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Original Research Paper

PROFILE OF STEROID HORMONES DURING OESTRUS AND EARLY PREGNANCY IN ARABIAN MARES

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Summary: Faecal and plasma steroid evaluations are well established approaches for monitoring reproductive function in mares. The purpose of this study was to detect the ovarian and uterine changes by transrectal ultrasonographic scanning, beside the estimation of gestagen and estradiol-17ß profiles in plasma and faecal samples of Arabian mares. Eight cyclic barren mares of different parities were used in the current work, and hormones were assayed using radioimmunoassay. The follicular growth was accompanied by a significant (P<0.05) decrease and increase in the profile of plasma progesterone (P4) and estradiol-17ß (E1-17ß), respectively. In addition, the minimum level of P4, and the maximum level of E1-17ß were detected at day 0 of ovulation. Similarly, the faecal progesterone metabolites (20∝-hydroxy-progesterone; i.e. 20∝-G) content showed a significant (P<0.05) decrease in its value starting from day -7 reaching its minimum level at second day post ovulation, meanwhile, the faecal E1-17ß content was reaching its maximum value on day 1 after ovulation. Following ovulation, the plasma P4 and faecal 20∝-G content showed a continuous significant (P<0.05) increase starting from the 3rd day post ovulation, and the levels of both faecal and plasma E1-17ß showed a continuous significant decrease. The levels of P4 in plasma and 20∝-G in faeces increased significantly (P<0.05) at day 14 to day 45 of gestation than those recorded at day 0 in nonpregnant mares. Moreover, the levels of E1-17ß in plasma and faeces increased significantly (P<0.05) at days 21 up to 45 of gestation than those estimated during 14th day of gestation as well as in nonpregnant mares. In conclusion, both ultrasonography and analysis of P4 and E1-17ß in plasma, and 20∝-G and E1-17ß in faeces have a predictive value for assessment of the follicular sizes, ovulation time and early pregnancy in Arabian mares.

Key words: arabian mare; estradiol-17ß; gestagens; ultrasonography; oestrus; gestation

Introduction

Determination of the reproductive status is one of the most important factors for effective management and efforts to use assisted reproductive techniques depend on the knowledge of the basic reproductive physiology of a given species (1) Several studies had been made to determine ovulation time in mares including the clinical and ultrasonographical examinations (2, 3, 4, 5). Ovulation was also predicated in oestrus mares by serial measurements of peripheral estrogen and progesterone concentrations (6). The maximum diameter of the follicle in mare was determined by detection of conjugated estrogens in blood (7); also, serum progesterone was evaluated before ovulation. Meanwhile, the blood steroids of pregnant (early and late gestation) in Arabian mares(8).

The growth of the dominant follicle was associated with certain intra-follicular E1-17ß and P4 levels in mares (9). Meanwhile, ovarian activity of cyclic mares was monitored by measurement of P4 and E1-17ß in plasma (5) and in follicular fluids (10) in transitional mares. The ovarian endocrine activity in the mare can be evaluated through the use of faecal steroids or their metabolities (6). Estrogens are end products of steroid metabolism and, therefore, the compounds in plasma and faeces are similar (1). Meanwhile, the faecal estrogens in relation to reproductive status in mare were demonstrated (11); they were also demonstrated in cows (12), in buffaloes (13), and in primates (14).

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Determination of the preovulatory faecal estrogens peak proved to be less successful as compared to pregnancy determination in mares (6,15). The faecal progesterone metabolites consist of several 5∝-, 5ß-pregnances and progestagens 20∝-G (1,16,17,18). They reported also that the faecal progesterone metabolites in the mare belong to the 5∝-pregnances progestagens. However, the faecal 20 ∝-hydroxyprogesterone concentration can be used to evaluate cyclic activity in mares (18). The present work aimed to monitoring the ovarian dynamics and pregnancy status using both transrectal ultrasonography and analysis of steroids in the plasma (P4 and E1-17ß) and faeces (20∝-G and E1-17ß) of Arabian mares.

