Nat. Volatiles & Essent. Oils, 2021; 8(4): 11484-11504

Study Of Histomorphological And Inflammatory Endometrial Changes And Circulating Hormones In Early Days Of Pregnancy In Mare

Tawhed Fadhil Jaber[1] , Haider Mohammed Haider[2] , Ahmed Abood ALkhalisi[3]

1 Faculty of Education, AL mustansiriyah University, Baghdad -Iraq,

2 Department of Dewan Affairs, Al mustansiriyah university, Baghdad -Iraq,

3 Chemical engineering,

Abstract

The goal of the current work was to establish histological changes in the endometrium, differences in serum concentrations of progesterone, 17β estradiol, insulin-like growth factor type 1, leptin and adiponectin of seas during early gestation compared to cyclic seas, and to verify associations between variables. During an empty period (cyclic mares) and a pregnant period. Radioimmunoassay, from ovulation to 13 days of estrous cycle and pregnancy, established hormones daily. In addition to quantifying the inflammatory cells in the endometrial tissue, endometrial biopsies were taken on days 7, 10 and 13 of the estrous cycle and pregnancy during which a histomorphological analysis of the endometrial glandular structure and luminal epithelium was performed. No variations were observed in the height of the luminal epithelium between the sampling days or between the groups within the histomorphological measurements; however, higher values were reported in pregnant seas than in cyclic seas at GE height, GD, GLdiameter and intraglandular secretions. Epithelium and glandular diameter decreased in both groups during the sampling days, while lumen and glandular secretions increased in the pregnant group during the days, not finding group differences. A greater number of eosinophils and lymphocytes were found in pregnant seas on day 7 than in cyclic seas, all cell types were decreased in pregnant seas during the sampling days. Of the serum hormones tested, no variations were observed between the study groups except for serum insulin, which appeared to be higher in the cyclical group than in the pregnant group; with higher concentrations in cyclical mares on days 8 and 12 than in pregnant ones. In conclusion, early pregnancy was associated with changes in endometrial and inflammatory cells in endometrial tissue. Changes in serum insulin were identified during early pregnancy. Only progesterone was associated with histomorphological changes in both classes of seas and with changes in inflammatory cells in pregnant seas, which in turn interrelated the glandular changes.

Keywords: Mares Early Days of Pregnancy, histomorphologicalanalysis; glandular structure; statistical analysis

1. INTRODUCTION

Pregnancy in the equine species has been studied in several aspects related to physiology, endocrinology and neurobiology with the aim of better establishing the mechanisms and biological activities related to this phase. In the mare, the gestational period is long, between 320 to 360 days, with the end of fetal maturation occurring only in the last 5 days before giving birth [1]. Another peculiar characteristic of this species is that the second stage of delivery is fast, lasting between 20-30 minutes. Mares are seasonal breeders with long days associated ovarian operation and an average 22-day estrous period length with an estrous of between 5 and 7 days. The pattern of estrous regulations depends upon the responsive equilibrium of pineal hormones, hypothalamus, cavities and endometrium. Hypothalamus, hypophysilic gland [2]. Knowledge about early pregnancy in equines is rudimentary in some respects compared to other domestic species. We believe that elucidating the questions about said period, before and during the RMP, knowing the changes that are generated and the requirements for the survival of the embryo, would help reduce part of the previously mentioned embryonic losses, thus generating greater productivity for the equestrian sector [3].

When the light hours are enough to suppress the inhibitory reflection induced by melatonin in the release of GnRH, the start of the reproductive stage is sufficient to stimulate the synthesis of FSH and LH in the circulation. The phases of the cycle are estrus, or the follicular phase, and right-handed, or the luteal phase [4].Estrus is the time when the stallion is sexually receptive to the stallion. In the absence of P4, it is stimulated by elevated E2 levels provided by an emerging dominant follicle. Ovulation normally occurs 24 to 48 hours prior to the end of estrus, with P4 being responsible for the end of estrousbehavior[5]. The right is the time when the stallion is not receptive and the reproductive tract is able to accept the embryo and feed the embryo; usually it takes 12 to 16 days. The luteal phase is triggered by ovulation and by the production of a CL [6]. In the mare, one or two major follicular waves may occur per estrous cycle; the primary follicular wave (wave that ends in ovulation during oestrus) originates in the right hand 7 to 8 days after ovulation, with FSH being responsible for the initial growth of the follicles for 6 to 7 days [7] Later, after the selection of the dominant follicle, FSH levels decrease as a consequence of the increase in follicular production of inhibin and E2. After the increase in E2, the levels of LH increase, the hormone responsible for the final maturation of the follicle and induction of ovulation [8]. The pattern of LH secretion during estrus is unique in the mare with a concentration peak 1 to 2 days after ovulation. The P4 produced by CL exerts negative feedback on LH. Of the few published works that link systemic maternal insulin with endometrial histomorphological changes, the one by Satué et al (2012) [9] stands out who, through the evaluation by electron microscopy of endometrial cells after insulin administration in rats, suggest that the hormone would stimulate cellular activity by identifying an increase in secretory vesicles and Golgi complexes compared to untreated animals. There are several ways to evaluate the uterus and endometrium, however many abnormalities can only be revealed by an endometrial biopsy [10]. Endometrial biopsy consists of the removal of a portion of an endometrial fold, without reaching the muscle, although occasionally it may include a portion of the inner circular myometrial layer. The technique has been widely described by various authors [11-12]. A proper endometrial sample requires a 70 cm long biopsy forceps with a sharp 20x3x3mm cutting surface to prevent bleeding and tissue distortion. Despite the large endometrial surface area (850- 1350 cm^2), this instrument provides a representative sample of its entire extension [13]. The biopsy is normally obtained from the dorsal wall of the uterine body or from the base of one of the horns. It is a diagnostic technique that allows evaluating the structural integrity of the endometrium by visualizing two types of pathological processes: inflammatory or endometritis and degenerative or endometrosis; at the same time, it allows predicting the ability of the endometrium to sustain a pregnancy at term and determining the stage of the estrous cycle in which the sample was taken. The equine endometrium undergoes histological changes according to the season and within the reproductive season during the different stages of the estrous cycle, these histological changes have been described in detail by several authors [14]. The most noticeable changes throughout the estrous cycle are seen in the configuration of the glands, the characteristics of the secretions, and the presence of edema in the lamina propria and the height of the luminal epithelium (the highest height reached during estrus). Very few works have been found in horses on the finding of changes in serum insulin concentration during early pregnancy, suggesting a possible use of maternal insulin during the first days of pregnancy by the endometrial embryo system as it occurs in other species [15].

The main objective of the current work to determine the histological measurements of the luminal epithelium and endometrial glands as well as the number of inflammatory cells in the endometrium at 7, 10 and 13 days after ovulation in healthy pregnant and cyclic mares and to determine by radioimmunoassay serum concentrations of progesterone, estradiol, insulin, IGF-1, leptin and adiponectin in healthy pregnant and cyclic mares from ovulation to 13 days of the cycle and pregnancy. Finally the work evaluate the existence of correlations between the morphometric variables of the endometrium, the number of inflammatory cells and the serum concentrations of the hormones under study.

