Universidad Católica de Valencia
San Vicente Mártir

TFG

TRABAJO FIN DE GRADO

GRADO EN VETERINARIA

Blood biochemistry and endocrinology during gestation in the Spanish Purebred mare

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1.- RESUMEN

La gestación es el período de tiempo comprendido entre la concepción y el parto, teniendo una duración de entre 330 y 345 días en la yegua. En otras especies se ha demostrado que los parámetros bioquímicos sufren variaciones durante el proceso de gestación. No obstante, en la especie equina hay escasa información al respecto.

La finalidad de este TFG es, mediante una revisión bibliográfica, determinar si en la yegua de Pura Raza Española hay variaciones durante la gestación en cuanto a la bioquímica sanguínea y la endocrinología se refiere.

En cuanto a los resultados, no se han encontrado diferencias significativas en los parámetros bioquímicos y aumentan los niveles de estrógenos, cortisol y progestágenos.

Como conclusiones de esta revisión, las variaciones observadas en las concentraciones plasmáticas de los parámetros estudiados entran dentro de la normalidad y son consecuencia de los procesos fisiológicos sucedidos durante el período de gestación.

PALABRAS CLAVE: Yegua, Gestación, Bioquímica sanguínea, Endocrinología.

2.- ABSTRACT

Gestation is the period of time between conception and delivery, lasting between 330 and 345 days in the mare. In other species it has been shown that the biochemical parameters undergo variations during the gestation process. However, in equine species there is little information in this regard.

The purpose of this Final Degree Project is, by means of a bibliographic review, to determine if in the Purebred Spanish mare there are variations during pregnancy in terms of blood biochemistry and endocrinology.

Regarding the results, no significant differences were found in the biochemical parameters and the levels of estrogens, cortisol and progestogens increased.

As conclusions of this review, the variations observed in the plasma concentrations of the studied parameters fall within normality and are a consequence of the physiological processes that occur during the gestation period.

KEY WORDS: Mare, Gestation, Blood Biochemistry, Endocrinology.

3.- INTRODUCTION

To carry out a successful gestation, important physiological and metabolic adaptations are required in the mother. Hormonal changes begin even before conception significantly alters blood biochemical and hematological parameters. Steroid hormones, various peptides and prostaglandins interact to modulate the maternal capacity to supply energy and nutrients to the fetoplacental unit. It has been observed that, as gestation progresses in humans, many hematological parameters and some blood biochemicals undergo very significant changes compared to the non-pregnant state. For this reason, special reference ranges have been established for pregnant women in the different periods of pregnancy, favoring a correct interpretation of the tests in the diagnosis and monitoring of pregnancy. The use of these diagnostic tests is of greater importance in the management of high-risk pregnancies.

There are numerous investigations on this subject in humans, and modifications in liver, kidney, protein, electrolyte and metabolic profiles have been described. An example is the evolution of glycemia in pregnant women. It is well defined that during the first weeks of gestation, the release of estrogens and progesterone (P4) leads to hyperplasia of the pancreatic β-cells. As a direct consequence, there is an increased secretion of insulin and a greater tissue sensitivity to this hormone. For this reason, a decrease in basal blood glucose at the beginning of gestation is common. On the contrary, postprandial glucose (GLU) concentrations increase to values of up to 140 mg/dl, due to the effects of the anti-insulin hormones produced by the placenta (1). On the other hand, maternal carbohydrate metabolism is enhanced at the end of gestation in response to increasing concentrations of placental lactogen and other hormones synthesized in the placenta. The sum of these hormonal changes results in insulin resistance of moderate intensity (2).

A second example is the great change experienced by various laboratory parameters used routinely for the diagnosis of liver injury and/or dysfunction. These parameters can be interpreted incorrectly during gestation associating them with various diseases, while they are normal changes induced by this physiological state. The most notable variation is due to the stimulation of protein synthesis by estrogens, with alteration in the concentrations of various protein fractions, such as coagulation factors, angiotensinogen, and some acute-phase proteins, such as ceruloplasmin and α1-antitrypsin (3).

As can be seen, the hematological and biochemical changes in blood that women experience during pregnancy are perfectly described and detailed, with the consequent ease and precision in their diagnostic application. Unfortunately, this does not happen in the mare.

Within equine medicine, the most important article on the matter studied mares of different breeds (Quarter horse, English Thoroughbred, Saddle Horses, Standardbred and Morgan) during gestation and lactation, analyzing numerous biochemical parameters. Not only did they find that there were variations in some variables such as triglycerides (TG), potassium (K), creatinine (CREAT) and total bilirubin (BIL) concentrations, but also that some of these changes were of sufficient magnitude to establish specific values of reference (4).

Two of the factors to consider in the hematological and clinical biochemical interpretation, within a given species, are the breed and the climatic or geographical conditions in which the animal lives. Therefore, the extrapolation of the data obtained by Harvey et al. (2005) in the United States to pregnant Purebred Spanish (PRE) mares does not have to be exact in Spain. If a correct and exhaustive interpretation of clinical blood tests is to be carried out, the range of variation should be reduced as much as possible, controlling as far as possible the factors that may condition or modify the interpretation. Because of the stated above, the blood laboratory variations associated with gestation in the PRE mare are evaluated in this Final Degree Project.

4.- OBJECTIVES

Based on the above-mentioned, due to the limited scientific information on the evolution of the biochemical and hormonal variables during the mare's gestation, the objectives proposed in this degree project are the following:

First. Identify the existence of variations in the biochemical parameters during gestation in PRE mares.

Second. Identify the variations in endocrine parameters depending on the different hormonal concentrations during gestation in PRE mares.

5.- METHODOLOGY

To perform this bibiographic research Final Degree Project, scientific articles and both veterinary and human medicine books were consulted.

5.1.- INCLUSION CRITERIA:

- Articles related to blood biochemistry, endocrinology and/or gestation in mares.
- Articles published in English or Spanish.

5.2.- EXCLUSION CRITERIA:

- Articles with restricted access to the full text.
- Articles that did not meet the parameters studied.

6.- RESULTS AND DISCUSION

6.1- RESULTS OF THE BIOMETRIC ANALYSIS

To carry out the Final Degree Project, 78 scientific articles, 13 books and 2 different publications from the previous ones were consulted, classified as “others”. Hence, the total number of documents used for the literature review of: "Blood biochemistry and endocrinology during gestation in the Spanish Purebred mare", were 93.

The section determined as “others” include publications referring to doctoral theses and conference papers.

