate the existence of fetoplacental signals that modulate maternal Fe homeostasis in pregnant mares although future studies would be needed to confirm it.

A successful pregnancy and the birth of a healthy neonate depend to a great extent on the controlled supply of essential nutrients via the placenta. Although the maternal iron status and erythropoietic activity during pregnancy, or unknown pregnancy-specific regulators could be involved in the regulation of Hepc synthesis and secretion, other data indicate that fetal factors might also exert influence on maternal Hepc production. Some human studies showed significantly lower levels of Hepc in maternal serum than in cord blood [65]. Although Hepc levels did not correlate between maternal and fetal blood, lower values in maternal blood would ensure maximal Fe delivery to the fetus [66] as maternal Hepc correlated with iron parameters in umbilical cord blood in the neonate [67]. In addition, Merhi et al. [67], examining the maternal absorption and placental transfer of isotopically labeled heme and non-heme iron, revealed that Fe transfer to the fetus was inversely correlated with maternal Hepc (non-heme Fe: $p = 0.002$, $r^2 = 0.43$; Fe heme: $p = 0.004$, $r^2 = 0.39$) and was directly associated with neonatal hemoglobin ($p = 0.004$, $r^2 = 0.39$; $p = 0.008$, $r^2 = 0.35$). These findings indicate that maternal Hepc determines, at least in part, fetal Fe homeostasis. However, iron in the developing fetus accumulates against a concentration gradient and, in the case of maternal iron deficiency, the placenta may protect the fetus [68]. This is due to serum Fe status markers such as fetal Hepc that might be able to regulate fetal Fe status independently of maternal Hepc [69]. In addition, it has been hypothesized that fetal liver Hepc controls the iron availability to the fetus by blocking Fpn-mediated Fe transport into the fetal capillaries of the placental syncytiotrophoblast [70].

In Spanish Purebred mares, minimum Fe, Ferr and estrogen values and maximum Hepc were found during the first period of gestation, and maximum levels of Fe, Ferr and estrogens, but minimum levels of Hepc, during the 2nd and 3rd periods were found. Although Fe and Ferr concentrations were inversely correlated with placental hormones during the 1st period of gestation

[24], clinical and experimental evidence suggest that estrogen manipulates intracellular iron metabolism and that elevated levels of estrogen are associated with increased systemic Fe availability. This has been attributed to the ability of estrogen to suppress Hepc synthesis, maintain Fpn integrity and enhance Fe release from iron-absorbing duodenal enterocytes and iron-storing macrophages and hepatocytes. These observations speak of a potential "estrogen-iron" axis that manipulates iron metabolism in response to hematologic (erythropoiesis) and non-hematologic (uterine growth, pregnancy, lactation) needs for Fe [71].

In postmenopausal women, it has been speculated that estrogen and Fe levels in the blood reverse during the menopausal transition. Thus, high estrogen levels are associated with low Fe concentrations in younger women while low estrogen levels and high Fe concentrations are found in older women [72]. Likewise, in women who use contraceptives, there is an estrogen-dependent increase in Fe stores compared to those who do not use this type of therapy [73]. Such axis could contribute to minimizing Fe deficiency in premenopausal women and Fe overload in postmenopausal women [21–23,71]. Furthemore, pregnant women often develop anemia, concomitant with the increase in serum erythropoietin levels, which are lower than those of nonpregnant anemic women due to the possible suppressive effect of endogenous estradiol on erythropoietin induction [74,75]. On the other hand, it is speculated that the increase in Fe stores is mainly attributed to the reduction in the volume of menstrual blood loss. However, this cannot explain the animal data, showing a significant increase in body Fe stores and absorption in estrogen-treated ovariectomized rats, since rodents do not menstruate [76,77] as it occurs in the mare.

In the mare, the decrease in Hepc, despite the effect exerted by ovarian and placental estrogens, and the increase in plasma Fe and Ferr, seems to suggest that the suppressive effect exerted by estrogens is not as marked or that the absorption of Fe from the diet is most efficient during gestation, as both circulating and reserve levels rise during the gestational period despite fluctuations in estrogen concentrations. According to results, in pregnant Spanish Purebred mares, there is clearly a relationship between estrogen and systemic Fe metabolism, although the molecular mechanism should be clarified.

