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RESEARCH ARTICLE

Uterine Involution, Follicle Development and Concentrations of Plasma Progesterone, 20α-OH-Progesterone and Total Estrogen Levels During the Postpartum Period in Anatolian Donkeys

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Summary

In this study, it is aimed to determine the length of estrus cycle, size of the preovulatory follicle, and ovulation time during the postpartum period (pp) in Anatolian jennies and to measure steroid hormone levels during the same period. Five healthy Jennies at late-stage of gestation were included in the study. During the post partum period, plasma progesterone (P4), total estrogen (E2) and 20α-OH-progesterone (20α-OH-P4) levels were studied by RIA. Ovarian activity and uterus involution were determined by rectal palpation and ultrasound examinations. The first postpartum behavioral estrus occurred as early as 6 days pp. Follicles were reached to a preovulatoric size of 30 to 35 mm during the 2nd day of estrus, during which plasma P4, E2 and 20α-OH-P4 concentrations were 0.9±0.40 ng/ml, 6.82±4.64 ng/ml and 0.98±0.21 ng/ml, respectively. The first ovulation occurred 10-15 days after parturition. During the first postpartum cycle, plasma P4 reached to a maximum concentration 14 days after ovulation. The second estrus was observed 32.2±1.58 days postpartum period (pp). The estrus behaviour lasted 6.0±1.00 days and 5.6±1.67 days in the first and second cycles, respectively. The uterus borders were detected by rectal palpation as early as 3 and 4 days postpartum period (pp). The uterus was totally palpable 6-9 days after parturition. Importantly, a high correlation was determined between the P4 and 20α-OH-P4 concentrations.

Keywords: *Jennies, Postpartum, Follicular Dynamics, Ultrasonography*

Postpartum Dönemdeki, Anadolu Eşeklerinde, Folliküler Gelişme, Uterus İnvolusyonu, Plazma Progesteron, 20α-OH-Progesteron ve Total Östrojen Düzeyleri

Özet

Bu çalışmada Anadolu eşeklerinde postpartum dönemde (pp) siklus uzunluğu, preovulatorik follikül büyüklüğü, ovulasyon zamanı ve bu dönemlerdeki steroid düzeylerinin ortaya konulması amaçlandı. Çalışmada gebeliğin son dönemindeki, beş adet sağlıklı dişi eşek kullanıldı. Postpartum dönemde (pp), plazma progesteron (P4), total östrojen (E2) ve 20α-OH-progesteron (20α-OH-P4) düzeyleri RIA ile ölçüldü. Uterus involusyonu ve ovaryum aktiviteleri rektal palpasyon ve transrektal ultrason muayeneleriyle belirlendi. İlk östrus davranışları en erken postpartum 6. günde gözlendi. Folliküller, preovulatorik büyüklükleri olan 30-35 mm ye östrusun 2. gününde ulaştı. Preovulatorik folliküller saptandığında, plazma P4, E2 ve 20α-OH-P4 düzeyleri sırasıyla, 0.90±0.40 ng/ml, 6.82±4.64 ng/ml ve 0.98±0.21 ng/ml, olarak ölçüldü. Postpartum ilk ovulasyonlar 10-15. günler arasında gözlendi. Postpartum ilk siklusda, plazma P4 düzeyi ovulasyondan 14 gün sonra en yüksek düzeye çıktı. Postpartum ikinci östrus 32.20±1.58 günde gözlendi. Östrus davranışlarının süresi, birinci siklusta 6.0±1.00 gün, ikinci siklusta 5.6±1.67 gün olarak belirlendi. Rektal muayenelerde uterusun, doğumu izleyen 3-4. günlerde sınırlarının belirlenebildiği, 6-9. günlerde ise tamamen palpe edilebilecek kadar invole olduğu saptandı. P4 ve 20α-OH-P4 düzeyleri arasında önemli derecede, yüksek korelasyon saptandı.