Figure 1 shows the proportion of publications used according to their literary typology, which shows a great predominance in the use of scientific articles.

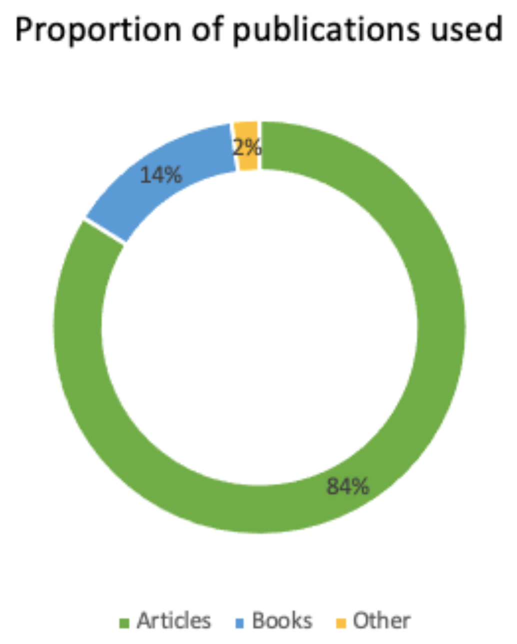

FIGURE 1. Proportion of publications used in the literature review, classified according to the type of bibliographic reference.

6.2.- GENERALITIES ABOUT THE MARE'S GESTATION

The gestation period in the mare is tipically between 330 and 345 days. After the oocyte has been fertilized, the zygote begins its journey down the oviduct and reaches the uterine horn around day 6 (89, 90).

Progesterone begins to increase after ovulation and regardless of the state of the mare, if she becomes pregnant or not, there is development of the corpus luteum. This initial increase in progesterone is linear for the first few days. If the mare is pregnant, the progesterone produced by the CL maintains the pregnancy and luteolysis does not occur, as it would in the non-pregnant mare. This primary CL is maintained and will continue to secrete progesterone.

The migration of the embryo between days 11 and 15 is necessary for the maternal recognition of pregnancy, which prevents the pregnant mare from returning to estrus. The signal for the maternal recognition is still unknown in the mare but migration of the embryo within the uterus is clearly required for it to occur, thus preventing the release of prostaglandin by the endometrium.

On day 16 or 17 after ovulation, the embryo stops migrating into the uterus and fixation occurs, usually at the base of one of the uterine horns. Fixation is believed to be caused by a combination of an increase in embryo size (diameter) as well as an increase in uterine tone, possibly caused by estrogen secreted by the embryo.

In pregnant mares, structures known as endometrial cups form around day 35 after ovulation. The cells that form the base of the endometrial cups come from the embryo and produce a hormone called equine chorionic gonadotropin (eCG). This hormone is systemically detectable for the first time between days 35 and 40 of gestation. They will secrete eCG until day 100 to 150 of gestation in most mares. This hormone is stimulating to the ovary and causes follicular development, followed by ovulation or luteinization (without ovulation) of these follicles. New luteal structures are produced, called supplementary corpus luteum. As a result of the increased number of corpus luteum in the ovaries, systemic progesterone increases. The primary CL is also stimulated by eCG and remains active secreting even more progesterone, with systemic concentrations reaching its maximum between days 60 and 120 of gestation. They are active until regression begins 150 to 180 days after ovulation. Progesterone production is quite low in the pregnant mare after 180 days of gestation. At this time, the fetus-placental unit takes over the production of progestogens (89, 90, 91).

Although sometimes overlooked, estrogen plays an endocrine role in early pregnancy. The embryo produces small amounts of locally active estrogens. However, it is not until about day 35 that systemic estrogen rises. The source of this estrogen is the ovary, more specifically the corpus luteum and the follicles. Stimulation of the ovaries by eCG is responsible for the timing of this increase in estrogen. It appears that estrogen is not actually necessary for the maintenance of pregnancy, because ovariectomized mares given exogenous progestogens will maintain pregnancy without estrogen administration (89, 90).

At the end of gestation, there is a dramatic change in the hormonal environment, and progestogens fall precipitously. This fall coincides with the maturation of the fetal hypothalamic-pituitary-adrenal axis, which occurs very late in gestation and requires the fetus to indicate its preparation for delivery. As fetal adrenocorticrophic hormone (ACTH) increases, the adrenal enzyme pathways are altered and the fetal adrenal decreases the production of pregnenolone and instead produces cortisol. Therefore, levels of progestogens fall a few days before delivery (92, 93).

6.3.- BLOOD BIOCHEMISTRY DURING GESTATION

6.3.1.- SUBSTRATES AND BLOOD METABOLITES DURING GESTATION

Parámetro	Efecto MS	Error MS	F	p
PPT	0,400	0,216	1,853	0,051 **n.s.**
ALB	457,1	23,51	19,52	0,000*
GLOB	38,18	24,76	1,542	0,093 **n.s.**
ALB/GLOB	0,758	0,132	5,734	0,008*
BIL	2,120	0,209	10,17	0,000*
CHOL	1513	368,7	4,103	0,000*
TG	2299	482,1	4,771	0,000*
GLU	310,9	201,8	1,541	0,100 **n.s.**
BUN	82,23	39,93	2,060	0,013*
CREAT	0,809	0,117	6,894	0,000*

Table T.1. Results of the 1-way analysis of variance of the concentrations of substrates and blood metabolites during pregnancy in PRE mares (*: significant differences due to pregnancy; n.s .: not significant); $p < 0.05$. (88)

6.3.1.1.- TOTAL PLASMA PROTEINS

Protein metabolism plays a key role in the regulation of physiological functions during pregnancy (5). Numerous studies carried out on protein metabolism in cows (6) and mares of various breeds (4, 7, 8), have failed to show significant changes throughout gestation. On the contrary, other studies have described cases with hypoproteinemia (9, 10) and hyperproteinemia (11, 12).

Hyperproteinemia has been associated with glucocorticoid secretion, increased liver synthesis, and estrogens secretion (12, 13). In fact, an increase in the secretion of estrogens, glucocorticoids and thyroid hormones intensifies the processes of protein synthesis and degradation during pregnancy (13). Glucocorticoids enhance the mobilization of extrahepatic proteins and the transport of amino acids to hepatocytes. The mobilized amino acids will later be used for the synthesis of GLU via gluconeogenesis, which is the primary source of energy for the embryo (14).

