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## Evaluation Of Cytological Picture Of Vaginal Smears And Plasma Progesterone Profile As A Tool For Monitoring The Reproductive Pattern In Normal Foaling Arabian Mares

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### ABSTRACT

Twenty one normal foaling Arabian mares were used in the present work. The alterations in the cells of vaginal smears and plasma progesterone profile were recorded during some reproductive phases in mares. There was an elevation in percentage of cornified cells and values of total leukocytic infiltration accompanied with the similarity in percentage of both small and large nucleated squamous cells were observed during puerperium. The relative increase in percentage of cornified cells, in addition to the similarity in percentage of both small and large nucleated squamous cells and the sharp decrease in values of total leukocytic infiltration were observed when the plasma progesterone concentration was sharply dropping during the 1<sup>st</sup> and 2<sup>nd</sup> postpartum estrus. The percentage of nucleated small squamous cells and values of total leukocytic infiltration, as well as the plasma progesterone concentration were increased during the 1<sup>st</sup> luteal phase, early stages of pregnancy and prolonged diestrus. The most important criterion predicting the mid and late stages of pregnancy are the increase in values of total leukocytic infiltration accompanied with the similarity in percentage of both small and large nucleated squamous cells which relatively enlarged in their size , meanwhile there was a non significant variation in plasma progesterone levels with advancement of gestation.

### INTRODUCTION

Reproductive performance is closely related to the balanced hormonal secretion during different reproductive phases. The plasma progesterone concentration should be determined, because it is one of the key hormone controlling reproductive function in mares (1). The low and high concentration of progesterone hormone plays an important role in embryonic losses in mares (2,3). The cytological picture of vaginal smears is greatly affected by ovarian hormones (4,5). However, the cytological features of vaginal epithelium and plasma progesterone profile were used as a diagnostic tool for assessment of the reproductive pattern in sheep and goats (5,6,7 and 8), cattle (9,10), camel (11) and bitch (12,13). Unfortunately, the vaginal smears from the mare is not distinctive during estrus as no cell type is characteristics for any stage of the cycle (14). Moreover, the cornification of the superficial layer of vagina is minimal during estrus and cannot be used as a cytological criterion for determining different stages of estrous cycle (15). The present investigation is a detailed trial to correlate the levels of exfoliated vaginal cells with plasma progesterone profile during different reproductive phases in normal foaling Arabian mares.

### MATERIAL AND METHODS

#### Animals

Twenty one normal foaling Arabian mares (Egyptian strains) of different parities (aged 6-18 years), belonged to El -Zahraa Stud for pure Arabian horses in Cairo were used in the present work. All the postpartum mares were rectally palpated to detect the uterine tone status and ovarian activity during puerperium, foaling heat, 1<sup>st</sup> luteal phase for all mares and in the 2<sup>nd</sup> post partum estrus for all returned females during one month. Heat signs were detected by the daily teasing with the stallion in the predicted time. The ovulation fossa was rectally palpated in 5 mares during foaling heat and 3 mares during 2<sup>nd</sup> postpartum estrus. All mares were naturally mated during foaling heat according to the routine program adapted in the farm. Females suspected to be non-pregnant were re-mated during the 2<sup>nd</sup> postpartum estrus. Expectation of early pregnancy was done by using diagnostic ultrasound 16 days post estrus and confirmed at days 35, 60 and 90. Rectal palpation was also done to detect the uterine tone status during pregnancy .

### Vaginal smears

For cytological examination, after dry cleaning of the vulva with dry cellulose paper and by the help of sterile vaginoscope samples from the roof of posterior vagina using a sterile long hand stainless steel spatula were obtained. The charged material in the spatula was spread on a clean dry microscope slide. The collecting vaginal smears representing the puerperium, foaling heat, 1<sup>st</sup> luteal phase, 2<sup>nd</sup> postpartum estrus and pregnancy (20<sup>th</sup> -30<sup>th</sup>, 31<sup>th</sup> - 40<sup>th</sup>, 150<sup>th</sup>-180<sup>th</sup> and 240<sup>th</sup> -300<sup>th</sup> days of gestation). Vaginal smears were also taken from the mares in which the ovulation fossa was rectally palpated during estrus and four times from the mare suspected to be had prolonged diestrus. All the collected smears were fixed in 95% ethyl alcohol for one minute and stained with a modified Papanicolaou's method (16). The cells of vaginal smears were evaluated according to *Ramachandraiah et al. (17)*. These cells were classified into nucleated small squamous, nucleated large squamous and cornified cells. In each smear, at least 200 cells were counted and the percentage of each type of cells was recorded in relation to the total number of cells. The values of total leukocytic infiltration was also observed in all counted fields in each smear. A prophylactic dose of antitetanic serum (1500 iu) was injected intramuscularly for each mare following collection of the vaginal smears .

