



The Investigation of Serum Estrogen, Progesterone, AMH and IGF-1 Profiles in Lactating and Non Lactating Mares after Altrenogest Administrations*

İbrahim Mert POLAT¹, Ömer KORKMAZ², Taha Burak ELİFOĞLU¹, İlknur Pir YAĞCI¹,
Hatice Esra ÇOLAKOĞLU³, Mert PEKCAN⁴

¹ Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale-TURKEY

² Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Harran University, Harran, Şanlıurfa-TURKEY

³ Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Ankara University, Ankara- TURKEY

⁴ Department of Biochemistry, Faculty of Veterinary Medicine, Ankara University, Ankara-TURKEY

Corresponding author: İbrahim Mert POLAT; e-mail: vethekert@gmail.com ORCID: 0000-0003-4029-1247

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Summary: The objective of this study is to follow up the follicular growth in ovaries until mating by ultrasonography to determine of pregnancy rates, serum estrogen, progesterone, AMH and IGF-1 concentrations in mares which synchronized with altrenogest and to compare the obtained data with non-lactating mares. The study was carried out during breeding season while ovaries were active; lactating mares were included in Group 1 (n = 8) and non-lactating mares in Group 2 (n = 8). In both groups, synchronization was performed by administration of altrenogest (Regumate®) and prostaglandin F 2 alpha (PGF2α). Dominant follicle diameters of non-lactating mares were found to be greater than those of lactating ones. During the study, estrogen, progesterone, AMH and IGF-1 levels were significantly lower in the lactating mares. The non- lactating mares were found to have a first pregnancy conception rate (75%), similar to that of lactating mares (62%) ($\chi^2=1.54$, $P=0.62$). It was concluded that in the mares synchronized with altrenogest, the presence of lactation and the foal are significantly influences ovarian activities, ovarian steroid levels, AMH and IGF-1 levels. Therefore, in order to increase pregnancy rates in lactating mare, sexual cycle synchronization with altrenogest are considered to be a suitable method.

Key words: Altrenogest, anti müllerian, estrogen, lactation, mare, progesterone

Laktasyonda Olan ve Laktasyonda Olmayan Kısraklarda Altrenogest Uygulaması Sonrası Serum Östrojen, Progesteron, AMH ve IGF-1 Düzeylerinin Araştırılması

Özet: Sunulan çalışmanın amacı, altrenogest ile senkronize edilen laktasyondaki kısraklarda aşım zamanına kadar geçen süreçte ovaryumlardaki folliküler gelişimlerin ultrasonografi ile takibi, serum östrojen, progesteron, AMH ve IGF-1 konsantrasyonlarının ölçülmesi ve gebelik oranlarının değerlendirilerek bu verilerin laktasyonda olmayan kısraklarla karşılaştırılmasıdır. Çalışma, üreme sezonun başladığı ve ovaryumların aktif olduğu üreme sezonunda gerçekleştirildi; laktasyonda olan kısraklar Grup 1'e (n=8) ve laktasyonda olmayan kısraklar Grup 2'ye (n=8) dahil edildi Her iki gruba altrenogest (Regumate®) ve prostaglandin F2α ile senkronizasyon gerçekleştirildi. Laktasyonda olmayan kısrakların dominant follikül çapları laktasyonda olanlara göre daha büyük bulundu. Çalışma süresince toplanan kan örneklerinde östrojen, progesteron, AMH ve IGF-1 düzeyleri laktasyondaki kısraklarda belirgin olarak düşük bulundu. Laktasyonda olmayan kısrakların ilk aşımında gebe kalma oranı (%75), laktasyonda olan kısraklarla (%62.5) benzer bulundu ($\chi^2=1.54$, $P=0.62$). Altrenogest ile senkronize edilen kısraklarda laktasyonun ve tayın varlığının ovaryum faaliyetlerine, ovaryum steroid düzeylerine, AMH ve IGF-1 düzeylerine önemli oranda etki ettiği kanısına varıldı. Bu nedenle laktasyonda olan kısraklarda gebelik oranlarını arttırmak amacıyla altrenogest ile seksüel siklus senkronizasyonunun uygun bir yöntem olduğu düşünüldü.