Similar responses have been seen in other animal species such as sheep (15). The elevated levels of total plasma proteins (TPP) maintained during the last period of gestation are indicative of the need of proteins for the secretion of gonadotropin releasing factors and other hormones (16).

Hypoproteinemia has been associated with hemodilution and increased metabolic requirements during gestation (17). Some studies with mares showed an increase in TPP at times close to delivery (9, 18). This elevation has been attributed to splenic contraction with hypervolemia (18).

In some species a reduction in proteinaemia has been described during pregnancy. However, in cows an increase in TPP has been found in the second third, followed by a decrease in the last third of gestation. These modifications could reflect changes in liver function due to the accumulation of, by fetal growth or especially by the use of amino acids for the synthesis of fetal proteins (16).

This decrease in the last third of gestation could also be the reflection of an adaptive mechanism to the high needs of mobilization of blood fluids for milk production at the end of gestation. The protein decline could be attributed to the fact that the fetus synthesizes all its proteins from the mother's amino acids and fetal growth increases exponentially during gestation, reaching a maximum level in the last months (19).

Pregnancy in the PRE mare did not condition appreciable changes in TPP concentrations, as it can be seen in table T.1. (88).

6.3.1.2.- ALBUMIN

Albumin (ALB) constitutes 50% of all protein fractions in domestic animals, maintains the oncotic pressure in the vascular compartment, and therefore regulates blood volume. This protein is also involved in the transport of molecules with low solubility in water, including fat-soluble hormones, bile salts, unconjugated BIL, Ca and ions. Although ALB plasma levels decrease mainly due to reduced hepatic synthesis and/or increased destruction or excessive excretion at the intestinal and renal level, ALB variations in mares could also be diet dependent (20).

ALB concentrations have been assessed in pregnant mares of various breeds with diverse results. Some of these studies describe the absence of changes in ALB throughout gestation (4). Other authors have found hyperalbuminemia or hipoalbuminemia, changes that could be given by the variation in the protein synthesis at the liver level (12, 18, 21).

On the other hand, hyperalbuminemia has also been described in pregnant ewes, being related to certain changes in glomerular functionality during late gestation (22).

As it is a transporter protein for a large number of chemical compounds, the increase in ALB can be explained by the higher concentrations of nutrients in the circulation during the gestational period (23).

Hypoalbuminemia during gestation has been described in various species, being associated with expansion of plasma volume and hemodilution (24, 25).

The results in studies with PRE mares are contradictory. Thus, while in the study by Satué and Montesinos (67) the levels did not change during pregnancy, in the mares in Calvo´s study (88) ALB experienced a significant increase from the 4th to the 10th month of gestation. This increase in ALB could suggest an improvement in protein synthesis by the liver in the pregnant mare, related to the energy demands of pregnancy.

6.3.1.3.- GLOBULINS

Globulins (GLOB) are a heterogeneous group of proteins that include antibodies and other inflammatory molecules, homeostatic and fibrinolytic proteins, transporters of lipids, vitamins, and hormones. The variability shown in the response of TPPs can also affect the concentrations of GLOB and the ALB/GLOB ratio, as they either do not change or increase during gestation (4, 22).

The increase in GLOB during gestation could represent the presence of a subclinical infection, although high levels of estrogens have an indirect effect on the control of infection by increasing uterine vascularization (26).

The reduction of TPP without modifications in ALB seen in some studies with sows, could reflect the decrease in GLOB during late gestation. In this case globulins could lead to the formation of immunoglobulins G and M, which progressively decline during late gestation and lactation due to the stress response (27).

The absence of changes in the ALB/GLOB ratio is considered an indirect reflection of the good nutritional status of mares throughout gestation (4).

In pregnant women and primates, the ALB/GLOB ratio decreases significantly towards the time of delivery, due to an increase in GLOB. This response is a reflection of the importance of maternal antibodies carried by colostrum in the protection of neonates against infectious diseases (13).

The concentrations of GLOB obtained in pregnant PRE mares were similar to the ones obtained in studies with other breeds. Pregnancy in the PRE mare did not condition appreciable changes in GLOB concentrations, as it can be seen in table T.1. (88)

6.3.1.4.- TOTAL BILIRUBIN

BIL is a bile pigment produced from the metabolism of various hemoproteins and subsequent conjugation at the liver. It is low sensitivity indicator of liver disease in equines. The evaluation of direct and indirect BIL levels helps in the diagnosis of liver dysfunction in this species. The three main groups of causes of increased BIL in equines are hemolytic anemias and hepatocellular and cholestatic diseases (28).

In the mare, a decreasing trend of BIL concentrations has been described throughout gestation. This fact could be subsequently related to the decrease in the jaundice index evident in PSI mares after calving. This circumstance has been associated with decreased liver uptake and changes in BIL absorption from the intestine during this period. Despite this, some studies showed an elevation of BIL at the end of gestation in PSI and Standardbred mares (9, 21, 29).

In sheep there is a significant increase in BIL throughout gestation. This response appears to come from the degradation of fetal HB or increased hepatic metabolism with inadequate glucuronic acid production (15,24).

In studies with PRE mares, BIL concentrations increased progressively and significantly from the 7th month to the end of gestation, with means significantly higher than those obtained in the first month of gestation. This increase could be related to the elevation of HCT, as well as to the greater degree of erythrocyte recycling during pregnancy (88).

6.3.1.5.- CHOLESTEROL

Gestation is a condition that involves a metabolic adaptation to meet the requirements of the developing fetus. These changes include the increase in circulating lipids due to hormonal variations. This increase in lipids represents a valuable energy source for the mother, necessary for the maintenance of basal metabolism, and favors the fetal development, which needs lipid sources for the formation of cell membranes. In contrast, the last third of gestation is a catabolic stage, with the release of fatty acids from adipocytes due to the stimulation of lipase enzyme.

These metabolic changes allow the pregnant to store energy in the first stage of gestation for the high energy requirements of the last stage. Furthermore, cholesterol (CHOL) is a precursor to steroid hormones and varies during the reproductive cycle in domestic females (30, 31, 32).

CHOL concentrations do not appear to change throughout gestation in the mare. However, in women and goats they increase significantly at the end of gestation. In rabbits, a reduction in CHOL levels has been shown at the beginning of gestation, followed by an increase at the end. Elevation of CHOL concentrations at the end of gestation is related to more intense transport of lipoproteins or with a deficit of energy in the diet. It has been speculated that the increase in CHOL could be a factor that contributes to the inhibition of GLU synthesis or could be responsible for the increase in its use by cells. This fact would reflect the synthesis of ovarian steroidal compounds (4, 25, 33, 34, 35).