2. MATERIALS AND METHODS

2.1 Experimental design

The present work was carried out in the period of 2018 to 2019, in the experimental field of the faculty of Veterinary medicine, Baghdad University, during the reproductive season, the project being previously approved by the animal experimentation commission. 30 mares, between 5 and 10 years old, without a defined breed (Caspian horse breed phenotype), with an approximate weight of 400 kg from a commercial herd utilised. They were held ad libitum in natural pastures.The animals presented body condition> 3 on a scale of 1 to 5 during the experiment, with no history of fertility problems. Their health status was assessed by routine physical and gynecological examination and their cyclicity was verified by gynecological examination and ultrasonography (due to the presence of a LC and / or a follicle greater than 25 mm) with a multi-frequency linear transducer (5-7.5 MHz). Upon ultrasonographic visualization of a corpus luteum, 250 µg (total dose) of dl-cloprostenol sodium (Estrumate™, India) was administered, in order to provoke a return to oestrus. The mares were monitored by daily ultrasonography; In the presence of a preovulatory follicle with a diameter ≥ 35 mm and grade 3 uterine edema, 2500 IU of intravenous hCG (Zydus Cadila, India) was administered. Daily ultrasound control was continued, detecting the day of ovulation (day 0 of cycle 1). Endometrial biopsies from both horns were collected in days 7 (Six mares), 10 (Six mares) and 13 (Six mares) in the first cycle after ovulation, constituting the group of cyclic mares. Endometrial biopsy samples were extracted according to the method described by Kenney (1978) using Yeoman forceps. A sample was taken from each uterine horn in the first dorsal third of the same after sterilization of the forceps by flaming; then the synchronization procedure was repeated (250 µg of synthetic PGF2α, ultrasound monitoring and ovulation induction with hCG). In the second cycle, the mares were served by natural mating 24 hours after the administration of hCG, in the presence of a preovulatory follicle with a diameter ≥ 35 mm and grade 3 uterine edema, for one of three fertile stallions, chosen at random. In parallel, a daily ultrasound control was carried out to detect ovulation (day 0). On days 7 (Six mares), 10 (Six mares) and 13 (Six mares) post ovulation, embryonic retrieval was done by uterine lavage. Mares with visualization of the concept by ultrasonography prior to washing or successful embryo recovery were assigned to the pregnant group. The concepts were collected by Tran’s cervical uterine lavage with 1 to 3 litres (according to gestational age) of lactated Ringer's solution at 37ºC. Day 7 and 10 embryos were recovered by the regular method. Day 13 embryos were recovered using a homemade uterine catheter with a 1.5 cm diameter silicone tubing. Immediately after embryo recovery, biopsies of both uterine horns were removed from all mares with the same methodology used for cyclic mares.

2.2 Sample processing

2.2.1 Endometrial biopsies: The specimens for the two ovarian horns (ipsilateral and contralateral to ovulation) were separately put in 5 mL test tubes and were then labelled for histological inspection in 4% tapered paraformaldehyde.After inclusion in paraffin, 5 µm sections were cut which were marked with H&E and PAS strain in the Department of Morphology of Baghdad University College of veterinary medicine, for their subsequent analysis. .

2.2.1.1 Histological analysis: Histological analysis was carried out in the Department of Animal Reproduction, Baghdad University College of veterinary medicine. The number of eosinophils, lymphocytes, neutrophils, hemosiderocytes and plasma cells present in both strata (stratum compact (EC) and stratum cancellous (EE)) were quantified in the haematoxylin-eosin stained slides. The analysis was carried out using an optical microscope at 400x magnification, through which the number and type of inflammatory cells present in 5 fields were quantified for each layer of the endometrium. Subsequently, an average of each cell type found in each stratum was made for each uterine horn.

2.2.1.2 Morphometric analysis: The histomorphometry research was conducted in the Baghdad University College of Veterinary Medicine, Department of Histology and a 400x high luminary and glandularepithelium (GE) height, Glandulardiameter(GD) and glandular diameter were used as part of an imagery analysis method associated with an Optical Microscope. Lumen glandular and PAS-stained sheets intraglandular secretion.

The altitude of the light epithelium from the crypt tissue to the apical tissue was reported in five random ways. Five sections were chosen. The remaining variables were reported in 10 randomly selected spherical EE glands. Two diameters of each diagnosis (from one crypt to the contrary) were restrained for its glandular diameter showing the average between both measurements and the stature of the glandular epithet from the cell's basement membranium to the apical cell membrane. The glandular lumen (GL) was measured to the same degree as the previous variable among the apical tissues of the epithelial cells. 10 spherical glands were analysed in the measurement of intraglandular secretions, in which intraglandular secretion was spherical, and a corresponding average measurements were taken for the two largest diameters of this secretion. Finally, the average records for each uterine horn have been registered for each variable; these values are the main objective of the statistical study.

2.2.2 Hormonal determination

- Blood trials were taken every day by jugular venepuncture in vacuum dry tubes from all mares from the day of ovulation to the day of endometrial sampling in both cycles. Within 15 minutes after extraction, the samples were centrifuged at 3000 g for 15 minutes. Serum was removed and stored in identified Ependorff tubes and in duplicate at -20C until hormonal determination.

- The determinations of the concentrations of progesterone, 17β-estradiol, insulin, insulin growth factor type 1, leptin and adiponectin were carried out using the radioimmunoassay (RIA) technique at the Baghdad university college of veterinary medicine.
- P4 was determined using the Quick P4/Cube Quantitative Equine Progesterone Kit. The detection limit of the analysis was 0.72 ng / mL with an intra-assay coefficient of variation (CV) for the low control (I) (0.84 ng / mL) and high (II) (2.65ng / mL) of 14 and 7.5% respectively, and an interassay CV of 4.8 and 8% for control I and II respectively.
- 17β-estradiol was determined using a double antibody RIA Kit (Bovine Estradiol ELISA Kits). The detection limit of the analysis was 2.6 pg / mL. The study presented an intra-assay CV for the control (5.9 pg / mL) of 10.8% and an inter-assay CV of 8.7%. Insulin was determined by immunoradiometric analysis (IRMA) (INS-IRMA KIP1251-KIP1254; DiaSource® ImmunoAssays SA, Louvain la Neuve, Belgium). The detection limit was 3.66 IU / mL with intra-assay CV for control I (15.5 ⍰IU / mL) and control II (70.8 ⍰IU / mL) of 8.7% and 7.9 %, respectively, and an interassay CV of 7.8 and 6.5% for control I and II respectively.
- IGF-1 concentrations were determined by Recombinant ovine leptin (RIA) using a commercial kit (Somatomedin ELISA Kit). The detection limit of the analysis was 0.57 ng / mL, with a CV of 6.9% and 4.1% for control I (51 ng / mL) and II (367 ng / mL) respectively.
- Leptin was determined by RIA using a commercial kit (Horse leptin ELISA Kit). The protocol was modified the amount of labeled hormone to 75 µl instead of 100 µl to achieve the detection of low serum values. The sensitivity of the analysis was 1.2 ng / mL, with a CV of 7.5% and 11.4% for control I (3.5 ng / mL) and II (9.6 ng / mL) respectively.
- Adiponectin concentrations were determined by the same method using a commercial kit (Canine Adiponectin ELISA Kit). The compassion of the analysis was 0.59 ng / mL, with an intra-assay CV of 8.8% and 18.6% for control I (8.9 ng / mL) and II (75.9 ng / mL) respectively, while the interassay CV was 13.1% for control I and 14.2% for control II.

2.3 Statistical analysis: Statistical analysis was performed with an ANOVA using the GLM procedure. The study of the correlation between the histomorphometric variables, the hormonal concentrations and the inflammatory cells was carried out by using CORR procedure of SAS (ver9.0;SAS, USA) using the hormonal values obtained on the days of endometrial sampling. The level of significance in all cases was considered as $P < 0.050$ and the trend when $0.050 > P < 0.100$.

3. RESULTS

3.1 Endometrial histomorphology: When comparing variables according to the ipsi horn or contralateral to ovulation, no substantial differences were found.