Anahtar sözcükler: *Dişi eşek, Postpartum, Folliküler dinamik, Ultrasonografi*

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INTRODUCTION

The Anatolian donkey, which has a coat of black and grey color variety, is a local breed of Anatolia and can be found throughout Turkey [1]. The jenny is very similar to the mare horse in many reproductive aspects [2,3], but it tends to have a longer breeding season than mares have [2,4]. Several studies [3-7] showed that the estrus cycle in jennies usually lasts about 20-25 days. Estrus usually lasts for 5 or 8 days, with ovulation in 1-2 days before the end of estrus [4,8,9]. During the estrus, the plasma P4 concentration dropped to 1 ng /ml [10].

The postpartum management of reproduction in horses has a great importance. The time from parturition to the resumption of cyclic ovarian activity is very short. The first fertile estrus occurs as early as 6 to 20 days after foaling [11]. Several factors may influence the ovarian activity after parturition. In case of lactational anestrus, mares fail to ovulate and/or show overt estrus during the pp period [12]. Galisteo and Perez [13] noted that, foal-heat was detected after parturition in Spanish donkey and ovulation occurred on day 13.2±2.7. The duration of foal-heat was 4.7±1.7 days. After parturition, the interval between the first and second ovulations and the duration of estrus were prolonged to 23.8±3.5 and 5.7±2.2 days, respectively.

Different methods have been used in mares to evaluate the pp period. Information about the uterine involution and follicular dynamics is essential to exploit the foal heat into a viable pregnancy. Like in other species, ultrasonography has also been used to evaluate the pp reproductive events. Importantly, basic information on various pp reproductive events in Jennies is still obscure [14].

It is well known that plasma progesterone is liver metabolized into 20α-hydroxyprogesterone and 20β-hydroxyprogesterone (20 β -OH-P4) before being partly eliminated in the urine and the faces. 20α-hydroxy-progesterone (20α-OH-P4) has been proposed to evaluate cyclic activity in mares. In cycling mares, the feces 20α-OH-P4 concentration is quite comparable to plasma P4 concentrations [15]. Schwarzenberger et al.[16] proposed to use 20α-hydroxyprogesterone concentrations in faces to evaluate luteinic activity. Steroid concentrations in feces exhibit a similar pattern to those in plasma.

The objectives of the present study were to investigate the relationship between follicular development and ovulation during the pp period in Anatolian donkey mares and to determine physiological parameters related to sexual cycles such as duration of sexual cycle and estrus during the pp period. Plasma progesterone (P4), total estrogens (E2) and 20α-OH-progesterone (20α-OH-P4) levels were also studied during the pp ovulatory cycles to support the ultrasonographic findings. The results can provide evidence if 20α-OH-P4 level is compatible with plasma P4 and can be used to evaluate the reproductive status of Anatolian donkey mares.

MATERIAL and METHODS

Experimental Animals

All experimental procedures were approved by the Review and Ethic Committee for Animal Experiments of Kırıkkale University (12/10, 06.02.2010). Five healthy and late-stage pregnant Anatolian donkey mares were included in the study. The age of the jennies ranged from 6 to 10 years. Jennies had free access to water and fed by a commercial diet in addition to grass and hay. Animals were maintained under natural day length of the spring season. The foals were kept along with their mothers during the study period and were free to suckle throughout the day.

Ultrasonographic Examinations

Transrectal ultrasound (US) examinations were undertaken using a B-mode real-time veterinary ultrasound scanner (Shimadzu, SDL-32; 5 MHz, linear-array, Maastricht, Netherlands) to characterize uterine involution and ovarian activity, as previously defined [14,17]. Rectal palpation (RP) and US were performed with 2-day intervals from the first day of the pp period until the day at which a follicle in a size of >20 mm was detected, and then US were conducted daily until the ovulation. The largest follicle was measured in all donkey mares and data were presented as mean±standard deviation as well as median (the lowest and the highest sizes). The development of corpus luteum (CL) was disregarded even though the CL was detected during the post ovulation period. The cornu uteri were divided into two regions: the region between tip-middle of the uterus and middle of the uterus-corporo-cornual junction (CCJ). The cross sectional diameter of each region was recorded. Uterine involution was considered to be completed when the CCJ became stable for three consecutive examinations.