Among the factors that contribute to the elevation of CHOL at the end of pregnancy, the use of fatty acids mobilized from fatty deposits and placental diffusion stand out. In rats, 50% of fetal fatty acid requirements have been considered to be derived from the mother. Other factors such as changes in basal metabolic rate, thermogenesis, and physical activity affect energy balance during pregnancy. On the other hand, the increase in CHOL reduces insulin sensitivity by interfering with the signal transduction mechanisms of the receptor for this hormone, inducing a state of peripheral resistance to insulin (31, 36).

A reduction in CHOL levels throughout gestation has been demonstrated in mares. On the other hand, CHOL levels rose significantly in the middle of gestation in goats. A strong reduction in lipogenesis and esterification with an increase in epinephrine and norepinephrine have been described, which could stimulate the release of fatty acids during pregnancy. In fact, the activity of lipoprotein lipase (LPL) in adipose tissue decreases, while in the mammary gland it increases. This metabolic change precedes the energy demands for lactation, which is stimulated by prolactin, the triggering factor for the beginning of the milk production phase (37, 38).

In the study carried out by Calvo, CHOL concentrations increased significantly during the fifth and sixth month of gestation, although they subsequently remained stable until the end of pregnancy. This response contrasts with that previously shown in mares of the same breed used in other studies, in which the cholesterol level did not change during pregnancy (7, 88).

6.3.1.6.- TRIGLYCERIDES

Triglycerides are indirect indicators of fat levels in the diet. In the horse, in addition, it has been suggested that TGs may be indirect markers of liver function, since prolonged hypertriglyceridemia is associated with lipid accumulation in the liver, interfering with its normal functioning.

In mares, TG concentrations rise during gestation. A similar response has been described in goats and in women in the last third of gestation. This increase in the pregnant woman is associated with an increase in liver synthesis (4, 28, 37, 39).

It is known that pregnancy can lead to hypertriglyceridaemia with hyperlipidemia in mares. In women, hyperlipidemia is characterized by an increase in all lipid components, although the TG fraction is the one that experiences the greatest elevation. This state of hypertriglyceridemia could be related to elevated estrogen and insulin resistance (40, 41).

On the contrary, in rabbits and sheep, pregnancy leads to a reduction in TG. This decrease has been related to lipolysis at the end of gestation. Finally, another study carried out in mares did not show any changes in TG levels throughout pregnancy (26, 42, 7).

The mean TG concentrations in the PRE mare were similar to those obtained in other breeds, increasing between 50 and 300% compared to the non-pregnant female. TG concentrations decreased in the third month of gestation, subsequently increasing from the 4th to the 9th month of gestation. (88).

6.3.1.7.- GLUCOSE

The glycemic response is subject to great variability, having detected increases, decreases and absence of modifications during pregnancy (21, 39, 4).

Infertile and pregnant mares have been documented to have a lower GLU concentration than cyclical mares. This lower amount of GLU in the pregnant mare is justified by the increase in the use of GLU by the gravid uterus, the fetus and the fetoplacental tissues. (10, 43).

Among the physiological mechanisms related to the decrease in GLU in pregnant mares the beginning of milk production stands out, since GLU is used for the formation of lactose in milk. This event takes place at the end of the gestational period, (44, 38).

On the contrary, the increase in GLU in other species could be a reflection of the increase in gluconeogenesis, as previously described in women. On the other hand, it could indicate alterations in liver function associated with fat accumulation, as has been suggested in cows. However, although glycemia increases in this last species in the middle of gestation, it progressively declines until the moment of parturition, reflecting its rapid use (16, 45).

Among other factors related to the increase in GLU, the influence of temperature is mentioned. Low winter temperatures induce the release of the phosphorylase enzyme via adrenaline activation, although hormonal modifications during the antepartum period also activate gluconeogenesis and glycogenolysis (26, 46).

Differences between pre and postpartum GLU levels represent the mobilization of GLU from the mother to the fetal circulation in the final stages of development and milk production. It should be noted that the stress present during the physical process of birth also predisposes to gluconeogenesis, thus raising blood glucose. However, the balance between synthesis and degradation of GLU is determined by factors of hormonal nature, such as insulin, growth hormone, insulin-like growth factor (IGF-1), thyroid hormones, sex steroids, glucocorticoids, glucagon and catecholamine, and by non-hormonal factors, such as nutrition, exercise, growth and infection, establishing complex interactions between them (35, 47).

At the beginning of gestation, the increase in estrogens and P4 favors the proliferation of maternal pancreatic beta cells and the increase of insulin secretion. Tissue sensitivity to insulin is normal or could be slightly increased, although due to the consumption of GLU by the fetus and the placenta there is a predisposition to hypoglycemia. In advanced stages of gestation, placental lactogen, characterized by its insulinotropic effect, and prolactin are increased. Prolactin decreases tolerance to GLU and cortisol (CORT), increasing insulin resistance. This state of insulin resistance is progressive from the second to the third period of gestation, and implies a state of less sensitivity to insulin in maternal muscle and adipose tissue. It is understood as a physiological adaptation to allow a preferential targeting of GLU and amino acids to the feto-placental unit. Insulin resistance promotes lipolysis and postprandial hyperglycemia, with a greater supply of nutrients to the fetus. The placental transport of nutrients stimulates the elevation of fetal insulin, favoring fetal growth due to the accumulation of fatty tissue and hepatic glycogen reserves. The development of maternal insulin resistance coincides with increases in serum concentrations of the lactogenic hormones, prolactin, placental lactogen and human placental growth hormone. Lactogens and somatogens reduce insulin sensitivity in adipocytes and skeletal muscle cells and stimulate beta-cell replication, insulin gene transcription, and GLU-dependent insulin secretion in the pancreatic islets, which will be responsible for the hyperinsulinemia present during late gestation. These facts favor the free availability of GLU in the maternal circulation to meet the needs imposed by the placental and fetal development (48, 49, 50).

Although the glycemic response to pregnancy in mares varies between studies, having detected an increase or decrease, in the study with PRE mares by Calvo GLU concentrations did not change throughout gestation, staying within the reference range described for healthy adult equidae (88).

6.3.1.8.- UREA

Urea or BUN (Blood ureic nitrogen) is the final product of protein metabolism and has a dual origin: protein degradation in the digestive tract and amino acid catabolism in the liver. Serum BUN concentrations determine the balance between endogenous and exogenous protein catabolism and renal excretory function (51).

