Plasma Estradiol-17β and Progesterone during Estrous Cycle in Caspian Mares

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Summary

Changes in diameters of the preovulatory dominant follicle (for 10 days before ovulation) and corpus luteum (during the estrous cycle) paralleling with plasma estradiol-17β and progesterone profiles were monitored in seven healthy Caspian mares, aged 8.8±1.53 years and weighed 198.6±0.9kg. The plasma concentration of estradiol-17β was progressively ($P<0.05$) increased from day 6 to day 2, when the peak value was attained. It then sharply decreased to the basal concentrations at the time of ovulation. The first significant ($P<0.05$) rise in plasma progesterone concentration was detected 24h after ovulation. It was steadily increased until day 4 after ovulation. The concentrations were remained elevated until day 12. From day 10 onward the gradual decrease in plasma progesterone concentrations was noticed with the first significant drop between days 12 and 13. There was a significant ($P<0.05$) correlation between plasma progesterone and estradiol-17β concentrations with cyclic changes in corpus luteum and preovulatory follicle, respectively. The results suggest that in Caspian mares as compared to other breeds, the progesterone levels began to decrease earlier, due to earlier occurrence of luteolysis, and regarding to estradiol-17β there was an extra minor peak that occurred at about day 8.

Key words: progesterone, estradiol-17β, Caspian, mare

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Introduction

In equine species the estradiol-17β concentrations begin to increase between days 14 and 16 after ovulation, 6 to 8 days prior to the next ovulation (Palmer & Terqui 1977, Adams & Bosu 1988, Ginther 1992). The peak values occur around 2 days prior to ovulation and thereafter decrease and attain the lowest concentrations by the end of estrus, and then remain variably low during diestrus (Meineck *et al* 1987, Adams & Bosu 1988, Bogh *et al* 2000).

During estrous cycle the concentrations of plasma progesterone fluctuate were measured between 0.1 to 11.5ng/ml (Tsunoda *et al* 1989, Weedman *et al* 1993, Bergfelt *et al* 1996, Nagy *et al* 1998). Plasma progesterone concentrations in the mare are well below 1ng/ml during estrus and then steadily increase within 12 to 24h after ovulation and reach to 5 to 8ng/ml between days 4 to 9 after ovulation (Meineck *et al* 1987, Plotka *et al* 1975, Adams & Bosu 1988, Ginther 1992). Following luteolysis, occurring on days 13 to 14 (Holtan *et al* 1975), the plasma progesterone concentrations decline rapidly and within 3 days (day 16 postovulation) reach to the basal concentrations, coinciding with the onset of estrus (Gunther *et al* 1980).

There are profound breed differences among horses in some reproductive parameters such as follicular wave patterns (Ginther 1992), multiple ovulations, the size of ovulatory follicle, ovarian response to exogenous gonadotropin stimulation, the incidence of diestrus ovulation and the pattern of antral formation. The Caspian horse, as one of the ancestor of all modern day breeds of horse, is extremely rare and barely pulled back from the edge of extinction in 1965 (Draper 2000). Presently, small number of them has been left (almost 70) in Iran. Apart from some physical differences between Caspian and other breed of horses, no relevant study regarding to the relation between cyclic changes in plasma concentrations of estradiol and progesterone with changes in dominant follicle and corpus luteum has been done in Caspian mare. This study was carried out to investigate the changes in plasma progesterone and estradiol-17β concentrations in parallel with cyclic changes in corpus luteum and preovulatory dominant follicle throughout estrous cycle in Caspian mare in order to detect any probable differences between Caspian and other types or breed of horses.

Materials and Methods

Experimental animals and location. Seven clinically healthy cycling Caspian mares, 8.8±1.53 years of age (range 4-15 years) and 198.6±0.9kg LW were studied. Mares were kept out doors and fed daily with Lucerne hay (1200gr), barley (665gr) and wheat straw (1500gr), consisting of 347gr crude protein and 7.4MJ digestible energy. This study was carried out at the Equine Research Centre of Natural Resources and Animal Affairs of Tehran province (Latitude 39'35"N, Longitude 44'51"E and Altitude 1320m) from late April to late June 2001-2002.