Material and methods

Animals

Eight cyclic barren mares of different parities (8 – 12 years/aged), belonged to El-Zahraa Stud for Arabian horses in Cairo were used in the current work. All the mares were free from internal and external parasites and in good health condition. At the beginning of the experiments all of the mares were nonpregnant.

Methods

Heat detection was done by day after day teasing with fertile stallion. The ovarian changes during oestrus period were detected by rectal palpation and transrectal ultrasonographic examination. All mares (n=8) were naturally bred every second day from the detection of growing follicles (>20 mm in diameter) until ovulation during oestrus (19). Pregnancy was diagnosed using transrectal ultrasound scanning of the uterus at day 14 post ovulation (20), and confirmed at 21st up to 45th day later (21). The ovarian and uterine scanning were done in the pregnant mares (n=8) and nonpregnant mares (n=3) using Pie-Medical Vet-200 ultrasound with transducer 5 and 7.5 MHz (Mitsobishi inc). The pregnancy was confirmed by rectal palpation on two months post service.

Sampling

Blood and faecal samples were collected daily from all mares starting on day -7 until ovulation which is represented as day 0 (n=8). All the samples were collected daily at day 14 later (post ovulation) from all mares. Samples were collected from pregnant mares on days 21 (n=8), 28 (n=5) and 45 (n=5) after last mating. Blood samples (10 ml) were collected by jugular vein puncture into heparinized vacutainer tubes, and were centrifuged at 3000 rpm for 20 minutes. The harvested plasma was stored in portions at -20°C until hormonal analysis. The faecal samples (20 gm) were collected from rectal balls and extracted (22,23), briefly: 0.5 gm faeces mixed and vortexed in 0.5 ml water and 4 ml methanol for 30 minutes, then 3 ml petroleum ether was added and vortexed for 10 seconds. After centrifugation at 1500 rpm/15 minutes, 0.2 ml of methanol extract was transferred into a new vial then diluted with 0.6 ml distilled water and 5 ml of petroleum ether/diethyl ether (v/v 9:1). The mixture was vortexed for 30 minutes then ether was evaporated at 40°C, later on the residue was diluted with 1 ml buffer and stored at -20°C until hormonal assay.

Hormonal assay

Progesterone in plasma was assayed (8), and faecal progestagen was assayed (8,18). Progesterone was measured using sold-phase125I-progesterone RIA (Coat-A-Count Progesterone; Diagnostic Product Corporation, Los Angeles, CA, USA). The assay sensitivity was 0.07 ng/ml (rang=0.03 to 0.16 ng/ml). The intra- and inter-assay coefficients of variation were 9.0 and 9.3% respectively. While, estradiol-17ß in plasma was assayed (24,25). Estradiol-17ß was determined by RIA using (Diagnostic Product Corporation, Los Angeles, CA, USA)125I-RIA Kits. The intra- and inter-assay coefficients of variation were 9.62% and 13.43% respectively. The concentration of standard estradiol ranged between 0 to 3600 pg/ml. The assay of faecal E1-17ß was performed (1,14).

Statistical analysis

Differences between comparable groups were demonstrated with Student "t" test. All computations were done using a personal computer, with the help of statistical program SPSS/PC 3.1 of SPSS Inc.

Results

The plasma P4 and E1-17ß, also the faecal 20∝-G and E1-17ß levels during the pre and post ovulatory period are shown in Table 1. The continuous significant ($P<0.05$) increase in follicular size starting from day –7 (16.50±1.0 mm) until reaching its larger size at 0-day of ovulation (40.12±1.4 mm) was accompanied by a continuous significant ($P<0.05$) decrease in the concentration of plasma P4 and increase in the concentration of plasma E1-17ß, starting from

day –7 (0.98±0.19 ng/ml and 24.00±2.00 pg/ml, respectively). The minimum level of P4 (0.18±0.05 ng/ml), and the maximum level of E1-17ß (78.75±4.20 pg/ml) were detected at 0-day of ovulation. A similar trend was observed for the faecal 20∝-G content that show decrease in its value starting from day –7 (196.85±15.67 ng/gm) reaching its minimum level at second day post ovulation (82.67±7.29 ng/gm). Meanwhile, the faecal E1-17ß content was reaching its maximum value on day 1 after ovulation (187.50±6.27 pg/gm).