However, P_4 levels appear to be positively correlated with Hepc and negatively with Fe and Ferr. Perhaps, P_4 (in addition to other factors) could explain the higher Hepc levels in the 1st and the 2nd month of gestation. The observation that P_4 increases Hepc levels, raises the possibility that Hepc levels may increase during states of high P_4 levels, such as during specific days of a woman's menstrual cycle [23]. Since this hormone is elevated during early pregnancy, it is tempting to speculate on the physiological role of P_4 in regulating Hepc serum levels during pregnancy. Hepc levels have been shown to be the highest in early pregnancy and decrease throughout pregnancy in woman [14]. Although in the mare the role of hormones on the concentration of Hepc is unknown, studies in ovariectomized mares showed that administration of P_4 increased uteroferrin in uterine secretions and that oestrogen synergistically acted with P_4 to augment the secretion [78]. Then, because uteroferrin controls the flow of Fe to the placenta, both estrogen and P_4 can function as a node to connect metabolic requirements and Fe availability during this period. Additional, studies will be needed to address the physiological role of these hormones on the regulation of Hepc biosynthesis at molecular level and, consequently, on the iron metabolism in the pregnant mare.

5. Conclusion

The increase in iron and ferritin concentrations suggests the existence of an efficient iron state in which estrogens are partially responsible for the suppression, and progesterone for the stimulation of hepcidin during pregnancy in the mare. Future research is needed to examine the relative contribution of maternal hepcidin in controlling iron transfer to the placenta, as well as optimizing maternal and fetal iron bioavailability in physiological equine pregnancies.

CRediT authorship contribution statement

Katiuska Satué: Conceptualization, Investigation, Writing – review & editing, Supervision. **Esterina Fazio:** Data curation, Writing – original draft. **Cristina Cravana:** Visualization, Investigation. **Pietro Medica:** Methodology, Validation, Software.

Declaration of competing interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the article.

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References

[1] Coffey R, Ganz T. Iron homeostasis: an anthropocentric perspective. J Biol Chem 2017;292(31):12727–34.
[2] Fisher AL, Nemeth E. Iron homeostasis during pregnancy. Am J Clin Nutr 2017;106:1567S–74S. 2017.
[3] Young MF, Griffin I, Pressman E, McIntyre AW, Cooper E, McNanley T, Harris ZL, Westerman M, O'Brien KO. Maternal hepcidin is associated with placental transfer of iron derived from dietary heme and nonheme sources. J Nutr 2012;142:33–9.
[4] Sangkhae V, Fisher AL, Wong S, Koenig MD, Tussing-Humphreys L, Chu A, Lelić M, Ganz T, Nemeth E. Effects of maternal iron status on placental and fetal iron homeostasis. J Clin Invest 2020;130:625–40.
[5] Hubbard AC, Bandyopadhyay S, Wojczyk BS, Spitalnik SL, Hod EA, Prestia KA. Effect of dietary iron on fetal growth in pregnant mice. Comp Med 2013;63:127–35.
[6] Gao G, Liu SY, Wang HJ, Zhang TW, Yu P, Duan XL, Zhao SE, Chang YZ. Effects of pregnancy and lactation on iron metabolism in rats. BioMed Res Int 2015;2015:105325.
[7] Gao G, Li J, Zhang Y, Chang YZ. Cellular iron metabolism and regulation. Adv Exp Med Biol 2019;1173:21–32.
[8] Ganz T, Nemeth E. Hepcidin and iron homeostasis. Biochim Biophys Acta 2012;1823:1434–43.
[9] Ganz T. The Discovery of the iron-regulatory ormone hepcidin. Clin Chem 2019;65(10):1330–1.
[10] van Santen S, Kroot JJC, Zijderveld G, Wiegerinck ET, Spaanderman MEA, Swinkels 553 DW. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective 554 longitudinal study. Clin Chem Lab Med 2013;51(7):1395–401.
[11] Bah A, Pasricha SR, Jallow MW, Sise EA, Wegmuller R, Armitage AE, Drakesmith H, Moore SE, Prentice AM. Serum hepcidin concentrations decline during pregnancy and may identify iron deficiency: analysis of a longitudinal pregnancy cohort in the Gambia. J Nutr 2017;147:1131–7.
[12] Guo Y, Zhang N, Zhang D, Ren Q, Ganz T, Liu S, Nemeth E. Iron homeostasis in pregnancy and spontaneous abortion. Am J Hematol 2019;94(2):184–8.
[13] Millard KN, Frazer DM, Wilkins SJ, Anderson GJ. Changes in the expression of intestinal iron transport and hepatic regulatory molecules explain the enhanced iron absorption associated with pregnancy in the rat. Gut 2004;53:655–60.
[14] Koenig MD, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. Nutrients 2014;6(8):3062–83.
[15] Petkova-Marinova TV, Ruseva BK, Paneva-Barzashka B, Atanasova MA, Dragomirova PV, Laleva PD. Relationships between hepcidin, interleuchin-6 and parameters of iron metabolism in pregnant women. Arch Balk Med Union 2020;55(4):564–72.
[16] Schulze KJ, Christian P, Ruczinski I, Ray AL, Nath A, Wu LS, Semba RD. Hepcidin