Estrous detection and ultrasonic examination. Daily observation for estrous detection including urination, winking, and lack of resistance of the mare in the presence of stallion was conducted for individual mare. The cyclic changes in size and echogenicity of corpus luteum, and diameter of the preovulatory follicles as well as time of ovulation and luteolysis were detected, throughout two complete interovulatory intervals, through daily monitoring of ovaries using real time B-mode ultrasound scanner (Pie-Medical 480, The Netherlands) equipped with 5 MHz, trans-rectal transducer.

Blood sampling and hormonal analysis. Daily blood samples, prior to ultrasound examination, were collected by jugular venipuncture throughout two complete interovulatory intervals at the morning time. The blood was drawn to the evacuated tubes, containing anticoagulant (EDTA) enough to produce about 0.5ml plasma. The plasma samples were separated by centrifugation at 2000g for 5min and stored in Eppendorf microtubes at–20°C until hormonal assay. The plasma estradiol-17β concentrations determined by double RIA using a coated tube radioimmunoassay kit

(Orion Diagnostica Spectra Co., Finland). The intra and inter-assay coefficients of variation ranged from 2.9 to 9.7% and 2.3 to 10.2% respectively, over the entire dose response curve. The sensitivity of the assay was 5.4pg/ml. Plasma progesterone concentration assayed by double antibody RIA using the Kavoshyar kit (Kavoshyar Co., Iran). The intra and inter-assay coefficient of variation ranged from 3.3 to 6.4% (0.77-5.75ng/ml) and 2.4 to 3.3% (0.81-11.4ng/ml) respectively. The sensitivity of assay was 0.10ng/ml.

Statistical analysis. The profile of progesterone and estradiol-17β concentrations and the changes in the diameter of corpus luteum and preovulatory dominant follicle were subjected to the repeated measures ANOVA test. Repeated measures data were analysed by either univariate or multivariate analysis. Multivariate analyses (Wilks' Lambda) were used where variance and covariance structures over time did not conform to analysis of variance assumptions, tested using the sphericity test (SAS institute Inc, 1990). The relation between the profiles of progesterone and estradiol-17β concentrations and the changes in the diameter of corpus luteum and preovulatory dominant follicle were assessed by Spearman correlation test. Data was presented as mean±SEM.

Results

The diameter of dominant follicle increased steadily ($P<0.05$) from 10 days (14.8±1.49mm) prior to ovulation (interovulatory interval: 22.1±0.43 days) and the maximum diameter of the preovulatory dominant follicle was achieved by day 1 (42.7±1.07mm). The cyclic changes in plasma estradiol-17β concentrations were positively correlated ($r=0.74$, $P<0.05$) with the changes in diameter of dominant follicle (Figure 1). The estradiol-17β concentrations steadily increased ($P<0.05$) from day 6 (27.8±2.24pg/ml) to day 2 (44.5±6.56pg/ml) and then rapidly declined to the lowest concentration (28.1±3.27pg/ml) within 2 days after ovulation. It was remained low until onset of the next estrus. However, two minor peaks occurred at days 4 (38.1±2.81pg/ml) and 14 (33.2±2.45pg/ml).

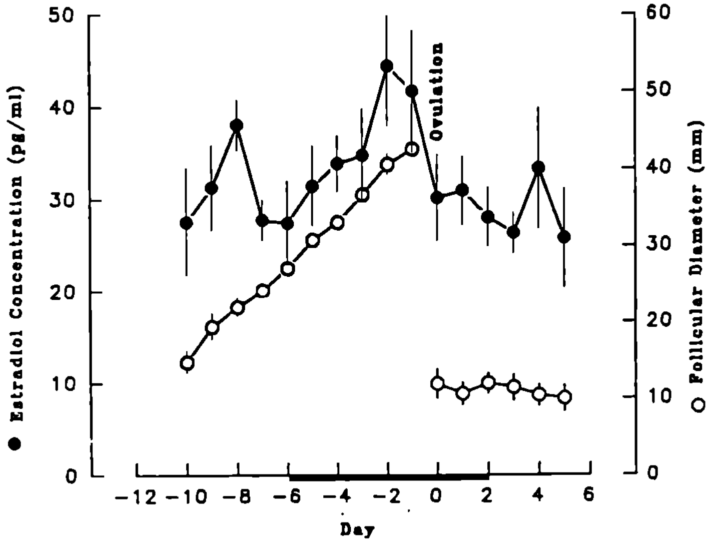

Figure 1. *Plasma concentrations of estradiol-17β and preovulatory dominant follicle diameters during the estrous cycle in Caspian mares (n=7). Each point represents a mean±SEM. The black horizontal bar represents the estrus period*