Numerous studies in mares have not detected changes in BUN concentrations during pregnancy. However, in women BUN levels decrease during the early stages of pregnancy, although they subsequently increase. Research in mares has described similar results. These differences are probably related to changes in dietary protein levels, to an increase in glomerular filtration rate or to an increase in tissue deposits. in the mother during the initial stages, since in the final stages of pregnancy, protein metabolism increases in both mother and fetus (4, 38, 39, 52).

In sheep and goats the levels of BUN increase significantly throughout gestation. In ewes, BUN production increases during gestation and decreases later after parturition. This response is related to the intensity of protein metabolism, nutritional management, CORT metabolism or the effect of thyroid hormones. The activity of thyroid hormones rises during pregnancy, favoring protein catabolism. The increased energy requirements from the second half of gestation could induce a notable increase in BUN, more evident at the end of gestation, as has been documented in sows (27, 37).

In PRE mares, BUN concentrations experienced a significant decrease from the 2nd to the 8th month of gestation. This results contrast with the obtained in older studies. Although we cannot specify exactly, these interspecific and research differences would probably be related to changes in protein levels in the diet, the time of the year, an increase in glomerular filtration rate or an increase in protein metabolism tissue in the fetus and mother during the final stages of pregnancy (52, 88).

6.3.1.9.- CREATININ

In mares, there is an increase in CREAT during gestation. Changes in this parameter have been suggested to be associated with increased muscle catabolism during the period of milk formation and with the production of CREAT by the fetus. However, other studies in mares did not find significant variations throughout gestation (4, 21).

In healthy individuals CREAT levels are constant and depend on the concentrations of its precursor, muscle creatine. At the same time, creatine levels depend on diet, on the synthesis of arginine and glycine from the kidneys, intestines, and pancreas, and on muscle mass as well. During gestation and more specifically towards the time of delivery, amino acids come from skeletal muscle metabolism, although uterine involution and myometrial protein degradation could have some contribution (51, 53).

In the PRE mare, although the CREAT concentrations were within the reference limits described for healthy adult equids, they increased significantly from the 9th month to the end of gestation. It has been suggested that the gestational increase is related to protein metabolism, catabolic processes during the period of milk formation in the mammary gland and/or with the production of fetal CREAT (88).

6.4.- ELECTROLYTES AND BLOOD MINERALS DURING GESTATION

In animals, improper feeding has been documented to affect reproduction, delay the onset of puberty in calves, induce anestrus in beef cows and prolong postpartum anestrus in dairy cows. The minimum nutritional requirements of elements such as Ca, P and Mg have been established in pregnant mares in relation to the increasing needs for fetal development. Some studies have also linked the deficiency of various minerals with certain reproductive disorders in mares. Thus, Mg deficiency delays uterine involution, and causes loss of the fetus and irregularity of estrous cycles in mares. Mg deficiency has also been related to sterility and to fetal malformation, reabsorption, and abortion (54, 55).

Parámetro	Efecto MS	Error MS	F	p
Na	80,26	26,47	3,034	0,000*
K	0,445	0,562	0,791	0,679 **n.s.**
Cl	27,85	10,42	2,672	0,001*
Ca	4,806	1,174	4,092	0,000*
P	49,53	5,544	8,933	0,000*
Mg	3,466	0,486	7,138	0,000*

Table T.2. Results of the 1-way analysis of variance of blood electrolyte and mineral concentrations during gestation in PRE mares (88). (*: significant differences due to gestation; n.s .: not significant); p <0.05

6.4.1.- SODIUM

During the last stages of gestation, nutritional requirements increase for fetal development and milk production, detecting a decrease in natremia in mares. However, the response of natraemia to pregnancy is highly variable, having detected elevation or absence of modifications (4, 7, 29).

In studies with pregnant PRE mares, natraemia showed significantly higher values in the 4th month compared to the 10th month of gestation. This fact has been partially related to the decrease in Na concentrations in mammary secretions at the end of pregnancy (88).

6.4.2.- POTASSIUM

While some studies have not shown changes in K concentrations in pregnant mares, other studies have shown higher concentrations during lactation when compared to pregnancy. These data have been related to the loss of K for the formation of colostrum at the mammary gland or with a greater peripheral demand due to the increase in the nutritional needs of the fetus. However, concentrations higher than those of mares in the postpartum period have been detected in pregnant mares (4, 44).

In studies with pregnant PRE mares, K concentrations were within the reference interval for healthy adult equids, not detecting any significant changes with not pregnant mares. This sets in opposition with the results obtained with other breeds mares, in which a reduction of K concentrations was described during the initial months of gestation (7, 88).

6.4.3.- CHLORINE

Regarding chloremia, it has been indicated that it is not subject to substantial changes during the gestation period in mares. These results contrast with other investigations, which have determined important elevations associated with hormonal variations (4, 7, 21).

In the study with PRE mares carried out by Calvo, an elevation of the mean concentrations of Cl was observed from the first to the fourth month of pregnancy, decreasing afterwards (88).

6.4.4.- CALCIUM

Ca and P represent 70% of the mineral content of the body. Approximately 90% of Ca and 80% of P are present in bones and teeth. The remaining 1% is distributed in various organs, such as muscle, blood and intercellular fluids, exerting vital functions such as muscle contraction, contraction and expansion of blood vessels, blood clotting, secretion of enzymes and hormones, and transmission of biochemical signals in the central nervous system (56).

Total Ca in blood represents the sum of its three fractions which are in dynamic equilibrium: ionic Ca, which constitutes 50-65%; Ca bound to plasma proteins, mainly ALB, which represents 30-45%; and Ca bound to various anions such as citrate or lactate, which comprises 10.5% of the total calcium (56).

In not pregnant mares, significantly higher total Ca concentrations have been shown compared to those pregnant. At the beginning of gestation, calcemia is slightly reduced in mares. The decrease in calcemia could reflect a greater degree of mobilization towards the fetus, necessary for bone development. However, other authors, have not evidenced modifications of this parameter during pregnancy (4, 10).