Anahtar kelimeler: Altrenogest, anti-müllerian, kısрак, laktasyon, östrojen, progesteron

Introduction

Estrus cycles of mares are controlled by the hormones which respond to photoperiod of season in mares. The photoreceptors of the retina turns light stimulus into signals and transfers these signals to

superior cervical ganglion which have adrenergic neuronal endings that reach the pineal gland and have action in different pathways of synthesis of melatonin. Thus, the increased exposure to photoperiod results in a decrease of melatonin secretion. As a result of decrease in melatonin secretion, gonadotropin releasing hormone (GnRH) is stimulated. The GnRH has a decent role in hypothalamic-pituitary axis and is transferred to adenohipophysis where it stimulates follicle stimulating hormone (FSH) and luteinizing hormone (LH) responsible of follicle growth

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and ovulation (Satue and Gardon, 2013). Lactating mares have a completely different reproductive metabolism from those of cyclic mares (Heidler et al., 2003). At early lactation, alteration of Growth Hormone (GH), Insulin-like Growth Factor 1 (IGF-1) and leptin have some roles in metabolism to regulate various mechanisms. These changes are beneficial to energy metabolism, apart from suppressing reproductive activity (Block et al., 2001). These hormonal alterations are most effective at early lactation right after delivery and their influence go down by time (Heidler et al., 2003). While GH is responsible for growth and development, it also mediates secretion of IGF-1 from liver and stimulates ovarian activity (Izadyar et al., 1998). Thus GH directly or with the help of IGF-1, in gonadotropin dependent or independent ways affects ovarian steroidogenesis (Hull and Harvey, 2000). Heidler et al. (2003) found that GH levels were lower in non-lactating mares than in lactating ones. However, researchers could not find any statistically meaningful difference in GH levels in cows at lactation than those in non-lactation period (Block et al., 2001). Estrogen is one of the determinative factors on IGF-1 levels and it elevates secretion of IGF-1. Also, estrogen augments number of GH receptors, so IGF-1 becomes more bio-effective (Ginther et al., 2002). The granulosa cells of pre-antral and small antral follicles secrete anti-mullerian hormone (AMH) (Ball et al 2008) and plasma concentration of AMH was found to be positively correlated with the number of follicles having a diameter of 6-20 mm in mares (Claes et al., 2014). AMH is secreted in minimal concentrations from follicles with a diameter greater than 30 mm (Ball et al., 2008) and its plasma concentration increases while follicles are developing (Vernunft et al., 2011). Although serum AMH concentrations may show individual variations among mares, these concentrations reportedly do not vary with the different stages of the estrous cycle or pregnancy (Ball et al., 2008; Almeida et al., 2011). Though many studies have shown the relationship between AMH and fertility, further studies are required to reveal the effects of lactation on AMH profile. The aim of this study is to monitor follicular growth in ovaries till mating and to investigate estrogen, progesterone, AMH, IGF-1 levels and pregnancy rates in mares which are synchronized with altrenogest, and to compare them with non-lactating mares which synchronized with altrenogest.

Material and Methods

Animals and study groups

The study was approved by Kırıkkale University Local Ethics Committee for Animal Experiments with the approval number 15/06. Sixteen mares with ages ranging between 4–10 years, a body condition score of at least 7, having given birth at least once, without any genital system infections, and with regular estrus

cycles were used. This study was carried out during breeding season while ovaries were active. The mares that lactate and were housed with their foals were included in group 1 (n=8), non-lactating mares were included in group 2 (n=8). Immediately before the study, USG examinations were performed, the mares with inactive ovaries or uterus pathologies were not included in the study. In this research, no artificial lighting, feed additive, hormone or drugs were used to stimulate ovarian activity

Ultrasonographic examinations

Ovaries and uterine horns were ultrasonographically examined daily with Pie Medical-Falco 100 device. Both uterine horns and ovarian follicle diameters were recorded. Examination of uterine horns with USG was performed using a linear transducer at the site of near corpus uteri via dorsal-ventral. Including both endometrial and stratum vascular layers, the distances between top and bottom serosal layers were measured as uterine horn diameter. Pregnancy examinations were performed with USG after mating at days 15, 35 and 60.

Drug administrations

For synchronization of estrus cycles; 0.044 mg/kg altrenogest (Regumate) administered orally daily for 12 days. At the 12th day of altrenogest administration, single dose of 12.5 mcg PGF2 α was administered intramuscularly.

Collection of blood samples and biochemical analyses

Blood samples were collected to plain vacuumed tubes from jugular vein right before drug administrations. Blood samples centrifuged at 3500 rpm for 15 minutes and stored at -20°C until analyses. Blood sera estrogen (Genway®), progesterone (Cayman®), AMH (Beckman®) and IGF-1 (IDS®) levels were measured with commercial ELISA kits and ELISA MAT-2000 microplate reader.

Statistical analyses

All the statistical analyses were performed using SPSS version 14.1. First, descriptive analyses of the parameters (mean and standard error) according to the experimental conditions were done. Then, the evaluation of normality by the Kolmogorov Smirnov test was performed. As all data were considered as nonparametric, Mann Whitney test was carried out. Statistical significance was considered when $P < 0.05$.