Following ovulation, the plasma levels of P4 and E1-17ß showed a continuous increase, while the faecal 20∝-G and E1-17ß showed a continuous decrease in their profiles. Meanwhile, the concentrations in plasma P4 and faecal 20∝-G increased starting from the 3rd day post ovulation, while plasma and faecal E1-17ß decreased starting from the 2nd day post ovulation.

The plasma P4 and E1-17ß and faecal 20∝-G and E1-17ß levels in pregnant and nonpregnant mares are showed in Table 2. The levels of P4 in plasma and 20∝-G in faeces were significantly (P<0.05) increased at day 14 up to day 45 of gestation than those recorded during ovulation (0-day). Meanwhile, the levels of E1-17ß in plasma and faeces was significantly (P<0.05) increased at day 21 up to day 45 of gestation than during 14th day of gestation

Table 1: Estimation the levels of progesterone and estradiol-17ß profiles in the plasma and faecal samples during pre- and post-ovulatory period in Arabian mares (Mean±S.E.)

Day related to ovulation n=8	Follicle size (mm)	Gestagens n=8		Estradiol-17ß n=8	
		Plasma P4 (ng/ml)	Faecal 20∝-G (ng/gm)	Plasma E1-17ß (pg/ml)	Faecal E1-17ß (pg/gm)
day –7	16.50±1.0	0.98±0.19	196.85±15.67	24.00±2.00	120.00±10.0
day –6	19.33±1.5	0.81±0.18	188.76±17.84	27.66±2.40	130.00±7.65
day –5	21.25±1.0	0.74±0.15	169.48±16.46	32.25±2.78	138.75±6.88
day –4	23.60±1.8	0.62±0.13	152.14±14.53	37.00±2.61	142.50±6.29
day –3	27.33±1.0	0.44±0.09	138.67±12.76	37.66±4.05	151.67±5.72
day –2	33.00±1.4	0.36±0.07	146.42±10.47	44.37±3.17	166.87±4.99
day –1	38.00±2.7	0.32±0.08	132.18±9.22	59.12±3.69	155.00±4.22
day 0*	40.12±1.4	0.18±0.05	122.34±8.73	78.75±4.20	181.87±6.81
day 1	---	0.58±0.14	118.56±8.14	40.25±2.55	187.50±6.27
day 2	---	1.16±0.35	82.67±7.29	39.25±2.82	142.50±3.78
day 3	---	1.68±0.44	136.14±13.56	35.87±1.82	122.50±2.83
day 4	---	2.17±0.58	198.25± 6.28	32.00±1.74	108.50±3.58
day 5	---	2.56±0.64	237.42±19.92	31.37±1.22	105.00±4.90
day 6	---	2.98±0.73	266.53±23.88	30.87±0.97	98.00±3.79
day 7	---	3.48±0.88	240.47±25.72	26.37±0.86	93.37±3.59
P value	P<0.05				

*day of ovulation
Means in all the columns are significantly different at level P<0.05

Table 2: Estimation the levels of progesterone and estradiol-17ß profiles in the plasma and faecal samples during early gestation period in Arabian mares (Mean±S.E.)

Day of gestation	Gestagens		Estradiol-17ß	
	Plasma P4 (ng/ml)	Faecal 20∝-G (ng/gm)	Plasma E1-17ß (pg/ml)	Faecal E1-17ß (pg/gm)
day 0* (n=8)	0.18±0.05 c	122.34±8.73 d	78.75±4.20 a	187.50±6.27 bc
day 14 (n=8)	3.86±0.82 a	440.84±31.82 b	38.37±1.94 d	163.75±16.35 c
day 21 (n=8)	4.11±0.94 a	490.36±38.48 ab	50.25±4.51 c	215.87±16.48 b
day 28 (n=5)**	5.02±0.86 a	524.56±43.12 ab	59.00±4.69 bc	285.20±19.31 a
day 45 (n=5)**	5.67±0.98 a	596.48±46.56 a	70.40±4.11 ab	317.00±22.22 a
non-pregnant day 28 n=3	1.22±0.36 b	212.63±16.68 c	33.00±1.52 e	147.33±23.67 c