Hormone Assay

Upon venipuncture of the jugular vein, blood samples (10 ml) were collected every other day, starting from the first day of the first estrus up to the detection the preovulatory follicle at the second estrus. Blood samples were collected from 4 jennies which exhibited clinical estrus. One jenny was excluded from the hormone assay because it showed no sign of estrus. The day at which the estrus behaviour was first detected was considered as day 0. The blood samples, which were previously collected into heparinized tubes, were centrifuged at 2500 rpm for 15 min. Their plasma were harvested and stored at -18°C until assayed. Plasma P4, E2 and 20α-OH-P4 were studied by RIA [15,16,18]. Due to high concentrations, E2 levels were measured and given in ng/ml.

Statistical Analysis

The mean and standard deviations were calculated by Descriptive Statistics. The bivariate correlate test was conducted in order to see the relationship between para-

meters tested. The linear regression test was applied to test relationship between blood parameters using their values at different days. The T-test was applied to show a statistical difference between two normally distributed groups. SPSS 10 (SPSS inc.) was used to perform the tests.

RESULTS

In all cases, the delivery was uneventful, and placenta was expelled within 30 min after foaling. No incidence of an abnormal condition occurred during the period of puerperium. The foals started suckling within 1 h after foaling. Plasma P4, E2 and 20α-OH-P4 values measured at the first day of the pp period were 37.9 (3.0-97.4) ng/ml, 23.6 (4.8-49.0) ng/ml and 189.5 (16.0-469.5) ng/ml, respectively.

Table 1 presents pp plasma hormone levels, follicular development and ovulation results in Jennies. The first behavioral estrus sign occurred as early as between the 6th and 10th days of the pp period, at which the largest follicle was 25.25 mm in diameter, and plasma P4, E2 and 20α-OH-P4 values were 1.16 ng/ml, 3.98 ng/ml and 1.28 ng/ml, respectively.

The circular and tense preovulatoric follicle became irregular and soft consistency prior to ovulation, and mild pain on palpation. Follicles reached to a preovulatoric size of 32.25 mm at 2 days after the first estrus detection time (9.60 days after parturition) during which plasma P4, E2 and 20α-OH-P4 values were 0.90 ng/ml, 6.82 ng/ml and 0.98 ng/ml, respectively. On the day of ovulation, plasma P4, E2 and 20α-OH-P4 values were 0.81 ng/ml, 3.78 ng/ml and 0.95 ng/ml respectively. The average ovulation day after parturition was 12.80. The earliest and latest ovulation days were 10 and 15, respectively. The signs of the first estrus ended about day 13.5±1.73 pp (the 12-16th day pp).

In the first pp cycles, P4 and 20α-OH-P4 value, increase after ovulation. The plasma P4 levels reached to a maximum concentration of 26.10±8.11 ng/ml and 27.72±14.02 ng/ml at the 10th and 14th days of ovulation, respectively. The 20α-OH-P4 level reached to a maximum concentration of 8.28±1.84 ng/ml and 8.78±2.04 ng/ml at the 10th and 14th days of ovulation, respectively. Starting from the 20th day of the cycle, both hormone levels began to decline. During the second estrus, they dropped down to their basal levels. There was a significant correlation between P4 and 20α-OH-progesterone values (r=0.986; P<0.001) during the entire cycle *(Fig. 1)*.

Plasma E2 levels reached to a maximum concentration of 6.82 ng/ml at the preovulatoric follicle detection time and then gradually decreased until the 20th day of the cycle. Plasma hormone profiles during the first pp estrus cycle are presented in *Fig. 1*.