Day (P = 0.39) or pregnancy (P = 0.86) were not affected by luminous epithelial heights; no group x day (P = 0.37) was observed (Figure 1 (left side). The height of the glandular (Figure 1 (right) was inclined by day (P < 0.002) and classes (P = 0.003). The GE was comparable between days 7 and 10 in both pregnant and cyclic mares, but on day 13, it deteriorated. Differences in height of the epithelium of glandular pigments were found at day 7 (P=0.008) and 10 (P=0.05) between the pregnant mares. No interactions group x day (P = 0.36) were observed.

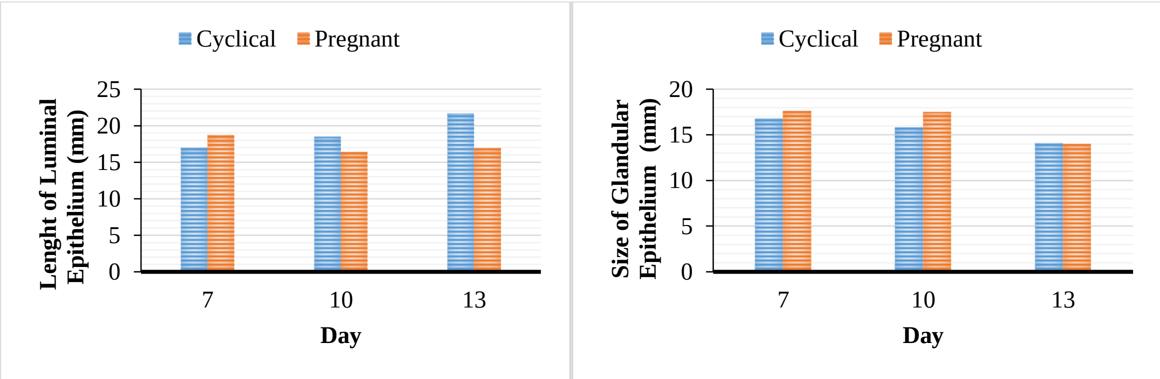
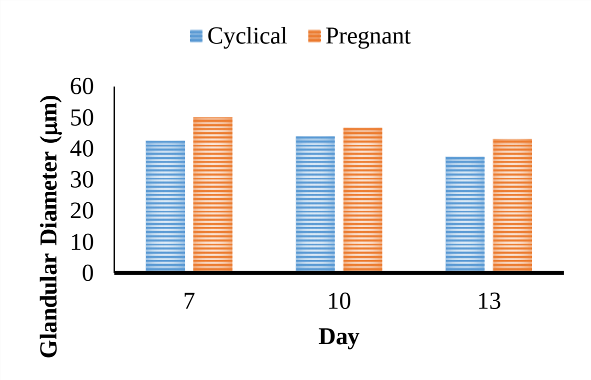


Figure 1: Bar Diagram for the height of the Luminal Epithelium (Left) and the GE(right) of cyclic and pregnant mares

The pregnant mares presented a greater glandular diameter in relation to the cyclic mares at 7 (P <0.001), at 10 (P = 0.05) and at 13 days (P <0.001) (Figure 2 (left)). The glandular diameter was affected by the day of sampling (P <0.001), decreasing with the passing of the days. Within each group, in pregnant mares differences were observed between days 7 and 13 ($P < 0.05$), while in cyclic mares between days 7 and 10 in relation to 13 (P <0.001 and P < 0.01 respectively). No group x day interaction was observed (P = 0.62). Influence of day (P <0.001), group (P = 0.01) and group-day interaction (P = 0.03) in relation to the diameter of the GL (Figure 2 (right)) was observed. In pregnant mares, the GL was similar (P> 0.05) between days 7 and 10, increasing (P <0.01) between days 7 and 13. In the same way, a greater size of the GL was detected on day 13 in pregnant mares than in cyclical ones. Within the group of cyclic mares, no changes in the GL (P> 0.05) were verified between days 7, 10 and 13.



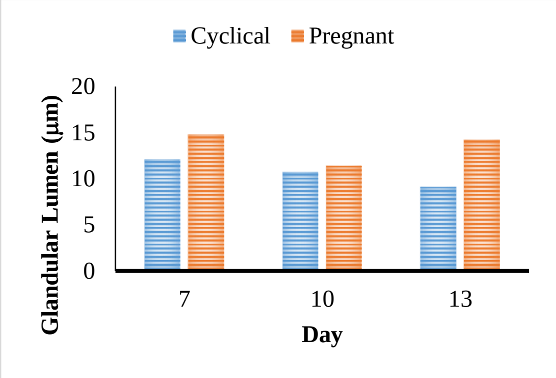


Figure 2: Bar diagram for the determination of GD (left) and GL (right side) of cyclic and pregnant mares

Glandular secretion was inclined by the cluster (P = 0.003) but it was not inclined by the day (P = 0.11) (Figure 3). An increase in secretion was observed at 10 (P = 0.02) and at 13 days (P = 0.02) in pregnant mares in relation to the same days in cyclic mares. A group x day interaction trend was observed (P = 0.07).

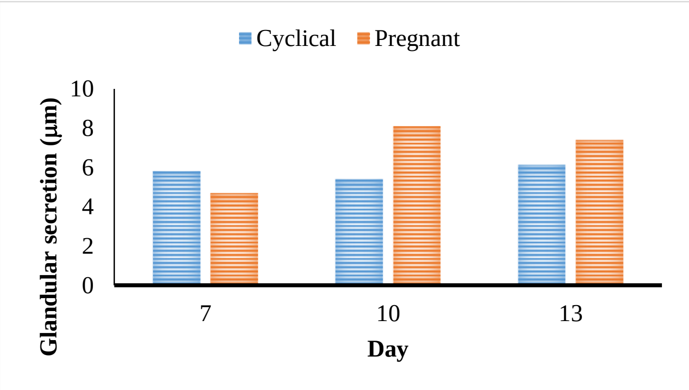


Figure 3: Bar diagram for the determination of the Glandular Secretion of cyclic and pregnant mares.

3.2 Endometrial inflammation

Eosinophils: The presence of eosinophils in the CS was inclined by the time of sampling (P = 0.004) and by the group sampled (P = 0.003). A greater (P = 0.005) presence of eosinophils was observed at 7 days post ovulation in pregnant mares than in cyclic ones. In pregnant mares, the number of eosinophils in the CE was lower on day 13 than on day 7 (P <0.02). Group x day interaction was verified (P = 0.009) (Figure 4(left)).

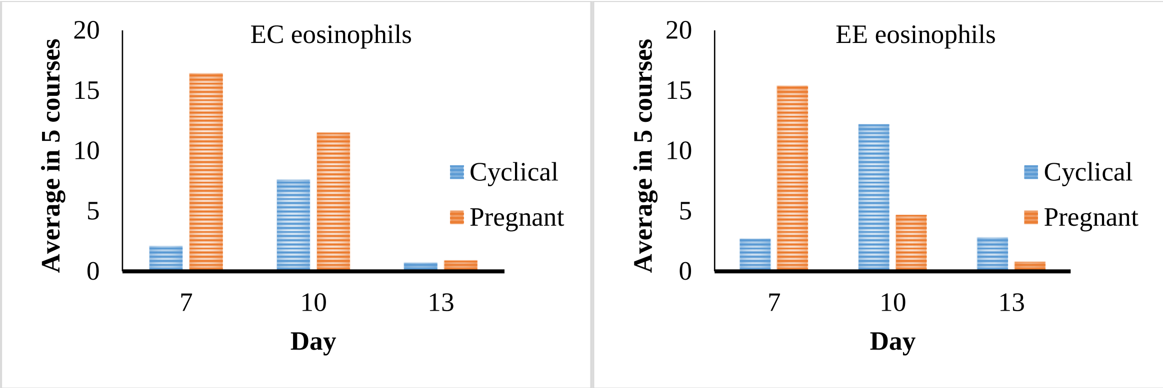


Figure 4: Bar diagram for the determination of the number of eosinophils in the EC (left) and EE (right) of the endometrium of cyclic and pregnant mares [EC = compact stratum, EE = spongy stratum]

In the EE, the day influenced the number of eosinophils (P = 0.04) but not the group (P = 0.2). A higher incidence was found within the group of pregnant mares at 7 days compared to day 13 (P <0.05). A group x day interaction trend was verified (P = 0.06) (Figure 4 (right side)).