*day of ovulation **3 mares were diagnosed nonpregnant
Means with different superscripts in each columns are significantly different at level P<0.05

Discussion

The hormonal profile is a reliable clinical investigation method of oestrus and pregnancy detection using analysis of progesterone and estradiol-17ß in mares (16). Meanwhile, the analysis of steroid hormones in plasma and faecal samples offer the potential of addressing many timely, integrative problems in reproduction and conservation biology (26). Our results provide evidence that plasma accompanied with faecal steroid analysis may be important for understanding the reproductive status in Arabian mares. However, the route of excretion of steroid hormones and its metabolites varies considerably among species, and also between steroids within the same species. Steroid concentrations in faeces exhibit a similar pattern to those in plasma, but have a lag time, which depending upon the species, can be from 12 to more than 48 hours (1,16). In most non-domesticated species, repeated blood sampling is not possible and, therefore, non-invasive faecal steroid evaluations are also used. Thus faecal samples are the most practicable choice beside to the plasma for this purpose.

In the present study, there was increase in follicular size starting from day -7 until reaching its larger size at day of ovulation, that accompanied by a continuous decrease and increase in the concentrations of plasma P4 and E1-17ß, starting from day –7. The minimum and maximum levels of P4 and E1-17ß reached at day of ovulation, respectively. Similarly, faecal 20∝-G content showed a decrease in its value starting from day –7 reaching its minimum level at 2nd day post ovulation, meanwhile, the faecal E1-17ß content was reaching its maximum value on day 1 after ovulation. Following ovulation, the plasma P4 and faecal 20∝-G levels showed a continuous increase (starting from the 3rd day post ovulation), and decrease in the profiles of plasma and faecal E1-17ß (starting from the 2nd day post ovulation). With increasing the follicular size from <30 mm to >30 mm diameter, there was a significant increase in the concentration of plasma E1-17ß and decrease in the concentration of plasma P4 (27). However, production of estrogen by the large follicles is consistent with the oestrus-like uterine echotexture which seemed approximately related to the growing phase of large follicle (7). A high relation between the ultrasonography findings and hormonal concentration, showing the increase of E1-17ß and the decrease of P4 concentration, corresponding to the days of the oestrus cycle at which the experiments were performed (2,28). In addition, the incidence of diestrous ovulations in mares is considerably higher (29), presumably because some breeds have more follicular activity and secretion of estrogen during the first half of dioestrus. This come in agreement with the findings in this study where the secretion of E1-17ß extended up to the second day post ovulation. Results from faecal hormone analysis indicated a useful characterizing and retrospectively predicting oestrus cyclicity and the occurrence of ovulation. Furthermore, cyclicity and ovulation were also confirmed by the rise and fall of the progestagens and E1-17ß excretion during the pre- and post-ovulatory periods (26).

For the study of ovarian activity in mares, several investigators have measured the concentration of

P4 in blood (30,31,32). There is agreement that concentrations below 1 ng/ml plasma (33) are indicative for oestrus or missing luteal activity. After ovulation, the values of P4 increase within 24-36 h, and remain high until day 14 or 15. Thereafter, in nonpregnant mares the values decrease rapidly to the low oestrus values. The plasma P4 and E1-17ß concentrations were similar to those found by others in the late luteal and follicular phases of the oestrus cycle of the mare (31,32,34). Moreover, large quantities of steroids are excreted in faeces largely because the principal means of excreting cholesterol (the progenitor of most steroids) is through the gastro-intestinal tract via bile (35). For this reason, some steroids and their metabolites may be excreted in faeces at concentrations that reflect biological events. The previous results indicated that the excretion of steroids into the gut is mainly through bile (1), but they have also shown that a small proportion of the circulating steroids is secreted through the mucosa of the large intestine (36). Furthermore, steroids might be unevenly distributed in the faecal balls of horses (37).