The second estrus was observed 32.2±1.58 days after parturition with an interestrus interval period of 25.0±1.73 days between the first and second cycles. The follicle size at the time of the first recognized sign of the second estrus was 24.00±2.07 mm (20-26 mm), which was comparable to

Table 1. *Plasma hormone levels and follicle size (X±Std) during postpartum period in jennies*
Tablo 1. *Dişi eşeklerde postpartum dönemde plazma hormon düzeyleri ve folliküler büyüklük (X±Std)*

Cyclic Situation	Days Postpartum [range]	Follicle Size (mm) [range]	P4 (ng/ml) [range]	20α-OH-P4 (ng/ml) [range]	E2 (ng/ml) [range]
First observed estrus time	7.50±2.06 [6-10]	25.25±2.87 [22-29]	1.16±0.37 [0.91-1.8]	1.28±0.52 [0.90-2.16]	3.98±1.64 [2.1-5.5]
Preovulatoric follicle detection time (firs cycle)	9.60±2.30 [7-13]	32.25±2.06 [30-35]	0.90±0.40 [0.75-1.21]	0.98±0.21 [0.80-1.60]	6.82±4.64 [2.57-11.61]
First postpartum ovulation time	12.80±1.92 [10-15]	-	0.81±0.39 [0.2-1.16]	0.95±0.14 [0.8-1.1]	3.78±1.28 [1.11-5.00]
Second observed estrus time	32.20±1.58 [30-34]	24.00±2.07 [20-26]	1.40±0.37 [1.0-2.1]	1.24±0.27 [1.-1.4]	3,14±2,01 [1.30-5.00]
Preovulatoric follicle detection time (second cycle)	34.00±1.58 [32-36]	31.40±3.43 [29-34]	0.96±0.42 [0.80-1.40]	1.12±0.48 [0.90-1.40]	4.54±3.52 [1.65-7.56]

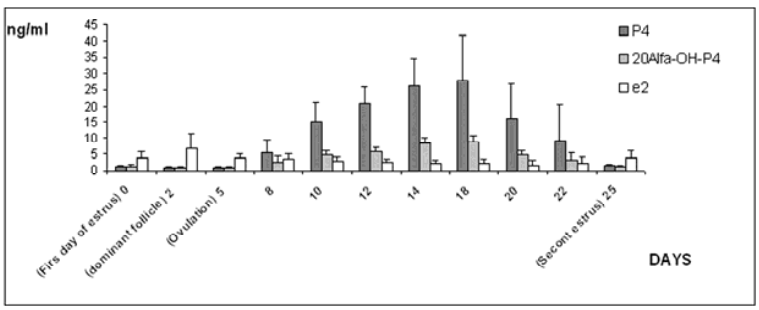

Fig 1. Plasma hormone profiles (X±Std) during first postpartum estrus cycle in jennies (P4 and 20α-OH-P4 r^2=0.986; P<0.001)

Şekil 1. Dişi eşeklerde, postpartum ilk östrus siklusunda plazma hormone düzeyleri (X±Std) (P4 ve 20α-OH-P4 r^2=0.986; P<0.001)

that of the first estrus *(Table 1)*. The behavioral estrus lasted 6.0±1.00 days and 5.6±1.67 days in the first and the second cycles, respectively.

The uterus borders could be detected by rectal palpation as early as the 3rd or the 4th day after foaling, and the uterus was totally palpable 6 to 9 days after foaling. The uterus horns became symmetrical 11-15 days after foaling, during which the diameter of the uterus horn was measured as 25.40±2.88 mm and 26.80±3.49 mm for the right and left horns, respectively.