Lymphocytes: The number of lymphocytes in the CS was inclined by the time (P = 0.007) and by the group (P = 0.041). A greater (P = 0.021) presence of lymphocytes was observed on day 7 in pregnant mares than in cyclic mares. In pregnant mares, a decrease in lymphocytes in the EC was observed from day 7 to day 13 (P = 0.05). Group x day interaction was found (P = 0.02) (Figure 5(left)). In the EE, there was no influence of the day (P = 0.23) or of the group (P = 0.23). There was a trend of group x day interaction (P = 0.09) (Figure 5(right)).

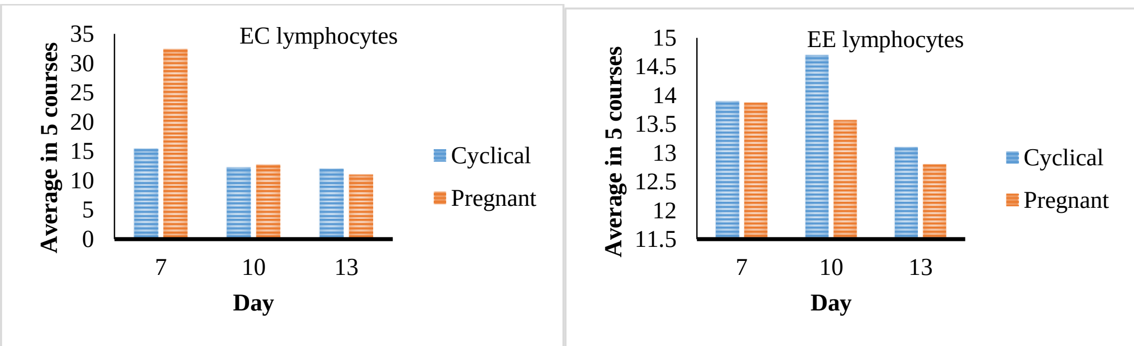


Figure 5: Bar diagram for the determination of the number of lymphocytes in the EC (left) and EE (right) of the endometrium of cyclic and pregnant mares [EC = compact stratum, EE = spongy stratum]

Other inflammatory cells: A minimal amount of neutrophils, hemosiderocytes, and plasma cells were observed (mean less than 1 cell among the 5 fields observed in both endometrial strata), with no differences between the days of sampling or between the different groups. No significant differences were observed when comparing inflammatory cells according to the ipsi horn or contralateral to ovulation.

3.3 Hormonal profiles: P4 concentrations were influenced by the sampling day ($P < 0.001$), but there were no differences between the study groups ($P = 0.54$) and no interaction between group and day ($P = 0.98$). The concentration of P4 increased progressively until day 5, remaining constant until day 10, at which point the concentration decreased by 30% towards the end of the sampling (Figure 6).

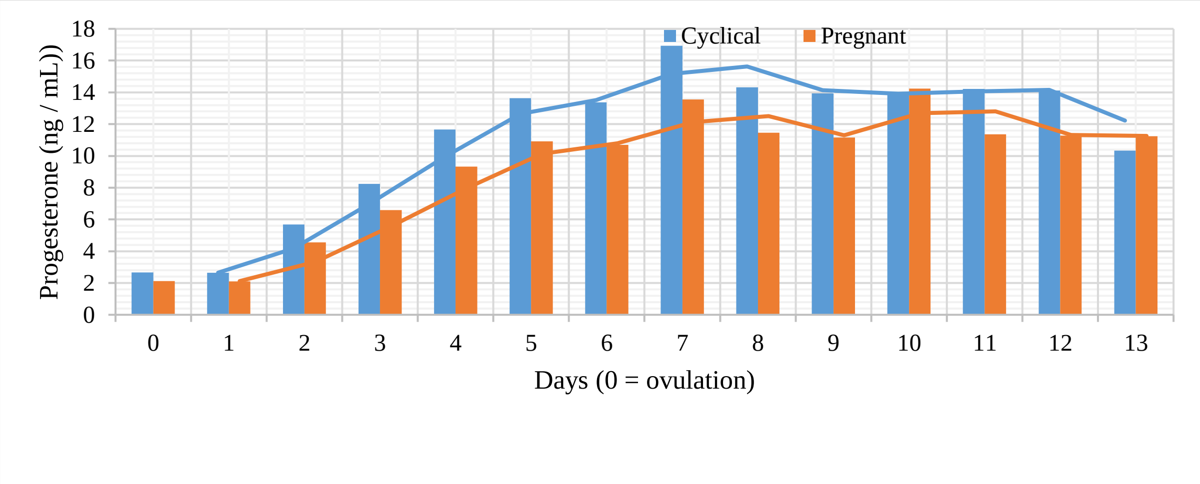


Figure 6: Determination of P4 serum concentrations (Mean ±SEM) (ng/mL) from ovulation [day 0 to the end of sampling (day 13)].

E2 serum levels were exaggerated by the day of sampling ($P = 0.02$), but not by the group (cyclical or pregnant) ($P = 0.41$). Neither interaction was observed between group and day. E2 concentrations were higher on days 0 and 1 than on days 5 to 8 inclusive ($P < 0.05$) (Figure 7).

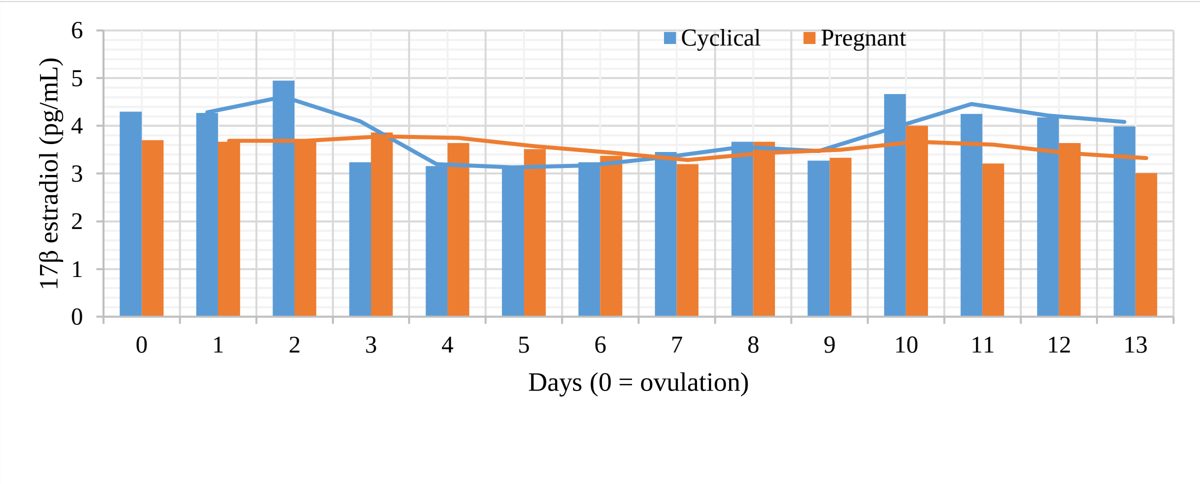


Figure 7: E2 serum concentrations (Mean ± SEM) (pg/mL) from ovulation [day 0 to the end of sampling (day 13)].