### Blood sampling

Blood samples were collected by jugular vein puncture in heparinized sterile evacuated tubes from all normal foaling mares during the 3<sup>rd</sup> day postpartum, foaling heat, 7<sup>th</sup> day later (1<sup>st</sup> luteal phase) as well as during the 2<sup>nd</sup> postpartum estrus and 7<sup>th</sup> day later from the returned females. It was also collected from the pregnant mares during early stages ( 20<sup>th</sup>, 30<sup>th</sup> and 40<sup>th</sup> days post mating), mid-stage (150<sup>th</sup> and 180<sup>th</sup> days of gestation) and late stage (240<sup>th</sup> and 300<sup>th</sup> days of gestation). Blood samples were also harvested from the mares in which the ovulation fossa was

rectally palpated during estrus and from the mare suspected to be had prolonged diestrus. The collected blood samples were chilled on ice and spun in cooled centrifuge at 3000 r.p.m. for 30 minutes. Blood plasma was separated and preserved at -20 °C until the time of progesterone assay.

### Determination of plasma progesterone

Plasma progesterone concentration was determined according to *El-Banna et al (18)* and *El-Banna and Gamal (19)* at the laboratories of Endocrinology Research Unit, belonging to Radio-biology Department, Nuclear Research Center, Atomic Energy Authority, Enshas, Egypt.

### Statistical analysis

Data were statistically analyzed according to the statistical analysis system (20).

## RESULTS

In clinically normal foaling mares, the uterine horns were detected to be distinctly tubular during puerperium, tubular during the 1<sup>st</sup> and 2<sup>nd</sup> postpartum estrus and tubular and firm during 1<sup>st</sup> luteal phase and in the mare suspected to be had prolonged diestrus. In pregnant mares, the uterine horns were detected to be distinctly tubular during early stages until day 40 of gestation, then it became flaccid with advancement of pregnancy. The ovulation fossa was rectally palpated in 5 mares during foaling heat and 3 mares during 2<sup>nd</sup> postpartum estrus. The time elapsed from foaling until the 1<sup>st</sup> postpartum heat was recorded to be  $7.52 \pm 0.65$  days. Only 6 mares out of 21 became pregnant after mounting during foaling heat, and 8 out of 14 mares became pregnant after remounting during the 2<sup>nd</sup> postpartum estrus. The 1<sup>st</sup> luteal phase was recorded to be shorter than 10 days in 4 mares, it was 10 to 16 days long in 7 mares and longer than 16 days in 3 mares. The duration of 2<sup>nd</sup> postpartum estrus was observed to be ranged from 5 up to 9 days in all returned females. One mare suspected to be had prolonged diestrus.

### Vaginal cytology in mares:

The mean percentage of various types of epithelial cells and values of total leukocytic infiltration in the vaginal smears of mares during puerperium, foaling heat, 7<sup>th</sup> day later (1<sup>st</sup> luteal phase), 2<sup>nd</sup> postpartum estrus, pregnancy (early, mid and late) and prolonged diestrus are presented in Table, (1) and Figures (1 & 2).

There was a relative increase in percentage of cornified cells and values of total leukocytic infiltration accompanied with many small and large nucleated squamous cells during puerperium. The vaginal smears appeared to have many nucleated small and large squamous cells and some cornified cells with few leukocytic infiltration during foaling heat (Figure, 3) and 2<sup>nd</sup> postpartum estrus. The small and large nucleated squamous cells in the vaginal smears were proliferated and increased

when the ovulation fossa was rectally palpated during estrus (Figure, 4). In contrast to that observed during estrus, there was numerous nucleated small squamous cells and leukocytic infiltration with few number of nucleated large squamous cells and cornified cells during 1<sup>st</sup> luteal phase (Figure, 5).

In pregnant mares, the vaginal smears shows many nucleated small squamous cells accompanied with numerous leukocytic infiltration during early stages of gestation (Figure, 6). The small and large nucleated squamous cells appeared to be nearly similar and relatively increased in their size and the values of total leukocytic infiltration was increased during the mid (Figure, 7) and late stages of gestation. In the mare suspected to be had prolonged diestrus, the vaginal smears shows numerous leukocytic infiltration and nucleated small squamous cells (Figure, 8).