Results

During the study, follicle sizes of both groups have been measured daily. In both study groups, generally dominant follicle used to develop at right ovary. It is

Table 1 Mean values for estradiol, progesterone, IGF-I and AMH serum concentrations of lactating and non-lactating mares

Days of Sampling	Parameter ($\bar{x} \pm S \bar{x}$)							
	E ₂ (pg/mL)		P ₄ (ng/mL)		IGF-I (ng/mL)		AMH (ng/mL)	
	LM	NLM	LM	NLM	LM	NLM	LM	NLM
0	7.6 ±0.87	8.72±1.4	1.14±0.1	2.80±0.4	27.3±1.9	48.7±4.1	0.32 ^a ±0.1	0.77 ^b ±0.0
3 rd	P=0.08		P=0.06		P=0.15		P=0.05	
	11.84±1.02	10.78±0.72	2.23±0.30	2.98±1.1	28.60 ^a ±2.26	54.86 ^b ±3.59	0.36 ^a ±0.20	0.62 ^b ±0.06
6 th	P=0.07		P=0.09		P=0.03		P=0.02	
	22.32±2.37	26.45±2.65	3.82 ^a ±0.12	7.52 ^b ±1.4	32.80 ^a ±2.70	62.24 ^b ±5.21	0.47 ^a ±0.12	0.84 ^b ±0.12
12 th	P=0.13		P=0.02		P=0.03		P=0.02	
	42.60±2.26	36.71±1.34	11.40 ^a ±1.12	16.40 ^b ±1.52	75.70 ^a ±4.21	88.77 ^b ±6.34	1.30 ^b ±0.45	1.11 ^c ±0.23
OD	P=0.16		P=0.026		P=0.05		P=0.012	
	64.62 ^a ±2.27	84.30 ^b ±1.67	0.32±0.11	0.48±0.03	108.50 ^b ±6.70	148.50 ^c ±7.65	0.56 ^a ±0.28	0.77 ^b ±0.27
	P=0.04		P=0.25		P=0.002		P=0.041	

LM: Lactating mares NLM: nonlactating mares. E₂: Estradiol, P₄: Progesterone, IGF-I: Insuline Like Growth Factor-I, AMH: Anti-mullerian hormone, OD: Day of ovulation

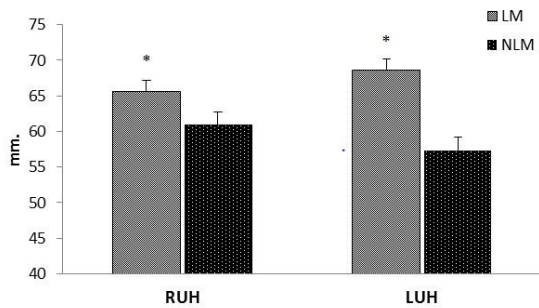


Figure 1. Mean diameters (standard error mean) of uterine horns during study period. **RUH:** Right uterine horn **LUH:** Left uterine horn. **LM:** Lactating mares **NLM:** Nonlactating mares. (* P<0.05)

found that, in lactating mares, dominant follicle sizes that ovulated are bigger than in non-lactating mares. It is observed that dominant follicle development used to take place on average 12-13 days after last drug treatment in both groups. Average dominant follicle diameter which will ovulate in lactating mares is measured as 43.2±0.82 mm and 52.6±0.63 mm in non-lactating mares (P<0,05). Even though ovarian activity was monitored with ultrasonography, the clinical signs of estrus in lactating mares were observed to be weaker than in non-lactating mares. The sizes of uterine horns in both groups have been shown in Figure 1.

In lactating mares, the diameter of right uterine horn

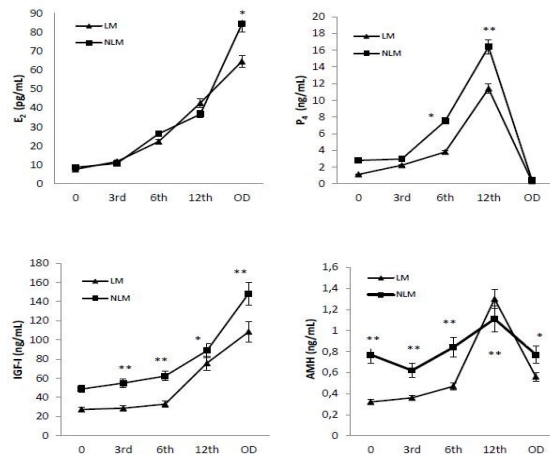


Figure 2. Mean values of estradiol, progesterone, IGF-I and AMH serum concentrations from day 0 to ovulation day of mares (*P<0.05, **P<0.01). **LM:** Lactating mares **NLM:** nonlactating mares. **E₂:** Estradiol, **P₄:** Progesterone, **IGF-I:** Insuline Like Growth Factor-I, **AMH:** Antimüllerian hormone. **OD:** Day of ovulation.

was observed to be bigger than in non-lactating ones. The results of blood serum analyses have presented in Figure 2 and Table 1.