Cyclic mares showed a trend (P = 0.07) to a higher insulin concentration than pregnant mares, presenting a higher concentration on days 8 and 12 (P <0.05) and a trend on days 6 and 13 (P = 0.06). Interaction between group and day (P = 0.03) was observed in insulin concentrations during the sampling days, but no influence of day was observed (P = 0.15) (Figure 8).

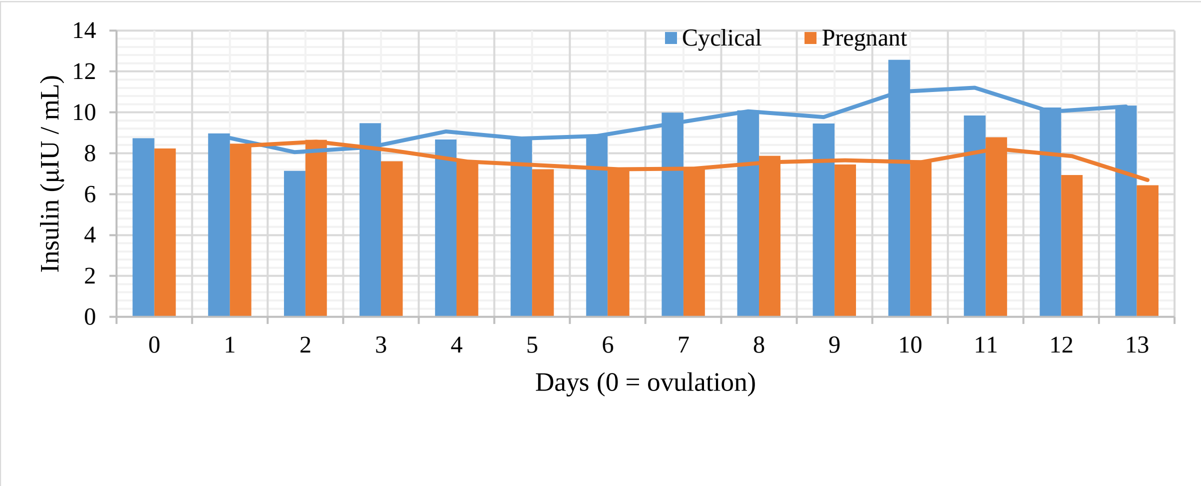


Figure 8: Serum insulin concentrations (Mean ± SEM) (µIU / mL) from ovulation [day 0 to day 13. *: P <0.05; +: 0.050> P <0.100].

IGF-1 levels were exaggerated by the day of sampling (P = 0.04), but not by the group (cyclical or pregnant) (P = 1.0). Neither was interaction detected between group and day (P = 0.36). Decreasing values were recorded in both groups from the zero day of ovulation until day 13, at which point the concentration decreased by 10% with respect to day 0 (Figure 9).

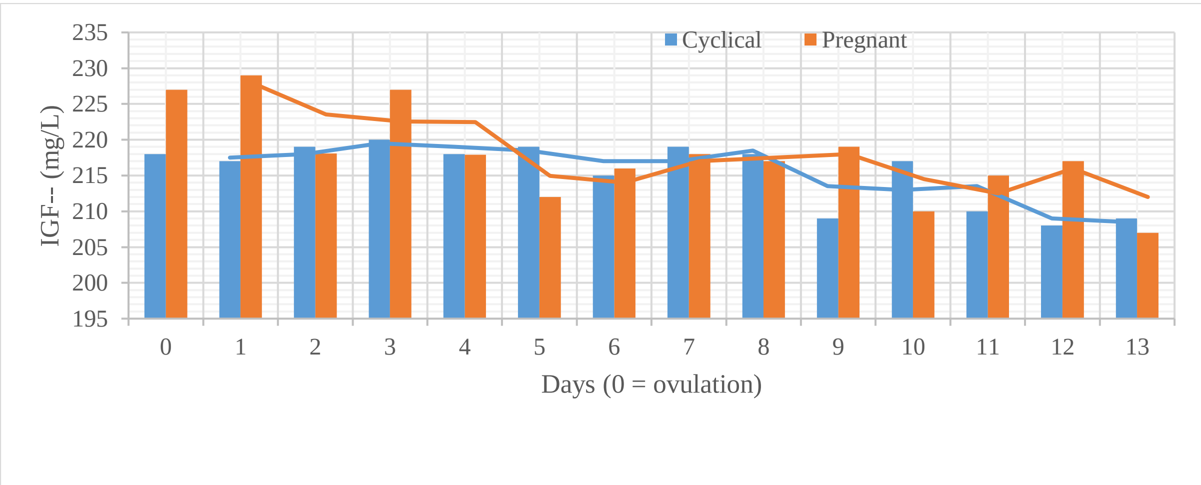


Figure 9: Serum insulin growth factor 1 (IGF-1; ng / mL) concentrations (Mean ± SEM) from ovulation from day 0 to day 13.

Figure 10 expresses the serum leptin concentrations in cyclic and pregnant mares. No group influence was seen (P = 0.69), daytime (P = 0.73) nor was group-day interaction observed (P = 0.27).

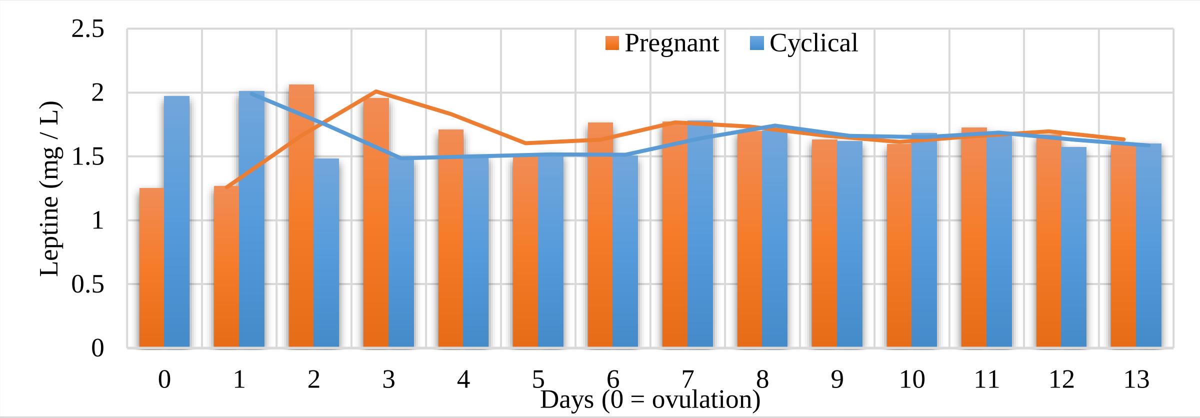


Figure 10: Serum leptin concentration (Mean ± SEM) from ovulation from day 0 to day 13.

In the serum concentration of adiponectin, differences were observed by the day of sampling (P = 0.03), but not by the group (cyclical or pregnant mares) (P = 0.6) nor was interaction group x day observed (P = 0.6). The daily values decreased 20% towards day 9 with respect to the day of ovulation (P = 0.03), remaining in decline until day 11 and recovering the values of day 0 from day 12 (Figure 11).