Following ovulation the follicular cavity was rapidly filled with the blood and within the first 2 to 3 days of ovulation the corpus heamorhagicum transformed to the corpus luteum. The maximum diameter of transforming corpus heamorhagicum was attained 2 days after ovulation (36.6±1.9lmm) (Figure 2). Concurrent with the formation of corpus luteum, over a few days, the luteal mass became more dense and smaller in diameter but more efficient in production of progesterone. The first significant (P<0.05) decrease in corpus luteum diameter occurred on day 11 and reached to its minimum size (16.9±1.20mm) by day 17. The plasma progesterone concentration during the estrous cycle was positively (P<0.0001) correlated (r=0.82) with the changes in corpus luteum diameter after thorough formation of corpus luteum (after day 4). The plasma progesterone concentrations declined steadily between days 10 and 14, with a significant (P<0.05) drop occurring between days 12 and 13. The minimum progesterone concentrations, less than 1ng/ml, were achieved after day 14 (2 days prior to the onset of estrus) and remained low until the day after the next ovulation. The first significant rise in plasma progesterone concentration occurred 24h after ovulation and reached to ≥8ng/ml by day 4 after ovulation and remained elevated until day 12. During estrous cycle two peaks of progesterone, at days 7 (11.1±1.39ng/ml) and 10 (11.6±1.37ng/ml) were detected. The average plasma concentration of progesterone over days 3 to 10 was 9.8±0.67ng/ml.

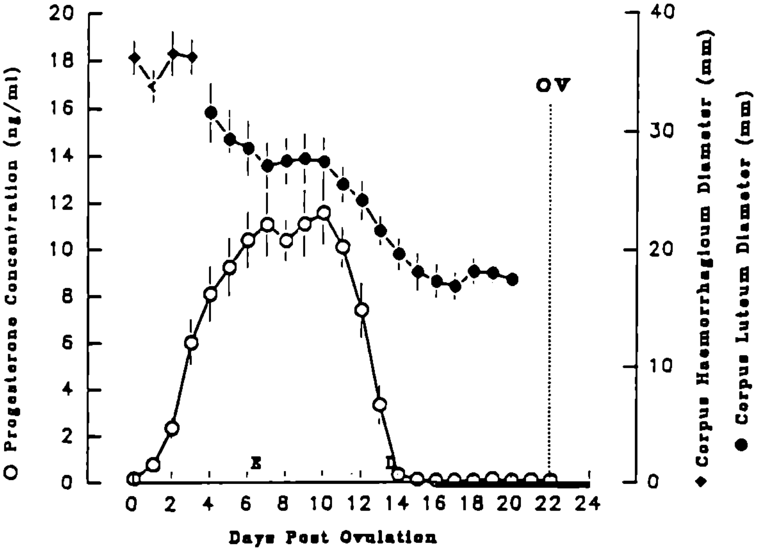

Figure 2. *Plasma concentrations of progesterone and corpus luteum diameter during the estrous cycle in Caspian mares (n=7). Each point represents a mean±SEM. The black horizontal bar represents the estrus period*