DISCUSSION

Postpartum determination of ovarian follicular size is important to determine ovarian activities during the pp period. Dadarwal et al.[14] noted that the follicle diameter in jenny ovaries varied from 10 to15 mm on the day of parturition. In the same study, they also found that at least one follicle >25 mm in diameter was present 5 to 12 days pp [14]. In this study, we found that follicles reached to an average preovulatoric size of 32.25 mm 9.60 days after parturition (2 days after clinically detectable estrus). These findings were quite comparable to the earlier report in jennies [14] as well to those of mares [12,19]. Galisteo and Perez [13] noted that the foal-heat in Spanish jennies lasted 4.7±1.7 days, and ovulation occurred 13.2±2.7 after parturition. In our study, the first sign of the foal-heat occurred 7.50±2.06 days of parturition, the foal-heat lasted 6.0±1.00 days, and ovulation occurred 12.80±1.92 days after parturition. We think that the ovulation times in foal-heat in Anatolian jennies are similar to those of Spanish jennies; however, the duration of foal-heat longer in Anatolian jennies. Reports by Meira et al.[8] and Henry et al.[9] indicated that ovulatory follicles of jennies could grow to a size more than 30 mm in diameter up to the day of ovulation, similar to the findings in the present study.

In this study, 4 out of 5 jennies included in this study ovulated regularly throughout the session, only one jenny showed no signs of estrus. Such observation was similar to an earlier report in jennies [13]. Several factors may influence the ovarian activity after parturition. In case of lactational anestrus, mares fail to ovulate and/or show overt estrus during the pp period [9].

As evidenced by the present study, the length of the estrus cycle pp in Anatolian jennies is about 25.0±1.73 days, which is similar to that indicated by Vandeplassche et al.[3], Miro´ et al.[5] and Trimeche and Tainturier [6]. However, Blanchard et al.[4], Nishikawa and Yamazaki [7] and Galisteo and Perez [13] have described a shorter period of estrus cycle (22.8, 23.3 and 23.8 days, respectively). The mean length of estrus cycle in Anatolian jennies is clearly longer than in mares (21 days) [20,21] but similar to that in ponies (25 days) [11]. The duration of estrus in the present study is similar to that reported for other breeds of donkey [4,8], but Henry et al.[9] reported a longer duration of estrus, 7.9 days. In the second cycle, we found that behavioral estrus lasted 5.6±1.67 days. Such observation was similar to an earlier report in jennies (5.7±2.2) [13] and comparable to that of mares [20,21].

The preovulatory follicles were characterized by their soft consistency, irregular shape, mild pain on palpation. The preovulatory follicle size we observed in the present study was also comparable to those previously reported in jennies [14] and mares [22].

Like in other species, a linear relation between ovarian structures and plasma progesterone concentrations is present in jennies [8,14]. Carluccio et al.[10] noted that the plasma P4 concentration dropped to 1 ng/ml during the estrus. In the present study, the ultrasonographic findings of the ovarian structures were supported with the plasma P4, E2 and 20α-OH-P4 values. Follicles reached to a preovulatoric size of 32.25 mm at 2 days after the first estrus detection time during which plasma P4 and 20α-OH-P4 values were below 1 ng/ml On the other hand, the E2 levels increased. In the mare, when the follicle size reached up to 30 mm, there is a significant increase in plasma E2-17β concentration of and a decrease in plasma P4 concentration of [23]. Our results indicated that hormone concentrations in Anatolian jennies are similar to those of mares. On the day of ovulation, no significant changes in plasma P4, 20α-OH-P4 concentrations were detected, and they were below 1 ng/ml. The E2 concentration on the day of ovulation (3.78 ng/ml) remained high although there was some decrease compared to that of the dominant follicle detection time. In mare, the P4 concentration decreases down to a minimum level during the ovulation period, but the estrogen concentration remains high levels. In the same study, the fecal 20-OH-gestagen concentrations in mare decrease down to minimum levels 2 days after ovulation [24]. In jennies we found that the plasma 20α-OH-P4 concentration decreased down to minimum levels on the day ovulation, suggesting that the decrease in 20α-OH-P4 levels occurs earlier in jennies compared to mares.