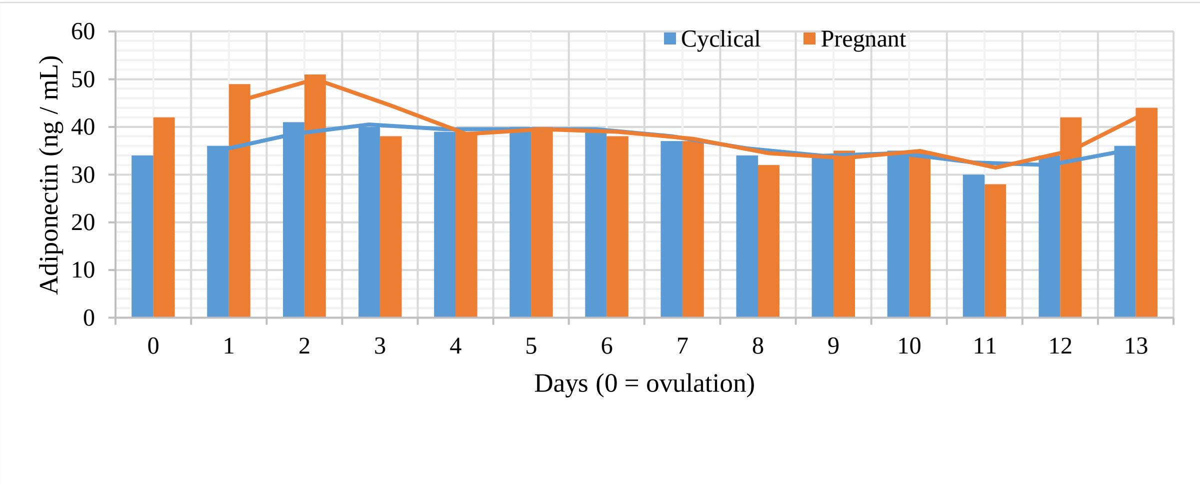


Figure 11: Serum concentrations (Mean ± SEM) of adiponectin (ng / mL) from ovulation [day 0 to day 13].

3.4 Correlation between hormonal concentrations, histomorphological variables and the number of inflammatory cells: The correlations of pregnant mares are shown in table 1, while those of cyclical mares are shown in table 2.

In both groups of mares, epithelium and glandular diameter were correlated with serum P4 concentrations. In turn, an association was observed between the GE and the glandular diameter. In both groups, a correlation was also observed between the height of the GE and the number of lymphocytes in CD.

Table 1: Correlation between the variables studied in the group of pregnant mares (n = 36).

	GE	GD	GL	Glandular secretion	EC-Lymphocyte	EC Eosinophils	EE Eosinophils
Progesterone	r = 0.33; P = 0.05	r = 0.35 ;P = 0.04	PZ*	PZ*	PZ*	r = -0.46 ;P = 0.004	r = -0.43 ;P = 0.0008
GE		r = 0.77 ;P = 0.0002	r = -0.5 ;P = 0.03	r = -0.45 ;P = 0.5	r = -0.5 ;P = 0.003	r = -0.53;P = 0.002	r = -0.45;P = 0.04
GD			PZ*	PZ*	PZ*	r = -0.51 ;P = 0.02	r = -0.51 ;P = 0.02
GL				r = -0.58 ;P<0.01	PZ*	PZ*	PZ*
Glandular secretion					PZ*	PZ*	PZ*
EC Eosinophils						PZ*	PZ*
EE Eosinophils							r = -0.88 ;P = 0.0001

*PZ: P>0.05

Table 2. Correlation between the variables studied in the group of cyclical mares (n = 36).

	GE	GD	GL	Glandular secretion	EC-Lymphocyte	EC Eosinophils	EE Eosinophils
Progesterone	r = 0.54; P = 0.01	r = 0.59 ;P = 0.01	PZ	PZ*	PZ*	PZ*	PZ*
GE		r = 0.85 ;P<	r = -0.39	PZ*	PZ*	PZ*	PZ*

		0.0001	;P = 0.03				
GD			PZ*	PZ*	PZ*	PZ*	PZ*
GL				PZ*	PZ*	PZ*	PZ*
Glandular secretion					PZ*	PZ*	PZ*
EC Eosinophils						PZ*	PZ*
EE Eosinophils							PZ*

*PZ: P>0.05

In pregnant mares, the lumen and glandular secretion were associated and both variables were negatively correlated with the GE. Serum P4 concentrations were related to the number of eosinophils in both strata. In turn, the eosinophils in both strata varied in relation to the height of the EG.

4. DISCUSSION

The present study has shown that early pregnancy is associated with histomorphological changes in the endometrium and an initial invasion of inflammatory cells with a subsequent reduction of these cells with advancing gestation. In turn, it showed that serum insulin was modified during early pregnancy, while the other hormones measured did not undergo changes in relation to cyclical mares. The histomorphological records found in cyclic mares coincide with those reported by other authors [16]. When comparing the endometrial records of cyclic mares with pregnant ones, we first observed the absence of differences in the luminal epithelium. This similarity observed between the groups coincides with that published by Aurich et al. (2015) [17], in whose work they found no differences in the height of the luminal epithelium from day 6 of gestation and right handed. However, in a study carried out with the same samples from this study, Camozzato, et al. (2018) [18] observed, by means of scanning electron microscopy, a decrease in the number of ciliated cells in the luminal epithelium of pregnant mares from 7 days of pregnancy in comparison with mares in right-handedness, which suggests that despite If there are no alterations in its height, it is possible that the epithelium is also undergoing modifications as a result of pregnancy.

The detection of differences in glandular measurements from the first day of sampling indicates the latter as a fundamental part of endometrial adaptation during gestation. This idea is supported by the fact that the uterine environment is critical for the continuous support and development of the concept from day 6 post ovulation and that prior to implantation, the concept is supported only by the secretions that accumulate in the uterine lumen, secretions originating from the endometrial glands. In our work, it was observed that pregnancy was associated with a dilation of the GL due to the accumulation of intraglandular secretion throughout the sampling days, while in cyclic mares no modifications were observed in either of the two variables through the days. This result coincided with that reported by Lefranc et al. (1991) [19] who, through electron microscopy observation, found a distention of the GL as a consequence of the accumulation of secretions in mares on day 12 of pregnancy, comparing them with cyclic mares on the same day of the cycle. The association between lumen enlargement and glandular secretion is supported in our study by the positive correlation found between both variables in pregnant mares.

Both the epithelium and the glandular diameter decreased through the sampling days in both groups. The decreases in these measurements were associated with serum P4 concentrations in both pregnant and cyclical mares; Considering that endometrial structures vary in relation to the concentration of steroid hormones, it could be assumed that the aforementioned effect at the glandular level would be promoted by the circulating concentrations of P4 that decrease towards the last day of sampling. Despite the decrease in both variables in both study groups, in pregnant mares a greater height of the epithelium and glandular diameter was observed from day 7 compared to the cyclical mares on the same day. These early changes in the endometrial structure would be reflecting rapid adaptations of the endometrium to pregnancy if it is considered that the equine embryo enters the uterus between 5 and 6 days after ovulation, or on the contrary they would suggest the existence of a systemic effect prior to the entry of the embryo into the uterus so that when it enters it is found with a uterine environment conducive to its survival. The decrease of both variables through the days in both pregnant and cyclical mares was previously reported [20], however, said authors did not detect differences between the study groups on sampling days (days 6 to 9 and 12 to 14). Given that the aforementioned study presents a relatively wide sample extraction window, the exact days of extraction being not clear, we believe that our work reflects a situation more adjusted to reality.

The decrease in the height of the GE in pregnant mares was associated with an increase in the lumen and glandular secretion. In addition to the effect of P4 mentioned previously, we believe in the possibility that the presence of the embryo could cause an increase in glandular secretion, and that this, through a physical effect, would lead to the dilation of the glands with the consequent reduction of the GE.