and iron status among pregnant women in Bangladesh. Asia Pac J Clin Nutr 2008;17:451–6.
[17] Kulik-Rechberger B, Kościesza A, Szponar E, Domosud J. Hepcidin and iron status in pregnant women and full-term newborns in first days of life. Ginekol Pol 2016;87(4):288–92.
[18] Santhakumar S, Athiyarath R, Cherian AG, Abraham VJ, George B, Lipiński P, Edison ES. Impact of maternal iron deficiency anaemia on fetal iron status and placental iron 2 transporters in human pregnancy. Blood Cells Mol Dis 2023;99:102727.
[19] Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood 2008;112:4292–7.
[20] Basu S, Kumar N, Srivastava R, Kumar A. Maternal and cord blood hepcidin concentrations in severe iron deficiency anemia. Pediatr Neonatol 2016;57(5):413–9.
[21] Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological changes in hematological parameters during pregnancy. Indian J Hematol Blood Transfus 2012;28:144–6.
[22] Hou Y, Zhang S, Wang L, Li J, Qu G, He J, Rong H, Ji H, Liu S. Estrogen regulates iron homeostasis through governing hepatic hepcidin expression via an estrogen response element. Gene 2012;511:398–403.
[23] Li X, Rhee DK, Malhotra R, Mayeur C, Hurst LA, Ager E, Shelton G, Kramer Y, McCulloh D, Keefe D, Bloch KD, Bloch DB, Randall T Peterson RT. Progesterone receptor membrane component-1 regulates hepcidin biosynthesis. J Clin Invest 2016;126:389–401.
[24] Wheeler S. Assessment and interpretation of micronutrient status during pregnancy. Proc Nutr Soc 2008;67:437–50.
[25] Oliveira-Filho JP, Badial PR, Cunha PH, Peiró JR, Araújo Jr JP, Divers TJ, Winand NJ, Borgese AS. Lipopolysaccharide infusion up-regulates hepcidin mRNA expression in equine liver. Innate Immun 2012;18(3):438–46.
[26] Oliveira-Filho JP, Badial PR, Cunha PHJ, Bordon AP. Freund's adjuvant-induced inflammation: clinical findings and its effect on hepcidin mRNA expression in horses. Pesqui Vet Bras 2014;34(1):51–6.
[27] Wijekoon S, Tsogbadrakh M, Sunaga T, Wang Y, Mwale C, Kim S, Alimaa D, Okumura M. Pentosan polysulfate regulates hepcidin expression in native Mongolian horses. J Vet Med Sci 2022;84(10):1437–41.
[28] Oliveira Filho JP, Badial PR, Cunha PHJ, Cruz TF, Araújo Jr JP, Divers TJ, Winand NJ, Borges AS. Cloning, sequencing and expression analysis of the equine hepcidin gene by real-time PCR. Vet Immunol Immunopathol 2010;135(1–2):34–42.
[29] Montesinos P, Satué K. Iron-related parameters in Spanish mares during pregnancy. Comp Clin Pathol 2011;22:21–7.
[30] Kozat S, Yüksek N, Göz Y, Keleş I. Serum iron, total ironbinding capacity, unbound iron-binding capacity, transferrin saturation, serum cooper, and hematological parameters in pregnant Akkaraman ewes infected with gastrointestinal parasites. Turk J Vet Anim Sci 2006;30:601–4.
[31] Yokus B, Cakir UD. Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. Biol Trace Elem Res 2006;109:255–66.
[32] Calvo JJ, Allue JR, Escudero A, et al. Plasma ferritin of sows during pregnancy and lactation. Cornell Vet 1989;1989(79):273–82.
[33] Tanritanir P, Dede S, Ceylan E. Changes in some macro minerals and biochemical parameters in female healthy siirt hair goats before and after parturition. J Anim Vet Adv 2009;8:530–3.
[34] Akhtar MS, Farooq AA, Mushtaq M. Serum trace minerals variation during pre and post-partum period in nili-ravi buffaloes. J Anim Plant Sci 2009;19:182–4.
[35] Milman N, Agger AO, Nielsen OJ. Iron supplementation during pregnancy. Effect on iron status markers, serum erythropoietin and human placental lactogen. A placebo controlled study in 207 Danish women. Dan Med Bull 1991;38:471–6.
[36] Milman N, Taylor CL, Merkel J, Brannon PM. Iron status in pregnant women and women of reproductive age in Europe. Am J Clin Nutr 2017;106(Suppl 6):1655S–62S.