Serum estradiol levels of lactating mares were observed to be lower than in non-lactating group. Moreover, though in both groups serum IGF-1 levels had sharp rises on ovulation day, a decrease was observed in serum AMH levels after selection of dominant follicle.

In the initial pregnancy diagnosis, similar pregnancy rates have been observed in both groups (5 at lactating mares, 6 at non lactating ones). One mare showed estrus signs at day 22 after coitus. USG examination showed that embryonic loss had occurred. Serum estradiol levels were found to be lower in lactating mares than non-lactating mares. The reason for this was thought to be that the dominant follicle diameters were smaller than the ones not in lactation.

Discussion and Conclusion

Maximum follicle sizes of ovaries could be 5 mm smaller in miniature breeds like pony and could be 10 mm bigger in giant breeds like Friesian. Also, in spring season, follicle size could be 5-8 mm bigger than in summer season (Brinsko et al., 2010). Moreover, A study by Koyla et al. (2000) reported that lactation had no effect on maximum follicle diameter in their research on five mares from two different breeds in a breeding season. In contrast to Koyla et al. (2000), the maximum follicle diameters differed between the groups in this study. Perkins and Grimmet (2001) reported that pregnancy rates in lactating and non-lactating mares (67 % vs. 66 %) were similar. In this study, similar pregnancy rates (6 in non-lactating mares and 5 in lactation) were obtained in both groups in the first pregnancy examination (day 15) after mating. One mare in lactation displayed estrus again on the 22nd day after mating. Ultrasonographic examination revealed an embryonic death. This finding was found to be similar to the one from the study by Van Niekerk and van Niekerk (1998a), which revealed a higher incidence of embryonic death in lactating mares. The primary source of progesterone is corpora lutea which is required for embryo-uterine interactions (embryo mobility and implantation) and uterine secretions. The progesterone levels were found lower in lactating mares (van Niekerk and van Niekerk, 1998b). It was thought that it may be a factor that may increase the risk of embryonic death in early pregnancy period. Plasma concentrations of progesterone gradually increased during altrenogest treatment from days 1 to 8 and rapidly decreased after the administration of prostaglandin (Korosua et al., 2012). In our study, similar to the study by Korosua et al. (2012), progesterone profiles rapidly increased on the 3rd days of altrenogest administrations. However, these increase were similar in both groups, the response of nonlactating mares was higher than lactating ones. Collins et al. (2014) compare the time of altrenogest therapy in Przewalski's mares. After altrenogest administrations for 12 days, estrogen and progesterone responses were higher than in a 14-day therapy.

Preantral and small antral follicles secrete AMH and the concentrations are positively correlated with the number of follicles and also 8 mm or larger follicles (Claes et al., 2014). AMH is secreted at low levels by

follicles with a diameter greater than 30 mm and its plasma concentration increases with the number of developing follicles. Although serum AMH concentrations may show individual variations among mares, these concentrations reportedly do not vary with the different stages of the estrous cycle, pregnancy and lactation period. In this study AMH levels were found significantly lower than in non-lactating mares. Whether these findings can be extrapolated to mares still remains to be determined. Further studies are required to determine AMH course during the whole lactation period. In addition, at 6th day of altrenogest treatment, AMH levels were rising in the same way in both groups when ovarian follicles reached 20-30 mm diameter. Even if AMH is low in lactating mares, it can be increased under the effects of progesterone treatment. Dominant follicles have higher IGF-1 levels than the largest subordinated follicles which are positively correlated with estradiol levels in mares. The increase in the IGF-1 concentration in the future dominant follicle reflects the follicle to decrease FSH sensitivity and increase LH availability. The other follicles in the follicular wave possess the same capacity for dominance but never reach similar sizes and the largest follicle alone continues developing, until it becomes dominant and ovulates (Salazar-Ortiz et al., 2014). IGF-1 has been shown to enhance FSH-induced granulosa cell differentiation, particularly LH receptor acquisition which is critical for adequate gonadotropin output because, prolactin can negatively regulate the LH response to GnRH especially in the presence of dopamine (Hodson et al., 2010). In a study in mare ovarian cell culture; prolactin stimulated cells were influenced from the GnRH response. Thus, in our study; the presence of lactation also prolactin and oxytocin can affect the maximum follicle diameter, negatively.

As a result, it is concluded that in mares which were synchronized with altrenogest, lactation and presence of foal have considerably effect on ovarian activity; especially on diameter of dominant follicle, ovarian steroids, levels of AMH and IGF-1. For this reason, it was thought that in order to increase pregnancy rates in lactating mares sexual cycle synchronization with altrenogest could be appropriate method. Furthermore, it was confirmed that altrenogest, in combination with PGF2 α , has tremendous potential for optimizing the fertility in lactating mares.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial rela-

tionships that could be construed as a potential conflict of interest.

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