**Table (1): Percentage of various types of vaginal epithelial cells and values of total leukocytic infiltration in the vaginal smears during some reproductive phases in Arabian mares (M ± SE).**

Types of cells Reproductiv stage	Nucleated small squamous cells (%)	Nucleated large squamous cells (%)	Cornified cells (%)	Total leukocytic infiltration per counted fields
Puerperium	45.54 ± 6.18 <sup>b</sup>	38.18 ± 4.18 <sup>b</sup>	16.28 ± 2.46 <sup>cd</sup>	118.64 ± 10.46 <sup>D</sup>
Foaling heat	36.78 ± 3.41 <sup>b</sup>	42.64 ± 5.18 <sup>b</sup>	20.79 ± 2.03 <sup>c</sup>	13.00 ± 1.83 <sup>E</sup>
1 <sup>st</sup> Luteal phase	78.36 ± 9.38 <sup>a</sup>	18.7 ± 1.27 <sup>de</sup>	2.89 ± 0.26 <sup>g</sup>	589.30 ± 18.46 <sup>B</sup>
2 <sup>nd</sup> postpartum estrus	36.97 ± 5.60 <sup>b</sup>	40.69 ± 6.78 <sup>b</sup>	22.34 ± 3.18 <sup>c</sup>	10.31 ± 1.69 <sup>E</sup>
Early pregnancy				
20 - 30 days	81.34 ± 11.62 <sup>a</sup>	16.53 ± 0.93 <sup>e</sup>	2.13 ± 0.16 <sup>h</sup>	593.20 ± 26.34 <sup>B</sup>
31 - 40 days	88.40 ± 12.38 <sup>a</sup>	7.28 ± 0.98 <sup>e</sup>	4.32 ± 0.36 <sup>f</sup>	821.36 ± 38.89 <sup>A</sup>
Mid pregnancy				
150 - 180 days	43.34 ± 6.32 <sup>b</sup>	48.63 ± 6.84 <sup>b</sup>	8.19 ± 1.26 <sup>e</sup>	833.28 ± 41.39 <sup>A</sup>
Late pregnancy				
240 - 300 days	41.34 ± 4.28 <sup>b</sup>	44.35 ± 7.31 <sup>b</sup>	14.35 ± 1.93 <sup>d</sup>	811.23 ± 46.40 <sup>A</sup>
Prolonged diestrus	80.3 ± 10.18 <sup>a</sup>	15.30 ± 1.82 <sup>ef</sup>	4.51 ± 0.23 <sup>gh</sup>	460.86 ± 22.84 <sup>C</sup>

Means with different small or capital alphabetic superscripts are significantly different from each other at level (P < 0.05).