[37] Vricella LK. Emerging understanding and measurement of plasma volume expansion in pregnancy. Am J Clin Nutr 2017;106(Suppl):1620S. –5S.
[38] Aguree S, Gernand AD. Plasma volume expansion across healthy pregnancy: a systematic review and meta-analysis of longitudinal studies. BMC Pregnancy Childbirth 2019;19(1):508.
[39] Kodali BS, Chandrasekhar S, Bulich LN, Topulos GP, Datta S. Airway changes during labor and delivery. Anesthesiology 2008;108(3):347–9.
[40] Byg KE, Milman N, Hansen S, Agger AO. Serum ferritin is a reliable, non-invasive test for iron status in pregnancy: comparison of ferritin with other iron status markers in a longitudinal study on healthy pregnant women; erythropoiesis. Hematology 2000;5:319–25.
[41] Borges AS, Divers TJ, Stokol T, Mohammed OH. Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. J Vet Intern Med 2007;21:489–94.
[42] Robles M, Gautier C, Mendoza L, Peugnet P, Dubois C, Dahirel M, Lejeune J, Caudron I, Guenon I, Camous S, Tarrade A, Wimel L, Serteyn D, Bouraima-Lelong H, Chavatte-Palmer P. Maternal nutrition during pregnancy affects testicular and bone development, glucose metabolism and response to overnutrition in weaned horses up to two years. PLoS One 2017;12(1):e0169295.
[43] Robles M, Peugnet P, Dubois C, Piumi F, Jouneau L, Bouchez O, Aubrière M, Dahirel M, Aioun J, Wimel L, Couturier-Tarrade A, Chavatte-Palmer P. Placental function and structure at term is altered in broodmares fed with cereals from mid-gestation. Placenta 2018;64:44–52.
[44] Upadhyaya C, Mishra S, Ajmera P, Sharma P. Serum iron, copper and zinc status in maternal and cord blood. Indian J Clin Biochem 2004;19:48–52.
[45] Adediran A, Gbadegesin A, Adeyemo TA, Akinbami A, Osunkalu V, Ogbenna A, et al. Cord blood haemoglobin and ferritin concentrations in newborns of anaemic and non-anaemic mothers in Lagos, Nigeria. Niger Med J J Niger Med Assoc 2013;54(1):22–6.
[46] National Research Council. Nutrient requirements of horses. 6th revised edition. Washington: National Academies Press; 2007.
[47] Wooding FB, Morgan G, Fowden AL, Allen WR. Separate sites and mechanisms for placental transport of calcium, iron and glucose in the equine placenta. Placenta 2000;21(7):635–45.
[48] Wooding FB, Fowden AL. Nutrient transfer across the equine placenta: correlation of structure and function. Equine Vet J 2006;38(2):175–83.
[49] Ellenberger C, Wilsher S, Allen WR, Hoffmann C, Kölling M, Bazer FW, Klug J, Schoon D, Schoon HA. Immunolocalisation of the uterine secretory proteins uterocalin, uteroferrin and uteroglobin in the mare's uterus and placenta throughout pregnancy. Theriogenology 2008;70(5):746–57.
[50] Carter AM, Enders AC. The evolution of epitheliochorial placentation. Annu Rev Anim Biosci 2013;1:443–67.
[51] Yokus B, Cakir D, Icen H, Durak H, Bademkiran S. Prepartum and postpartum serum mineral and steroid hormone concentrations in cows with dystocia. YYÜ Veteriner Fakultesi Dergisi 2010;21:185–90.
[52] Fisher AL, Sangkhae V, Balušíková K, Palaskas NJ, Ganz T, Nemeth E. Iron-dependent apoptosis causes embryotoxicity in inflamed and obese pregnancy. Nat Commun 2021;12:4026.
[53] Coutinho da Silva MA, Canisso IF, MacPherson ML, Johnson AE, Divers TJ. Serum amyloid A concentration in healthy periparturient mares and mares with ascending placentitis. Equine Vet J 2013;45(5):619–24.
[54] Satué K, Calvo A, Muñoz A, Fazio E, Medica P. Interrelationship between reproductive hormones and acute phase proteins during estrous cycle and pregnancy in Spanish purebred broodmares. Vet Anim Sci 2021;26:100212.
[55] Loux SC, Dini P, El-Sheikh Ali H, Kalbfleisch T, Ball BA. Characterization of the placental transcriptome through mid to late gestation in the mare. PLoS One;14;14:e0224497.