In women, pregnancy can cause hypocalcaemia. This hypocalcemia is usually more marked after the 18th week of gestation and suggests that maternal bone tissue contributes almost 80% to the formation of the fetal skeleton, especially during the third trimester. On the contrary, in goats, an increase in Ca has been shown throughout gestation. The Ca peak during pregnancy is attributed to increased PTH activity, with activation of osteoclasts and increased calcium levels after mobilization of bone Ca reserves. Mobilization is necessary to meet the Ca demands of the fetus for the development of its skeleton and for the formation of milk during lactation. The Ca requirements during the last trimester of gestation for the correct mineralization of the fetal skeleton have been estimated in a Ca/P ratio of 3: 1 in mares (37, 38, 39).

In pregnant PRE mares, calcemia increased significantly during the forth, fifth and seventh month of gestation compared to the first month of gestation, and it remained constant during the last third of gestation (88).

6.4.5.- PHOSPHORUM

Inorganic P is an essential component of phospholipids, a structural component of cell membranes that is necessary for the transport of lipids, energy metabolism and a constituent of several enzyme systems. Although some studies in mares have shown that P levels decrease towards the middle of gestation, other investigations have not shown significant modifications of this parameter throughout gestation (4, 10).

In other species, such as humans and rabbits, decreases in P have also been detected during pregnancy. On the other hand, studies in sows, goats and cows have shown that these levels do not vary. This fact possibly indicates a balanced diet, with efficient metabolism and phosphocalcic homeostasis (6, 25, 39, 57).

In the mares of Calvo's study, the P concentrations increased significantly during the sixth and seventh month of gestation. These results contrast with the absence of modifications experienced by this parameter in previous studies in PRE mares. The divergences between the results of the various investigations could be related to factors such as age and diet, as well as the degree of bone resorption and renal excretion (58, 88).

6.4.6.- MAGNESIUM

Although Mg deficiency is not the most common cause of infertility in mares, hormonal dysfunction, genetic problems, and management conditions such as nutrition are important factors to take into account. Studies with mares of various breeds have not been found significant modifications of Mg concentrations during pregnancy. However, other studies with mares and women have shown an increase in magnesemia at the beginning of gestation. In women, this increase was initially discrete, returning to baseline levels at the end of gestation. In cattle, a reduction in Mg has been described during pregnancy, with a significant increase during lactation. It is known that Mg is necessary for the development of the skeleton and it is one of the activators of the enzymatic chains, therefore its requirements increase during the period of fetal development (22, 29, 59, 60).

In pregnant PRE mares, Mg concentrations reached their maximum value in the third month of gestation. This evolution coincides with that shown in PSI mares and women, showing a significant elevation at the beginning of gestation and returning to baseline levels at the end (29, 88).

6.5.- ENZYMATIC ACTIVITY IN BLOOD DURING GESTATION

Parámetro	Efecto MS	Error MS	F	p
CK	15805	1561	10,13	0,000*
AST	2618	1269	2,062	0,000*
FAL	9409	3696	2,546	0,002*
GGT	629,5	184,3	3,414	0,000*

Table T.3. Results of the 1-way analysis of variance of enzymatic activities in blood during pregnancy in PRE mares (88). (*: significant differences due to pregnancy; n.s .: not significant); $p < 0.05$

6.5.1.- CREATIN KINASE

Creatine kinase (CK) catalyzes the reversible phosphorylation of creatine to creatine phosphate, the form of energy phosphate storage required at muscular level. Studies with mares showed a decrease in CK activity during gestation. These changes had been previously documented in mares and women, being associated with hemodilution. However, at moments close to parturition the activity of this enzyme increases notably (12, 18, 61).

It is known that reducing protein intake through diet increases tissue metabolism. Therefore, the elevation of plasma CK activity could be related to protein deficiency in the last stages of gestation and/or to an increase in metabolism and tissue and muscle destruction, due to the physical effort inherent in the physical process of parturition. In contrast, another study carried out in women showed no changes in the blood enzyme activity of CK during the gestational period (62, 63).

In relation to gestation, the mean CK activity experienced a progressive decrease in PRE mares, although the differences reached significant values in the seventh month of gestation (88).

6.5.2.- ASPARTATE AMINOTRASFERASE

Cytosolic and mitochondrial aspartate aminotransferase (AST), which catalyzes the reaction of aspartate from carbohydrates, has a wide tissue distribution, being released into plasma from damaged cells from various organs, such as the liver, skeletal muscle, myocardium, intestine and kidney (28, 64).

The activity of the AST enzyme in mares does not appear to undergo significant changes throughout gestation. However, some studies have shown decreases in AST towards the end of gestation, recovering later at the beginning of lactation (4, 7).

On the contrary, an increase in AST has been shown in goats and cows during pregnancy, although with different evolutions during the peripartum period. Thus, while some showed an elevation, the others described a decrease in AST activity at the time of delivery. The increased activity of AST has been related to gluconeogenesis induced by the stress during gestation. In fact stress, regardless of the cause, predisposes to gluconeogenesis, with increased liver enzymes (57, 65).

In the mares in Calvo's study, AST activity was higher in the second month of gestation than in the eighth, ninth and tenth months. During the period of intense fetal development, metabolic activity is intensely focused on the constitution of fetal tissues, since amino acids are destined for the protein constitution of various tissues, with the consequent decrease in AST in the mother (21, 88).

6.5.3.- ALKALINE PHOSPHATASE

Although some investigations in mares have not revealed significant variations in the activity of the alkaline phosphatase (ALP) enzyme throughout gestation, others studies in rats and goats have observed an increase in ALP activity at the end of gestation. This elevation has been related to the hormonal influence of pregnancy. More specifically towards the time of delivery, relating this increase with the increase of enzyme production due to fetal bone growth and placental secretion of its isoenzymes (21, 27, 65, 66).

On the contrary, some studies found that ALP activity decreases throughout gestation in sows and mares, reaching minimum levels at the time of parturition (27, 67).

The mean FAL activity in the PRE mare presented mean values within the reference range for healthy adult equids. FAL activity was higher in the fifth month compared to the second, seventh and eighth months of gestation (88).

6.5.4.- GAMMA-GLUTAMYL TRANSFERASE

Gamma glutamyl transferase (GGT) is a highly specific enzyme in the diagnosis of cholestatic liver processes in equines, as it is specifically located in the cell membranes of the bile canaliculi, associating with inflammation and/or epithelial destruction and cholestasis. In equines, elevated GGT is related with liver damage associated with toxic liver failure, subclinical liver disease, or hyperlipidemia in the presence of cholestasis (68).