The increase in P4 was also related in pregnant mares with an increase in the number of eosinophils. Said association would be generated as a consequence of some external factor, such as the presence of the embryo, since the same concentration of P4 was observed in cyclic mares in the absence of an increase in inflammatory cells. Supporting what happened in cyclic mares, Krakowski et al, (1999) [21] reported a lower amount of immune cells on day 7 after ovulation, assigning an inhibitory effect to the higher plasma progesterone levels found that day. The higher number of inflammatory cells observed in pregnant mares compared to cyclical ones on day 7 could be indicative of a maternal immune response to the presence of the embryo in utero. For that day, the embryo descended into the uterus from the uterine tube and the formation of the capsule is being completed with the consequent reduction of the zona pellucida, which would make the embryo immunologically active, considering that mammalian embryos they are immunologically inert until released from the zona pellucida [22]. However, we cannot assert this fact since we do not have a sampling prior to the descent of the embryo into the uterus or prior to the formation of the capsule.

The inflammatory cells observed in greater numbers in the endometrium of pregnant mares in the initial sampling were eosinophils and lymphocytes. This observation was previously reported by Krakowski et al, (1999), where the authors point out that the presence of lymphocytes may suggest a maternal immune response towards the embryo during its initial contact with the endometrium and that the increase in eosinophils may be given to moderate this response. Subsequently, through the days of sampling, a reduction in the number of both inflammatory cells is observed, which could indicate an immunomodulation in the maternal tissue to avoid possible rejection in the presence of an element genetically foreign to the mother. The reduction of endometrial inflammatory cells by day 13 was previously reported; Krakowski et al, (1999) detected a decrease in these cells at 12 to 14 days, and later Noronha et al. (1993) [23] reported that the number of T lymphocytes does not differ between pregnant and right-handed mares at day 14. This finding coincides with what happens in other species such as sows in which a reduction in the number of lymphocytes is observed from the day 10 of gestation. The increase in eosinophils also coincides with that found in the pregnant sow by Harvey et al. (2005) [24] in the pre-implantation stage. Furthermore, it is possible that, as was postulated in the sow, eosinophils may be associated with dynamic changes in endometrial structure and function that would be preparing the uterine environment to house the embryo.

The inexistence of morphometric differences and of inflammatory cells in the endometrium when comparing the ipsi horn or contralateral to ovulation leads us to consider the possibility that, given the unique migratory characteristics of the equine embryo, it would be stimulating the entire uterine extension equally, thus causing rapid transformations at the endometrial level; however, again, it leads us to suspect the existence of systemic processes that would be preparing the uterus to receive the embryo before its entry.

In relation to the hormonal levels found in the mares in this study, the serum insulin levels yielded novel results that had not been found in any previous study so far. Lower insulin concentrations were observed in pregnant mares compared to cyclical ones from day 6 and 8 post ovulation, a moment that coincides with blastulation, with a pronounced increase in glucose consumption by the embryo and with a rapidly developing blastocyst cavity expansion with high energy demands. Given the facilitating role of insulin in embryonic development, the theory that maternal insulin affects the embryo directly by increasing the consumption of glucose, amino acids and proteins as well as the synthesis of proteins and RNA by the embryo, and that possibly as happens in the mouse, maternal insulin could be internalized by the pre-implantation embryo in the equine, it could be thought that the decrease in serum levels of maternal insulin in pregnant mares could be the result of its consumption by the embryo. Differences in serum insulin concentrations could also be generated as a consequence of embryonic consumption of maternal glucose, which could cause a decrease in systemic concentrations of the hormone; or as a third possibility, that insulin could be being used at the endometrial cell level, and that as happens in Horse, the hormone would be stimulating cellular activity in the endometrium. Observing the serum values of the other hormones measured in this work, the serum profiles and concentrations of P4 in both study groups are in agreement with that reported by other authors [25]. In the case of E2, the secretion patterns coincide with that reported by Sander, et al.,(1979) [26] and by TalebkhanGaroussi et al. (1991) [27], who did not find differences between pregnant and right-handed mares within the sampling days of the present study, however they differ from that published some other authors [28]who reported lower values in pregnant mares during the same study period.

The lack of differences in serum IGF-1 concentrations between pregnant and cyclic mares suggests the absence of an effect of pregnancy on systemic IGF-1. This finding agrees with that found by other authors, Ali et al., (2013) conclude that the blood levels of IGF-1 during early pregnancy do not reflect the high endometrial and embryonic production of the factor in this period. Local secretion has been reported by several authors, it is known that both the embryo and the endometrium produce significant amounts of IGF-1, that the peptide is secreted in an autocrine-paracrine manner during the pregnancy early pregnancy and that IGF-1 has been detected in uterine washings of pregnant mares at 12, 13, 14, 16 and 17 days of gestation. Harvey et al. (2005) highlight the low probability that systemic maternal IGF-1 is directly involved in stimulating early growth of the concept during the early stages of pregnancy in the mare. In parallel, dissimilar that reported by NAMBOet al. (2009) who found no differences in IGF-1 concentrations between 7 and 14 days of the luteal phase, in our work a slight drop in its serum concentration was observed from ovulation until the end of sampling.

Regarding the results obtained from adipokines measured in serum, serum leptin agrees with that published by NAMBO et al. (2009), finding no differences between the days and sampling groups. Adiponectin did not reveal differences between the groups either, given that the hormone circulates under different isoforms, there is the possibility that, as postulated by Ali et al., (2013), the biological activity of the hormone is modified but that no changes are observed in its total concentration in serum. However, we believe it is more likely that given the absence of differences between cyclic and pregnant mares in the serum concentrations of both adipokines, and given the abundance of information in the literature on the local role of these hormones on the endometrium and the embryo, pregnancy early would not be generating a maternal systemic effect on its secretion, but rather its roles would be local like IGF-1.

In summary, from the observed changes, we believe that the decrease in P4 generated, in both groups of mares, a decrease in the GE and that it promoted a decrease in the glandular diameter over the days. The decrease in the GE in pregnant mares was in turn associated with an increase in the lumen and glandular secretions, probably as a consequence of the presence of an external factor, such as the presence of the embryo, and by a physical effect of distention of the gland by secretions. On the other hand, the increase in P4 associated with an external factor (embryo), promoted the increase in inflammatory cells in pregnant mares, cells that decreased over the days, probably as a consequence of maternal immunomodulation. Given that the endometrial changes were observed from the first day of sampling, when the embryo entered the uterus just hours ago, we ask ourselves whether the observed changes are the result of the presence of the embryo that is rapidly causing changes in the structure endometrial or if the changes are the product of systemic processes that prepare the uterus to receive the embryo before it leaves the uterine tube and enters the uterus. In parallel, we detect modifications in the concentration of systemic insulin that leads us to suspect that it has a role in embryonic and / or endometrial cell activity.

5. CONCLUSIONS

Early pregnancy is associated with changes in the glandular structure. However, the height of the luminal epithelium does not undergo modifications from 7 to 13 days of the estrous cycle and pregnancy. At day 7 there is a greater amount of eosinophils and lymphocytes in pregnant mares than in cyclic mares, these cells are reduced in pregnant mares by day 13.Serum insulin decreases in pregnant mares compared to cyclical ones from ovulation to day 13. No differences associated with pregnancy were observed in the remaining hormones determined. P4 correlates with histomorphological changes in both groups of mares and with changes in inflammatory cells in pregnant mares. There is no correlation between the other hormones measured with endometrial changes. The glandular changes are interrelated. Therefore, we understand that the information generated provides valuable data that can complement future studies on the changes that occur during the first days of pregnancy in the mare. In turn, it leads us to ask ourselves the question of the existence of structural changes and inflammatory cells, prior to the entry of the embryo into the uterus, which would imply that it would be sending signals from the uterine tube, and that through a systemic process it would be preparing the uterus for its survival.

References

[1]. Taklan, Aeman&Alwan, A & Nada, S. (2016). Induce estrus with ultrasonography examination and progesterone hormone assay for pregnancy diagnosis in Iraqi goats. Iraqi Journal of Veterinary Medicine. 40. 89-93. 10.30539/iraqijvm.v40i2.118.