Discussion

Plasma estradiol-17β and progesterone concentrations positively (P<0.05) correlated with the cyclic changes in the ovarian follicle and corpus luteum diameters, respectively (Figures 1, 2). It has been documented that concurrent with the growth of ovarian follicles, at about day 12, the plasma estradiol-17β concentrations increased and attained the minor peak by day 8, at about the time of selection of dominant follicle (Ginther 1992). Recent studies however, have indicated that corpus luteum may also have a minor contribution to the rise of estradiol-17β during diestrus (Daels *et al* 1991a, b). Near the time of follicular selection the selected dominant follicle inhibits the growth of other follicles by secreting hormones (e.g. inhibin, estradiol, or other proteinaceous products) that suppress FSH secretion (Miller & Ginther 1980, Lacker *et al* 1987, Ying 1988) which in turn leads to the demise of all follicles except the selected one. In the present study, concurrent with progressive increase in diameter of the dominant follicle and its prominent role in production of estradiol (Collins *et al* 1997, Gastal *et al* 1997, Gastal *et al* 1999, Gerard *et al* 2002) the plasma estradiol-17β concentrations were increased at first slowly then rapidly, and the peak values were then attained by day 2 (major peak). Since then the concentrations, rapidly declined and reached to the lowest values by the time of ovulation, which was comparable to that of, reported in other breeds (Meineck *et al* 1987, Adams & Bosu 1988). As known the pattern of estradiol-17β, secreted by the large granulosa cells, will be changed concurrent with the decrease in aromatase activity, which is in association with follicular maturation (Belin *et al* 2000, Davidson *et al* 2002). Therefore, the granulosa cells become primarily more progesterone-secreting cells during that period. This suggests that in the mare granulosa cells luteinize prior to ovulation and it may be the reason for the decline in plasma estradiol-17β concentrations at the same time (Tuker *et al* 1991). In the current study the occurrence of an extra minor peak of estradiol-17β on day 4, might be due to the follicular growth that usually occur in some instances in early diestrus or might be as a reflection of estradiol production by developing corpus luteum, which has been reported by Ginther (1992).

In mares progesterone concentrations begin to rise soon after ovulation and the first significant increase in the values occur between 12 and 72h after ovulation

(Meineck *et al* 1987). Preliminary results have indicated, however, that in the mare, granulosa cells luteinize prior to ovulation implying the production of progesterone before ovulation (Tuker *et al* 1991, Collins *et al* 1997). Thereafter the progesterone progressively ($P<0.05$) increase to the high diestrus values (4 to 22ng/ml) by days 5 to 7 of the cycle (Adams & Bosu 1988), coincided with the full function of cropus luteum that appears to be attained by day 6. These levels remain elevated until days 13 or 14 followed by a rapid decrease in progesterone from days 14 to 16 (4 days before ovulation) (Holtan *et al* 1975). In the current study the first significant increase in progesterone was first detected 24h after ovulation that was in agreement with other reports (Meineck *et al* 1987). The time period in which the progesterone concentrations were remained elevated (days 10 to 12) was similar to that of reported in other breeds (days 13 to 14). From day 10 onwards progesterone was gradually declined which was preceded by a decrease in corpus luteum diameter, and subsequently followed by an increase in estradiol-17β concentrations due to progressive growth of preovulatory dominant follicle. The low levels, less than 1ng/ml, were achieved on day 14 (2 days before onset of estrus) and remained low until 24h after the next ovulation. These findings indicate that in Caspian mares the low progesterone levels (<0.1ng/ml) achieve approximately 2 days earlier in the cycle, in contrast to the other breeds (day 10 vs day 14), which in other hand indicating the earlier occurrence of luteolysis in this breed (Holtan *et al* 1975). This may be the reason for prolonged duration of estrus period in Caspian in contrast to the other breeds of mares. The time period (early season) in which this study has been performed, however, could be another possible reason for prolonged estrus period. During the estrous cycle two peaks of progesterone were detected, at days 7 and 10, which was comparable with the reports (days 4 to 6 and days 8 to 10 postovulation) in other breeds of mares (Ginther 1992).

In conclusion in Caspian mares the progesterone and estradiol-17β concentrations were closely followed the cyclic changes, which occurred in macro-morphology (detected by ultrasonic examinations) of the ovarian corpus luteum and follicular activity, respectively. The profile of plasma progesterone with slightly differences was similar to the patterns, which had been previously identified in other breeds, except for the earlier decrease in progesterone concentrations due to earlier occurrence of luteolysis in Caspian mares. With respect to the pattern of estradiol-17β, except for the occurrence of an extra minor peak, occurred on day 8, the plasma profile was similar to that of observed previously by investigators in other breeds and types of mares.

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