GGT activity in pregnant mares appears to be no different from that found in lactating mares. However, some studies observed slight decreases in GGT towards the end of gestation, with a return to normal values near delivery. It has been suggested that this increase in GGT could derive from a greater degree of clearance at the peripheral level and/or from release by the biliary epithelium in pregnant mares. However, the glucocorticoids released at the time of delivery could have caused an increase in the activity of the enzyme in the liver (4, 7, 69).

In PRE mares, GGT activity showed a progressive decrease during pregnancy, although significant differences were only seen when comparing the second month with the eighth, ninth, tenth and eleventh months of gestation. It has been suggested that this reduction in GGT could derive from the elevation of the degree of clearance at the peripheral level and/or from a lower release by the biliary epithelium in pregnant mares (67, 88).

6.6.- ENDOCRINOLOGY DURING GESTATION

6.6.1.- HORMONAL CONCENTRATIONS DURING GESTATION

Parámetro	Efecto MS	Error MS	F	p
EST	25674	3920	6,550	0,000*
P4	725,8	137,6	5,275	0,000*
CORT	6765	2896	2,336	0,004*

Table T.4. Results of the 1-way analysis of variance of blood hormone concentrations during pregnancy in PRE mares (*: significant differences due to pregnancy; n.s .: not significant); p <0.05. (88)

6.6.1.1.- ESTROGENS

The fetoplacental production of estrogens in the pregnant mare differs significantly from that of P4. Around day 80 of gestation, the interstitial cells of the fetal gonads undergo hypertrophy and hyperplasia, reaching their maximum size around 230 and 260 days of gestation. At this time, the fetal gonads occupy one third of the total volume of the fetal abdominal cavity. Later, they begin to return, becoming imperceptible at the end of the gestation (70, 71).

A series of experiments established that the estrogens present in the pregnant mare were produced by aromatization of high amounts of dihydroandrosterone (DHA), dihydroepiandrosterone (DHEA) and precursors of DHEA (3β-hydroxyl C-19), all of them synthesized from the interstitial cells of the fetal gonad. In fact, the fetal gonad contains the necessary enzymes ($P450_{SSC}$ and 17α-hydroxylase) for the conversion of CHOL to DHEA. Dihydroepiandrosterone is aromatized in the placenta to phenolic estrogens, estrone (EST), and estradiol 17α and β. The β-unsaturated estrogens (equilin and equilenin and their derivatives) are derived from farnesyl pyrophosphate, through a non-CHOL-dependent pathway. These compounds have been isolated from maternal blood, urine, feces, and amniotic fluid, in addition to other hydroxylated derivatives (17β-hydroxyequineline) and sulfoconjugates (72, 73, 74).

The estrogen concentration varies throughout gestation in the mare. During the first 35 days levels are similar to diestrus in nongravid mares, increasing around day 40, due to follicular development prior to the formation of the corpora lutea, and they are maintained up to day 60 or 70 of gestation. It should be noted that the mare's primary corpus luteum produces E2 at this time, in response to the hormone equine chorionic gonadotropin (eCG) from day 80, and concentration increases reaching maximum levels around 210 days of gestation. This second peak in estrogen concentration is not affected by ovariectomy, although it is affected by spontaneous fetal death, reflecting the fetoplacental origin (75, 76, 77, 78).

Around 290 days, estrogen levels fall again until delivery. The biological role of estrogens secreted by the fetus-placental unit has not yet been elucidated. They are known to play an important role in the regulation of uterine and placental flow to facilitate the exchange of nutrients and waste products between mother and fetus (74, 79).

Estrogens are important in active labor processes. At the time of delivery they promote the synthesis of prostaglandins and increase endometrial sensitivity to oxytocin, thus stimulating the contractile activity of the myometrium. It is known that females carrying gonadectomized fetuses show an immediate decrease in estrogen between 197 and 253 days of gestation. Parturition in these mares occurs spontaneously, preceded by strong myometrial contractions, with a significant decrease in the synthesis of prostaglandin F2α (PG2Fα) and leads in most situations to placental retention. Furthermore, gonadectomized animals have a reduced birth weight and show less muscle development than non-gonadectomized animals. On the other hand, it has been documented that the concentration of EST decreases significantly in serum after fetal expulsion or elimination, while the levels decrease transiently after ovariectomy (74, 80, 81).

In the studies with PRE mares the EST concentration increased significantly in the fifth month, compared to the first months of gestation, and reached the maximum level in the sixth month. From the seventh month on it progressively decreased, so that concentrations from the eighth month were similar to those of the initial months of gestation (88).

6.6.1.2.- PROGESTERONE

At the beginning of gestation, various waves of follicular growth lead to the development of medium-sized (10-20 mm) and large (> 20 mm) follicles. This ovarian response is mainly due to the increase in the concentration of follicle-stimulating hormone (FSH) during the first 20 or 30 days of gestation. The prevention of regression of the primary corpus luteum, in association with the mobility of the concept at the uterine level, has been considered as the maternal recognition of pregnancy in the mare. The maximum mobility interval of the embryo (11-16 days) corresponds to the period of luteolysis blockage and, consequently, the primary corpus luteum continues to synthesize P4. Both the size of the corpus luteum and the production of P4 are minimal during the first 15 days of gestation (82).

From this moment on, the size of the corpus luteum increases and with it the concentration of P4, leading to the re-emergence of the primary corpus luteum, which corresponds to the “second luteal response of pregnancy”. At around 30 to 40 days, the endometrial calyces begin to synthesize eCG. The increase in the systemic levels of eCG and P4 occurs in parallel with the increase in the size of the primary corpus luteum (70, 82).

High follicular activity during the 40 and 60 days of gestation is associated with the formation of the secondary corpus luteum. The formation of these luteal glands corresponds to the “third luteal response of gestation”. These supplementary corpora lutea produce P4 during this period. Initially, the formation of these structures results from the luteinization of the ovulatory follicles (secondary corpus luteum) and later, of the anovulatory follicles (accessory corpus luteum) due to the luteotropic effect of eCG. This type of endocrinological dynamics leads to an increase in P4 secretion around day 75 of gestation (82).

The regression of the endometrial calyces causes the involution of all the corpora lutea around 120-180 days of gestation, inducing a drastic decrease in blood concentration of P4. Some experimental studies in mares have confirmed that bilateral ovariectomy at 75 days of gestation induces abortion in the period between 75 and 150 days. The degree of abortion is variable, and after day 150 ovarian P4 is not necessary, since the corpora lutea have already regressed and there is no evidence of abortion. Therefore, these experiments reflected the role of the placenta in the maintenance of pregnancy based on the regression of the endometrial calyces (74, 80).