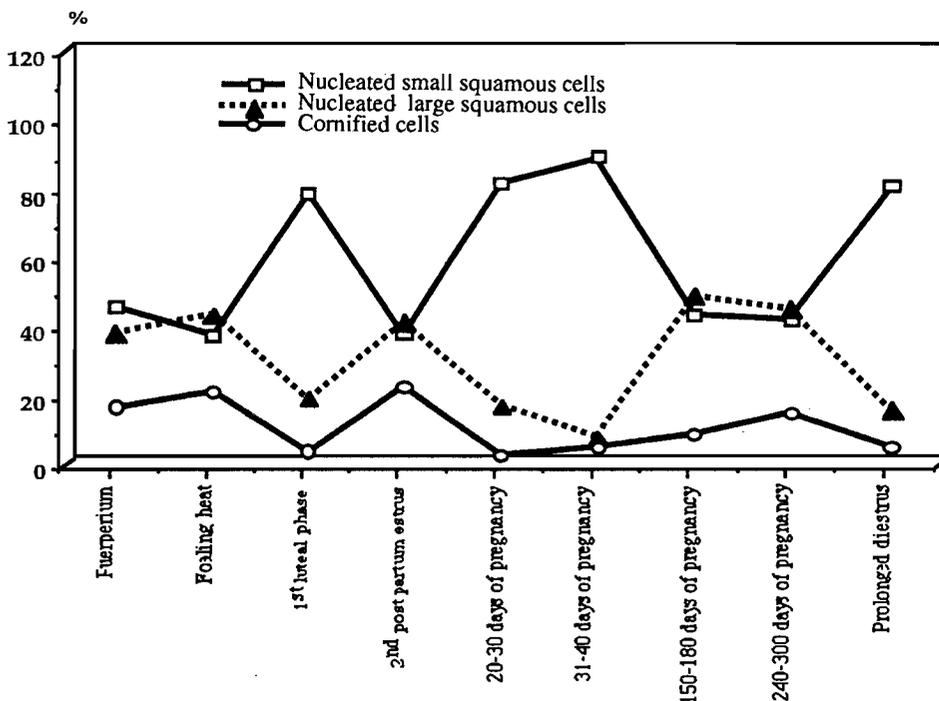


Figure (1): Percentage of Various types of vaginal epithelial cells in the vaginal smears during some reproductive phases of Arabian mares. Note the relative increase in percentage of cornified cells and the similarity in percentage of both small and large nucleated squamous cells during puerperium and estrus ; the increase in percentage of nucleated small squamous cells during 1<sup>st</sup> luteal phase, early stages of pregnancy and prolonged diestrus, in addition to the similarity in percentage of both small and large nucleated squamous cells during mid and late stages of pregnancy .

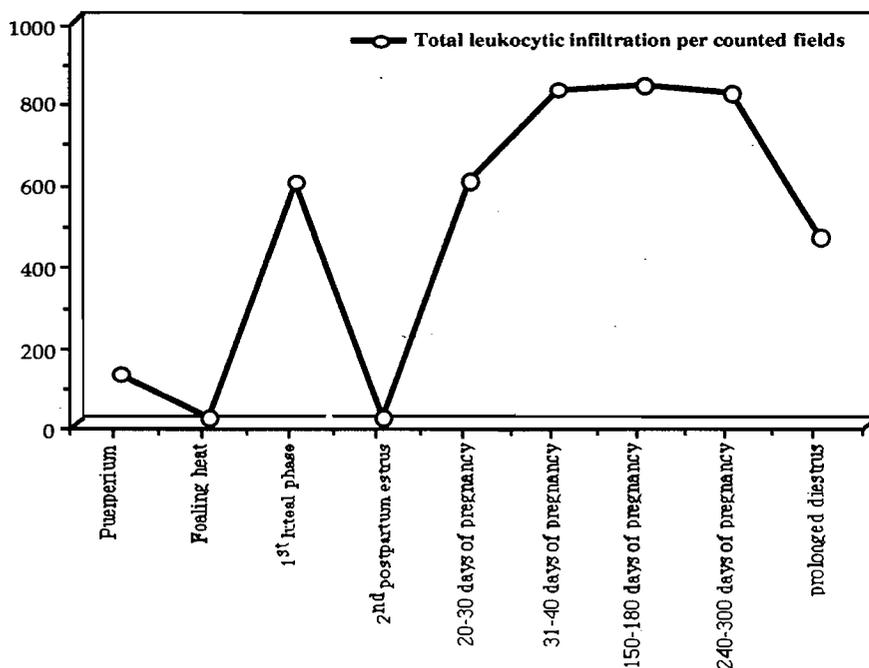


Figure (2): Values of total leukocytic infiltration in the vaginal smears during some reproductive phases of mares. Note the sharp increase in the values of total leukocytic infiltration during pregnancy, as well as their relative elevation during 1<sup>st</sup> luteal phase and prolonged diestrus .

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Figure(3): Vaginal smears of mares after mating during foaling heat showing small and large nucleated squamous cells accompanied with some cornified cells and some spermatozoa (arrow). Papanicolaou's stain X 600



Figure(4): Vaginal smears of mares when the ovulation fossa was rectally palpated during the 2<sup>nd</sup> postpartum estrus showing numerous small and large nucleated proliferated squamous cells. Papanicolaou's stain X 400.



Figure(5): Vaginal smears of mares during 1<sup>st</sup> luteal phase showing numerous leukocytic infiltrations and nucleated small squamous cells. Papanicolaou's stain X 600



Figure(6): Vaginal smears of pregnant mares during early stages showing numerous leukocytic infiltration and nucleated small squamous cells. Papanicolaou's stain X 600