While progesterone concentration in the jennies having preovulatory follicle was <1.0 ng/ml, it was >2 ng/ml when the jennies had CL. Similar relation of ovarian structures with plasma progesterone concentrations in jennies had been reported earlier [25]. In this study after ovulation, the values of P4 increased within 24-36 h, and remained high until day 14 or 15.

Fecal 20α- OH-P4 concentrations in mares have been determined and the fecal 20α- OH-P4 concentration is also quite comparable to that of plasma P4 concentrations [15,16]. In this study we also studied plasma 20α- OH-P4 concentrations in jennies. In the present study, there was increase in follicular size starting from the first estrus time and reached the largest size at the day of preovulatory detection time. As expected [26], the gradual increase in the follicular size in the present study was accompanied by a continuous decrease in the concentrations of plasma P4 and plasma 20α-OH-P4. Importantly, the high correlation between the P4 and 20α-

OH-P4 concentrations (0.986; P<0.001) indicates that the 20α-OH-P4 concentration can be used in jennies for determination of follicular development and ovulation as plasma P4 and 20α-OH-P4 concentrations are quite comparable.

The uterine involution, the day after which no further reduction in the uterine diameter is observed, was completed 22.5±1.7 days after foaling in jennies (ranging from 18 to 27 days) [14]. In the present study, the uterine involution was completed 14.0±1.7 days after foaling (ranging from 11 to 15 days). Several factors (climate, feeding, etc) may cause minor differences between our and previous studies in terms of the involution period. In a future study, such a difference should be investigated further what are the factors for the shorter involution period in Anatolian jennies.

In conclusion, the postpartum reproductive events in Anatolian jennies seem to be similar to those reported for other breeds. The plasma steroids (P4 and 20α-OH-P4.) analyses are reliable and diagnostic tools to study the fundamental reproductive endocrinology and provide information regarding the estrus cycle in the Anatolian jennies. Furthermore, ultrasonography is a safe and reliable tool to evaluate physiological alterations in the reproductive organs of jennies.

REFERENCES

1. Mason IL: A World Dictionary of Livestock Breeds, Types and Varieties. Fourth ed., p. 273. C.A.B International, Philadelphia, 1996.

2. Ginther OJ, Scraba ST, Bergfelt DR: Reproductive seasonality of the jenney. *Theriogenology,* 27, 587-592, *1987.*

3. Vandeplassche GM, Wesson JA, Ginther OJ: Behavioral, follicular and gonadotropin changes during the estrous cycle in donkeys. *Theriogenology;* 16, 239-249, *1981.*

4. Blanchard TL, Taylor TS, Love CL: Estrous cycle characteristics and response to estrus synchronization in mammoth asses *Theriogeneology*, 52, 827-834, 1999.

5. Miro´ J, Lobo V, Medrano A, Rigau T: Dina´mica folicular en la burra catalana. **In,** *Comunication, IV Congreso Ibe´rica de Reproduccio´n Animal, July 11-12, Julio Arucas, Spain,* 2003.

6. Trimeche A, Tainturier D: E ´ tude e´chographique de la cine´tique folliculaire des baudettes du Poitou pendant l'oestrus au cours du printemps et de l'e´te´. *Revue Me´d Ve´t*, 146, 743-874, 1995.

7. Nishikawa Y, Yamazaki Y: Studies on reproduction in asses. I. Breeding season, oestrous cycle and length of heat. *Jpn J Zootech Sci,* 19, 119-124, 1999.

8. Meira C, Ferreira JCP, Papa FO, Tornero MTT, Bicudo SD: Study of the estrus cycle in donkeys *(Equus asinus)* using ultrasonography and plasma progesterone concentrations. *Biol Reprod (Mono),* 1, 403-410, 1995.

9. Henry M, Figueiredo AEF, Palhares MS, Coryn M: Clinical and endocrin aspects of the oestrus cycle in donkeys *(Equus asinus). J Reprod Fert,* 35, 297-303, 1987.