Studies in mares showed that the placenta begins to synthesize appreciable amounts of progestogens around day 70 of gestation. Progestins are similar in structure to P4, they bind to its receptors and are majorly present in plasma (74, 80).

Colesterol

↓ (1)

P_5

↓ (2)

P_4

↓ (3)

5α-DHP → (4) → **20α5P**

↓ (5)

3α5P → (4) → **β αdiol**

Abreviaturas	**Nombre común**	**Nombre sistemático**
P_5	Pregnenolona	3β-hidroxi-5 pregnan-20-ona
P_4	Progesterona	4-pregnan-3,20-diona
5αDHP	5α-dihidroprogesterona	5α-pregnan-3, 20 diona
20α5P	-	20α-hidroxi-5-pregnan-3–ona
Bαdiol	-	5α-pregnan-3β, 20αdiol
3α5P	-	3α-hidroxi-5-pregnan-3–ona

Enzimas:

1.-$P450_{SCC}$, citocromo de clivaje de la cadena lateral del colesterol (feto y placenta)

2.-3β-HDS, 3β deshidrogenasa hidroxiesteroide (feto y placenta)

3.-5α-reductasa (endometrio y placenta)

4.-20α-reductasa (endometrio)

5.- 3β-oxidoreductasa (placenta)

FIGURE 2. Metabolic pathways for the synthesis of progesterone and progestogens and their enzymatic conversions (74).

The fetal adrenal gland synthesizes high amounts of pregnenolone (> 10 µmol / min), which is the precursor of all progestogens during late gestation. A side chain cleavage enzyme named $P450_{SCC}$, necessary for the conversion of CHOL, is present in the equine placenta towards mid-gestation and appears widely distributed in the uteroplacental tissues towards the end of gestation (74, 80).

The most important progestogens in maternal plasma during this period are 5α-DHP and its derivatives, 20-α5P and βα-diol. These steroids are produced from pregnenolone and reach values greater than 500 ng/ml at term. Pregnenolone at the level of the uteroplacental tissues is also transformed into P4 and is released exclusively to the umbilical circulation, while 20α5P and 5α-DHP are secreted both into the umbilical and uterine circulation. These steroids are thought to be important within the fetoplacental unit in maintaining uterine quiescence during the second half of gestation (74, 83).

In the study carried out by Calvo (88), the mean level of P4 in the PRE mare reached its maximum value in the third month of gestation. A decrease was later observed in the forth month, which remained stable until the end of pregnancy. These observations confirmed the results previously obtained in other studies with pregnant PRE mares. This high values of P4 during the first months of gestation indicates the secretory activity of the primary corpus luteum and the secondary and accessory corpus luteum, which continue to produce P4 until day 110 of gestation (88).

6.6.1.3.- CORTISOL

Most research suggests that the metabolism of CORT is significantly altered during pregnancy. Therefore, it is logical to assume that the functional activity of the hypothalamic-pituitary-adrenal axis would also be modified. It is known that total CORT and free CORT appear increased in most females during gestation. Specifically in the mare, variations in cortisol levels throughout gestation have been studied, concluding that CORT levels during the first half of gestation are higher than those in the second half. Other research described that the CORT level in empty mares was significantly higher than in pregnant mares (84, 85).

The effect of gestation in mares has been suggested to mask the presence of circadian rhythms of CORT during the second half of pregnancy. This process appears to be the consequence of hormonal changes. On the contrary, it has been shown that in people the circadian rhythms of CORT during pregnancy are not altered (86, 87).

Among the factors that could decrease the concentration of plasma CORT we can find the reduction of its production and the increase in the volume of distribution. These could be counteracted by certain modifications during pregnancy, which would lead to an increase in the levels of the cortisol transporter protein (CBG), such as a decrease in metabolic clearance and/or an increase in its production.

The mean CORT levels in the PRE mare were higher in the forth month than in the first month of gestation. Similar modifications have been described in mares of the same breed and in other equine breeds, such as Standardbred and Arabs (88).

The increase in plasma CORT levels during pregnancy could be caused by the dual effect of adrenocorticotropic hormone (ACTH) synthesis. In women, the increased secretion of adrenal steroids occurs in the third third of pregnancy and is related to the secretion of corticotropin-releasing hormone (CRF) at the placental level. In any case, this observation seems controversial as placental-derived CRF levels affects adrenocortical function in this same period, although they do not exclude such effects. In fact, CRF is not the only factor that regulates the pituitary-adrenal axis in the last third of gestation. The nervous system also controls the circadian rhythms of CORT secretion (86, 87).

7.- CONCLUSIONS

The main conclusions of this Final Degree Project are the following:

First: The progressive increase in plasma concentrations of albumin and creatinine would result from an increased protein metabolism in the mother for the supply of amino acids to the fetus and, in the case of creatinine, the excretion of this metabolic residue after its fetal production. On the other hand, the decrease in CK activity seems to indicate a lower mobilization of this enzyme from the muscle, possibly in association with a restriction in motor activity throughout gestation.

Second: The absence of clear variations in Ca and P concentrations during gestation despite the increased needs for milk production and fetal growth, reveals efficient phosphocalcic homeostasis.

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9.- ANNEXES

9.1.- ABBREVIATION LIST

- ACTH; adrenocorticotropic hormone
- ALB; albumin
- ALP; alkaline phosphatase
- AST; aspartate aminotransferase
- BIL; total bilirubin
- BUN; blood ureic nitrogen
- Ca; calcium
- CBG; cortisol transporter protein
- CHOL; cholesterol
- CK; creatine kinase
- CL; corpus luteum
- CORT; cortisol
- CREAT; creatinine
- CRF; corticotropin-releasing hormone
- DHA; dihydroandrosterone
- DHEA; dihydroepiandrosterone
- dl; deciliters
- eCG; equine chorionic gonadotropin
- EST; estrone
- FSH; follicle-stimulating hormone
- GGT; gamma glutamyl transferase
- GLOB; globulins
- GLU; glucose
- HB; hemoglobin
- K; potassium
- LPL; lipoprotein lipase
- Mg; magnesium
- mg; milligrams
- ml; milliliters
- mm; millimeters

- Na; sodium
- ng; nanograms
- P; phosphorum
- P4; progesterone
- PGF2α; prostaglandin F2α
- PRE; spanish purebred horse
- TG; triglycerides
- TPP; total plasma proteins