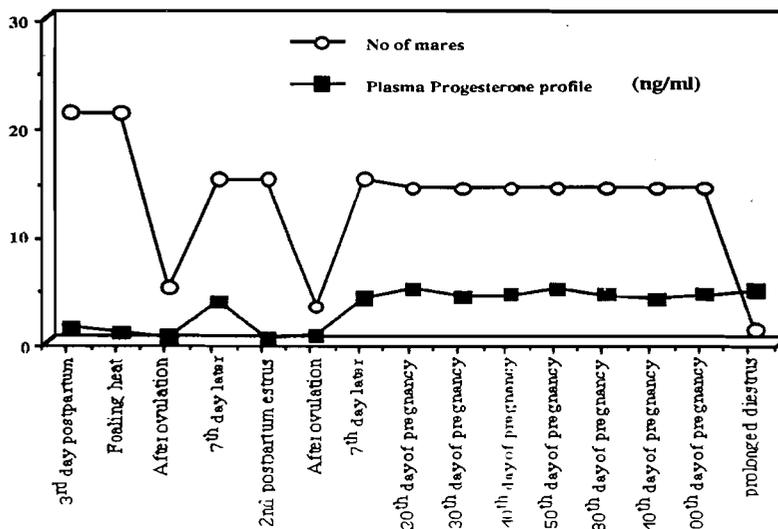
Figure(7): Vaginal smears of pregnant mares at mid gestation showing numerous leukocytic infiltration accompanied with small and large nucleated squamous cells which relatively increase in their size. Papanicolou's stain X 600

Figure(8): Vaginal smears of the mare suspected to be had prolonged diestrus showing numerous leukocytic infiltration and nucleated small squamous cells. Papanicolou's stain X 600

**Plasma progesterone concentration in mares**

As shown in Figure (9), the plasma progesterone profile was recorded to be  $1.18 \pm 0.32$  ng/ml at the 3<sup>rd</sup> day postpartum. It significantly decreased during the 1<sup>st</sup> and 2<sup>nd</sup> postpartum estrus ( $0.68 \pm 0.12$  and  $0.26 \pm 0.08$  ng/ml, respectively), as well as after ovulation during estrus ( $0.36 \pm 0.09$  -  $0.46 \pm 0.11$  ng/ml). While it significantly increased at the 7<sup>th</sup> day

after the end of 1<sup>st</sup> and 2<sup>nd</sup> postpartum estrus ( $3.66 \pm 0.72$  and  $3.92 \pm 0.84$  ng/ml, respectively), as well as in the mare suspected to be had prolonged diestrus (4.70 ng/ml). Also, the plasma progesterone concentration was significantly increased during early stages of pregnancy ( $3.35 \pm 0.96$  -  $4.86 \pm 0.98$  ng/ml) than that recorded during estrus. No significant change was observed in plasma progesterone profile with advancement of gestation.



Figure(9): Plasma progesterone profile during some reproductive phases in Arabian mares.

## DISCUSSION

The distinctly tubular uterine horns during puerperium is a reflection of good uterine involution (21). Moreover, the uterine horn became tubular during foaling heat and the 2<sup>nd</sup> postpartum estrus. A finding which came in consistent with that reported previously (22). However, during the 1<sup>st</sup> luteal phase and in the mare suspected to be had prolonged diestrus, the uterus was found to be tubular and firm. This may be due to the lack of any secretion in the uterus at this time (23). In pregnant mares, the distinctly tubular uterine horn was detected during early stages up to 40 days, thereafter no tone was detected with the advancement of gestation by day 50. This may be attributed to the increase of fetal fluid with the progress of pregnancy (24). The relatively low conception rate in females mated during foaling heat may be returned to the incomplete endometrial involution at this time (25).

In contrast to the previous statement concerning the cytological picture of vagina in mares that reported by *Coles (14)* and *Prabhakar et al., (15)*, our findings indicated that the vaginal cytological examination is a rapid reliable method for assessment the reproductive pattern in mares. The relative increase in percentage of cornified cells and values of total leukocytic infiltratoin accompanied with the similarity in percentage of both small and large nucleated squamous cells were observed during puerperium. This may be attributed to the decrease in plasma progesterone concentration as presented herein and indicated the defense mechanism of the mare during this stage. Many small and large nucleated squamous cells and cornified cells with few leukocytic infiltration were found during 1<sup>st</sup> and 2<sup>nd</sup> postpartum heat. Moreover, the small and large squamous cells were proliferated and increased when the ovulation fossa was rectally palpated - during estrus . It is worthy to note that the plasma progesterone concentration was sharply decreased during the 1<sup>st</sup> and 2<sup>nd</sup> postpartum estrus, as well as after ovulation during estrus. However, the vaginal cornification could be used as a tool in recognition of biological activity exerted by steroid hormones (26). In estrous ewes, there was a moderate number of RBCs and large number of cornified cells and moderate number of intermediate cells with no polymorphonuclear leukocytes and no basal or

parabasal cells (4), they reported that the vaginal epithelium is greatly affected by ovarian hormones .