[2]. Yoon, Min. (2012). The Estrous Cycle and Induction of Ovulation in Mares. Journal of Animal Science and Technology. 54. 10.5187/JAST.2012.54.3.165.

[3]. Foote, Christopher & Block, William & Crane, Keith & Gray, Simon. (2004). Economic Policy and Prospects in Iraq. The Journal of Economic Perspectives. 18. 47-70. 10.1257/0895330042162395.

[4]. Ishak, Ghassan & Bashir, Shah Tauseef &Gastal, Melba &Gastal, Eduardo. (2017). Pre-ovulatory follicle affects corpus luteum diameter, blood flow, and progesterone production in mares. Animal Reproduction Science. 187. 10.1016/j.anireprosci.2017.09.003.

[5]. Bollwein, Heinrich & Mayer, R & Weber, F &Stolla, R. (2002). Luteal blood flow during the estrous cycle in mares. Theriogenology. 57. 2043-51. 10.1016/S0093-691X(02)00705-7.

[6]. Satué, Katy &Gardón, Juan. (2020). Physiological and Clinical Aspects of the Endocrinology of the Estrous Cycle and Pregnancy in Mares. 10.5772/intechopen.90387.

[7]. Nett, Terry & Shoemaker, C & Squires, ed. (1989). Changes in Serum Concentrations of Luteinizing Hormone and Follicle-Stimulating Hormone Following Injection of Gonadotropin-Releasing Hormone during Pregnancy and after Parturition in Mares. Journal of animal science. 67. 1330-3. 10.2527/jas1989.6751330x.

[8]. Beyer, Teresa & Rink, B. Elisabeth & Scarlet, Dragos & Walter, Ingrid &Kunert, Steffen &Aurich, Christine. (2018). Early luteal phase progestin concentration influences endometrial function in pregnant mares. Theriogenology. 125. 10.1016/j.theriogenology.2018.11.018.

[9]. Satué, Katy &Gardón, Juan. (2012). Pregnancy: Risk factors, management and recovery in mares. Pregnancy: Risk Factors, Management and Recovery. 1-54.

[10]. Allen, W.. (2000). The Physiology of Early Pregnancy in the Mare. Proc. Am. Ass. Equine Practnrs.. 46.

[11]. Ricketts, Sidney. (2010). The Technique and Clinical Application of Endometrial Biopsy in the Mare. Equine Veterinary Journal. 7. 102 - 108. 10.1111/j.2042-3306.1975.tb03243.x.

[12]. Snider, T.A. & Sepoy, C &Holyoak, G.. (2011). Equine endometrial biopsy reviewed: Observation, interpretation, and application of histopathologic data. Theriogenology. 75. 1567-81. 10.1016/j.theriogenology.2010.12.013.

[13]. Yong, H.. (2007). Application of endometrial biopsy in the horse. Journal of Veterinary Clinics. 24. 292-294.

[14]. Pazinato, Fernanda & Curcio, Bruna & Fernandes, Cristina & S Feijo, Lorena &Schmith, Rubia & Nogueira, Carlos. (2016). Histological features of the placenta and their relation to the gross and data from Thoroughbred mares. PesquisaVeterináriaBrasileira. 36. 665. 10.1590/S0100-736X2016000700018.

[15]. Cole, H. & Howell, C. & Hart, G.. (2005). The changes in the ovary of the mare during pregnancy. The Anatomical Record. 49. 199 - 209. 10.1002/ar.1090490305.

[16]. Burger, Dominik &Wohlfender, Franziska & Imboden, Isabel. (2008). Managing a mare for breeding and sport. Pferdeheilkunde. 24. 10.21836/PEM20080122.

[17]. Aurich, Christine, and Sven Budik. "Early pregnancy in the horse revisited - does exception prove the rule?." Journal of animal science and biotechnology vol. 6 50. 2 Dec. 2015, doi:10.1186/s40104-015-0048-6

[18]. Camozzato, G.C. & Martinez, M.N. & Bastos, Henrique &Fiala Rechsteiner, Sandra & Meikle, Ana &Jobim, M.I.M. & Gregory, R.M. & Mattos, Rodrigo. (2018). Ultrastructural and histological characteristics of the endometrium during early embryo development in mares. Theriogenology. 123. 10.1016/j.theriogenology.2018.09.018.

[19]. Lefranc, A & Allen, W. (2007). Influence of breed and oestrous cycle on endometrial gland density in the mare. Equine veterinary journal. 39. 506-10. 10.2746/042516407X235812.

[20]. Smits, Katrien& Nelis, Hilde &Steendam, Katleen&Govaere, Jan &Roels, Kim &Ververs, Cyriel&Leemans, Bart &Wydooghe, Eline & Deforce, Dieter &Soom, Ann. (2016). Proteome of equine oviducal fluid: Effects of ovulation and pregnancy. Reproduction, fertility, and development. 29. 10.1071/RD15481.

[21]. Krakowski, Leszek &Krzyżanowski, Jan & Wrona, Zygmunt &Siwicki, Andrzej. (1999). The effect of nonspecific immunostimulation of pregnant mares with 1,3/1,6 glucan and levamisole on the immunoglobulins levels in colostrum, selected indices of nonspecific cellular and humoral immunity in foals in neonatal and postnatal period. Veterinary Immunology and Immunopathology. 68. 1-11. 10.1016/S0165-2427(99)00006-9.

[22]. Risvanli, Ali &Çetin, Hayrettin &Gulacti, Irem&Yuksel, Murat &Saat, Nevzat& Korkmaz, Ömer& Osman, M. (2006). The presence of anti-zona pellucida antibodies in blood serum of pregnant and infertile mares and cows. Animal science papers and reports. 24. 153-158.

[23]. Noronha, Leela &Antczak, Doug. (2012). Modulation of T-cell Reactivity During Equine Pregnancy is Antigen Independent. American journal of reproductive immunology (New York, N.Y. : 1989). 68. 107-15. 10.1111/j.1600-0897.2012.01154.x.

[24]. Harvey, John & Pate, Melanie &Kivipelto, Jan & Asquith, Richard. (2005). Clinical biochemistry of pregnant and nursing mares. Veterinary clinical pathology / American Society for Veterinary Clinical Pathology. 34. 248-54. 10.1111/j.1939-165X.2005.tb00049.x.

[25]. Nambo, Yasuo &Tatee, Hiroaki &Kotoyori, Yasumitsu&Komano, Michio & TANAKA, Hidetoshi. (2009). Weekly Changes in Serum Progesterone Concentrations in Pregnant Thoroughbred Mares, in Comparison with Seven Mares with Early Pregnancy Loss. J. Jpn. Vet. Med. Assoc. 62. 630-635. 10.12935/jvma.62.630.

[26]. Sander, Valeria &Piehl, Lidia L. &Facorro, Graciela &Celis, Emilio & Motta, Alicia. (2008). Regulation of functional and regressing stages of corpus luteum development in mice. Role of reactive oxygen species. Reproduction, fertility, and development. 20. 760-9. 10.1071/RD08051.

[27]. TalebkhanGaroussi, Massoud&Farzaneh, Nima&Gallehdar, Ehssan&Mohri, Mehrdad. (2012). Reproductive performance in out-of-breeding season of fatty ewes using implant norgestomet with or without PMSG. Tropical animal health and production. 44. 965-968. 10.1007/s11250-011-9993-z.

[28]. Ali, Farah & Lodhi, Laeeq& Qureshi, Zafar & Ahmad, Ishfa& Hussain, Riaz. (2013). Serum Mineral Profile in Various Reproductive Phases of Mares. Pakistan Veterinary Journal. 33. 296-299.