[56] Ball B, Loux SC, Dini P, Murase H, El-Sheikh H. Genomics, transcriptomics and proteomics of the normal and abnormal equine placenta: a better understanding of late gestational function and dysfunction. Rev Bras Reprod Anim 2021;45:369–75.
[57] Sangkhae V, Nemeth E. Placental iron transport: the mechanism and regulatory circuits. Free Radic Biol Med 2019;133:254–61.
[58] Koenig MD, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. Nutrients 2014;6:3062–83.
[59] Ganz T. Hepcidin—a regulator of intestinal iron absorption and iron recycling by macrophages. Best Pract Res Clin Haematol 2005;18:171–82.
[60] Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003;102:783–8.
[61] Ganz T. Hepcidin and its role in regulating systemic iron metabolism. Hematology Am Soc Hematol Educ Program 2006;507:29–35.
[62] Murphy A, Witcher DR, Luan P, Wroblewski VJ. Quantitation of hepcidin from human and mouse serum using liquid chromatography tandem mass spectrometry. Blood 2007;110:1048–54.
[63] Darshan D, Anderson GJ. Interacting signals in the control of hepcidin expression. Biometals 2009;22:77–87.
[64] Nemeth E, Ganz T. Hepcidin-ferroportin interaction controls systemic iron homeostasis. Int J Mol Sci 2021;22(12):6493.
[65] Van Santen S, de Mast Q, Luty AJ, Wiegerinck ET, van der Ven AJ, Swinkels DW. Iron homeostasis in mother and child during placental malaria infection. Am J Trop Med Hyg 2011;84:148–51.
[66] Rehu M, Punnonen K, Ostland V, Heinonen S, Westerman M, Pulkki K, Sankilampi U. Maternal serum hepcidin is low at term and independent of cord blood iron status. Eur J Haematol 2010;85:345–52.
[67] Merhi ZO, Seifer DB, Weedon J, Adeyemi O, Holman S, Anastos K, Golub ET, Young M, Karim R, Greenblatt R, Minkoff H. Circulating vitamin D correlates with serum antimullerian hormone levels in late-reproductive-aged women: women's interagency HIV study. Fertil Steril 2012;98:228–34.
[68] Cetin I, Berti C, Mandò C, Parisi F. Placental iron transport and maternal absorption. Ann Nutr Metab 2011;59(1):55–8.
[69] Ru Y, Pressman EK, Guillet R, Katzman PJ, Vermeylen F, O'Brien KO. Umbilical cord hepcidin concentrations are positively associated with the variance in iron 45 status among multiple birth neonates. J Nutr 2018;148(11):1716–22.
[70] Kämmerer L, Mohammad G, Wolna M, Robbins PA, Lakhal-Littleton S. Fetal liver hepcidin secures iron stores in utero. Blood 2020;136(13):1549–57.
[71] Hamad M, Bajbouj K, Taneera J. The case for an estrogen-iron Axis in health and disease. Exp Clin Endocrinol Diabetes 2020;128(4):270–7.
[72] Jian J, Pelle E, Huang X. Iron and menopause: does increased iron affect the health of postmenopausal women? Antioxidants Redox Signal 2009;11:2939–43.
[73] Casabellata G, Di Santolo M, Banfi G, Stel G, Gonano F, Cauci S. Evaluation of iron deficiency in young women in relation to oral contraceptive use. Contraception 2007;2007(76):200–7.
[74] Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological changes in hematological parameters during pregnancy. Indian J Hematol Blood Transfus 2012;28:144–6.
[75] Horiguchi H, Oguma E, Kayama F. The effects of iron deficiency on estradiol-

induced suppression of erythropoietin induction in rats: implications of pregnancy-related anemia. Blood 2005;106(1):67–74.
[76] Haouari M, Haouari-Oukerro F, Alguemi C, Nagati K, Zouaghi H, Kamoun A. Effects of oestradiol-17β on small intestine iron absorption and iron uptake into blood and liver. Horm Metab Res 1994;1994(6):53–4.
[77] Borràs M. Hormone dependency of splenic iron stores in the rat: effect of oestrogens on the recuperation of reserves in ferrodeficient subjects. Lab Anim 1998;32:290–7.
[78] Hinrichs K, Kenney RM, Sharp DC. Differences in protein content of uterine fluid related to duration of progesterone treatment in ovariectomised mares used as embryo recipients. Equine Vet J 1989;21:49–55.