Numerous nucleated small squamous cells and leukocytic infiltration accompanied with few number of nucleated large squamous cells and cornified cells were observed, when the plasma progesterone level was elevated during 1<sup>st</sup> luteal phase and prolonged diestrus. In ewes, small spherical or irregular epithelial cells with higher leukocytic infiltration were detected during luteal phase (27). In buffaloes, the basophilic intermediate epithelial cells were predominant during luteal phase (28). In goats, the number of larger cells were sharply reduced when the progesterone was predominant during metestrus and diestrus (7). Contrary to the current study, *El-Sayed and Abdel-Ghaffar (5)* found that the leukocytic infiltration was decreased, when the plasma progesterone level was elevating in diestrus ewes.

The results reported herein reveal the presence of many nucleated small squamous cells with higher leukocytic infiltration during early stages of pregnancy. The increase in values of total leukocytic infiltration accompanied with the similarity in percentage of both small and large nucleated squamous cells which relatively increased in their size are consider the most predominant feature during mid and late gestation. These finding goes in a harmony with the increase of plasma progesterone concentration in pregnant mares. The variation in the vaginal epithelial cell types along the whole gestation period may be attributed to the simultaneous effect of estrogen and progesterone (29, 30). In sheep, a great percentage of large nucleated spherical cells were recorded in pregnant than the non-pregnant ones (31, 32). Moreover, the increase of small spherical cells in the vaginal smears of ewes indicating positive pregnancy (6). In goats, pregnancy was diagnosed positively when there was an elevation in the intermediate cells (33) or small spherical cells (6). In cattle and buffaloes, the intermediate cells were predominated during pregnancy (28). The increase in values of total leukocytic infiltration during pregnancy may be indicate the defense mechanism against any infection during pregnancy.

In conclusion, an evaluation of the cytological picture of vaginal smears might be provide an additional simple helpful tool in prediction of the 1<sup>st</sup> and 2<sup>nd</sup> postpartum estrus as well as pregnancy in Arabian mares.

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## الملخص العربى

### تقييم الصورة الخلوية للمسحات المهبلية ومستوى هرمون البروجيستيرون فى البلازما كأداة لمراقبة النمط التناسلى للأفراس العربية ذات الولادة الطبيعية

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أستخدم فى هذا العمل عدد إحدى وعشرون من الأفراس العربية ذات الولادة الطبيعية. حيث تم تسجيل التغيرات فى الخلايا الموجودة فى المسحات المهبلية ومستوى هرمون البروجيستيرون فى البلازما خلال بعض المراحل التناسلية لتلك الأفراس العربية . وقد تم ملاحظة وجود ارتفاع فى نسبة الخلايا المتقرنة وقيم إرتشاح كرات الدم البيضاء مصاحب بوجود تماثل فى نسبة الخلايا الحرشفية الصغيرة والكبيرة ذات الأنوية خلال مرحلة النفاث . كذلك تم ملاحظة وجود زيادة نسبية فى نسبة الخلايا المتقرنة بالإضافة إلى وجود تماثل فى نسبة كلاً من الخلايا الحرشفية الصغيرة والكبيرة ذات الأنوية مصاحب بوجود نقص شديد فى قيم إرتشاح كرات الدم البيضاء عندما إنخفض بشدة تركيز البروجيستيرون فى البلازما خلال فترة الشيع الأولى والثانية بعد الولادة . كما وجدت زيادة فى نسبة الخلايا الحرشفية الصغيرة ذات الأنوية وقيم إرتشاح كرات الدم البيضاء وكذلك فى تركيز هرمون البروجيستيرون فى البلازما خلال أول مرحلة لطور الجسم الأصفر والمراحل الأولية للحمل وكذلك فى أثناء إمتداد فترة وجود الجسم الأصفر وقد وجد أن أهم الصفات المميزة للمراحل الوسطى والأخيرة للحمل هى الزيادة فى قيم إرتشاح كرات الدم البيضاء مصاحبة بوجود تماثل فى نسبة كلا من الخلايا الحرشفية الصغيرة والكبيرة ذات الأنوية التى يكبر حجمها نسبياً ، بينما كان هناك إختلاف غير معنوى فى مستويات هرمون البروجيستيرون فى البلازما مع تقدم الحمل .