10. Carluccio A, Tosi U, Contri A, Amicis I De, Veronesi MC: Corpus Luteum Sensitivity to PGF2*α* Administration in the Martina Franca Jenny. *Vet Res Comm*, 30 (1): 171-173, 2006.

11. Ginther OJ: Reproductive Biology of the Mare: Basic and Applied Aspects. 2nd ed., pp. 75-232. Wisconsin: Cross Plains, Equiservices Publishing, 1992.

12. Allen WR: Practical control of anestrus in thoroughbred broodmares**. In,** Ellendorff F, Elsaesser F (Eds): Endocrine Causes of Seasonal and Lactational Anestrus in Farm Animals. pp. 98-107, Dordrecht, Martinus Nijhoff Publishers, 1985.

13. Galisteoa J, Perez-Marinb CC: Factors affecting gestation length and estrus cycle characteristics in Spanish donkey breeds reared in southern Spain *Theriogenology*, 74, 443-450, 2010.

14. Dadarwal D, Tandon SN, Purohit GN, Pareek PK: Ultrasonographic evaluation of uterine involution and postpartum follicular dynamics in French Jennies *(Equus asinus). Theriogenology*, 62, 257-264, 2004.

15. Schwarzenberger F, Mostl E, Bamberg E, Pammer J, Schmehlik O: Concentrations of progestagens and oestrogens in the faeces of pregnant Lipizzan, trotter and thoroughbred mares. *J Reprod Fertil*, 44 (I1): 489-499, 1991.

16. Schwarzenberger F, Mostl E, Bamberg E, Van Hegel G: Monitoring of corpus luteum progestagens in faeces of non-pregnant mares *(Equus caballus)* and Przewalsky mares *(Equus przewalskii). Anim Reprod Sci*, 29, 263-273, 1992.

17. McKinnon AO, Squires EL, Harrison LA, Blach EL, Shideler RK: Ultrasonographic studies on the reproductive tract of mares after parturition: effect of involution and uterine fluid on pregnancy rates in mares with normal and delayed first postpartum ovulatory cycles. *J Am Vet Med Assoc*, 192, 350-353, 1988.

18. Möstl E, Choi HS, Bamberg E: Stimulation of androgen and oestrogen concentrations in plasma of cows after administration of a synthetic glucocorticoid (flumethasone) at the end of gestation. *J Endocrinol*, 105, 121-126, 1985.

19. Koskinen E: Postpartum ovarian activity in Finnhorse mares with special reference to seasonal effects. *Acta Vet Scand*, 32, 313-318, 1991.

20. Ginther OJ, Pierson RA: Regular and irregular characteristics of ovulation and the interovulatory interval in mares. *J Eq Vet Sci*, 9, 4-12, 1989.

21. Renaas KG, King SS, Nequin LG: Follicular growth and estradiol concentrations in foaling and nonparturient mares. *J Eq Vet Sci*, 20, 266-268, 2000.

22. Pierson RA, Ginther OJ: Ultrasonic evaluation of the corpus luteum of the mare. *Theriogenology*, 23, 795-806, 1985.

23. Koskinen E, Kuntsi H, Lindeberg H, Katila T: Predicting ovulation in the mare on the basis of follicular growth and serum oestrone sulphate and progesterone levels. *J Vet Med A*, 36, 299-304, 1989.

24. Amer HA, Shawki G., Randa I: Pro file of steroid hormones during oestrus and early pregnancy in Arabian mares. *Slov Vet Res,* 45 (1): 25-32, 2008.

25. Carluccio A, Contri A, Mattioli M, Vilani M, Veronesi MC: Progesterone. LH plasma concentrations during the estrous cycle inMartina Franca donkeys. *Reprod Dom Anim*, 40 (348): 18-24, 2005.

26. Illera, JC, Illera MJ, Silvan G, Illera M: Correlations between ultrasonography findings and hormonal profiles at oestrus in pure Spanish breed mares. *Aust Vet Assoc,* 70, 273-275, 1993.