The levels of P4 in plasma and 20∝-G in faeces was significantly increased at days 14 up to 45 of gestation than those recorded during at ovulation in nonpregnant mares. Meanwhile, the levels of E1-17ß in both plasma and faeces was increased at days 21 up to 45 of gestation than those estimated during 14th day of gestation. However, faecal progesterone metabolites and estrogen determination proved to be reliable indicators for pregnancy diagnosis in the species in which the foeto-placental unit is the source of large quantities of estrogens (1). The differences in these two variables between pregnant and nonpregnant mares reflect the first luteal response to pregnancy and could be an expression of the maternal pregnancy recognition mechanism (3). During the oestrus cycle and pregnancy, P4 is produced by corpus luteum and its metabolities circulated in the peripheral plasma and may be excreted via faeces (38), that could be used for monitoring the growth, maintenance and regression of corpus luteum, and thus, as a tool to confirm oestrus cyclicity and possible pregnancy. However, faecal progestagen values increased at luteal phase within 10 days after fertilization and remained in this range for the first 2 months of pregnancy (17). Likewise, plasma P4 concentrations were measured in 179 mares bled on alternate days commencing with a positive pregnancy diagnosis on day 17 to 18 after ovulation and concluding on days 42 to 45 (40). Similar to our findings, faecal progestagen analysis has been successfully used for monitoring corpus luteum function and pregnancy (14,16,23,41). Although some studies reported the determination of the preovulatory oestrogen peak in mares, these methods proved to be less successful as compared to pregnancy determination, peak concentrations of faecal estrone conjugates during the follicular phase was very low (6,15). So, for a reliable analysis of the preovulatory estrogen peak in faecal samples, more extraction and clean up procedures of the samples and sensitive assays would be necessary. Moreover, follicular waves occurred periodically until the corpus luteum regressed, and in the absence of luteolysis (pregnant mares) the periodicity continued (42).

The difference in the excretion time of steroids between the oestrus cycle and pregnancy is probably caused by the very high concentrations present during pregnancy and by the enterohepatic circulation, which retards the excretion (16,17). Subsequently, the differences between the concentrations of both P4 and E1-17ß during oestrus and early gestation period could predicate the reproductive status of the mare. Subsequently, more research and coordination between researchers and biotechnology industries are required before any on-farm or field type faecal progestagen kits can be developed.

In conclusion, plasma and faecal steroid analysis can be used and accepted as a reliable and a diagnostic tool to study the fundemental reproductive endocrinology and provide information regarding the oestrus cycle and early pregnancy. Meanwhile, the E1-17ß and progesterone metabolites might be more accurate for monitoring the reproductive performance of mares. Subsequently, the ultrasonography accompanied with the estimation of steroid levels in plasma and faeces has a predictive value for the assessment of follicular sizes, ovulation time and early pregnancy in Arabian mares.

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PROFIL STEROIDNIH HORMONOV MED ESTRUSOM IN ZGODNJO BREJOSTJO PRI ARABSKIH KOBILAH

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Povzetek: Za ugotavljanje reproduktivnega ciklusa pri kobilah predstavlja ovrednotenje steriodnih hormonov v krvni plazmi in blatu že ustaljeno metodo. Namen naše študije je bil primerjati vrednosti gestagenov in estradiola 17ß v plazmi in blatu s spremljanjem sprememb na jajčnikih in maternični sluznici s transrektalno ultrazvočno preiskavo pri arabskih kobilah. Študija je zajela 8 kobil arabske pasme z normalnim ciklusom. Hormone smo določali radioimunsko. Ugotovljeno rast folikov je spremljal statistično značilen (P<0.05) padec gestagena P4 in porast estradiola 17ß (E1-17ß) v krvni plazmi. Poleg tega smo ugotovili najnižjo raven P4 in najvišjo E1-17ß na dan 0 – čas ovulacije. Podobno je progesteronski metabolit v blatu 20∝-hydroxy-progesterone (20∝-G) kazal statistično značilen (P<0.05) padec ravni od sedmega dneva pred ovulacijo z najnižjo vrednostjo dva dni po ovulaciji, medtem ko je E1-17ß v blatu dosegel najvišjo vrednost 1 dan po ovulaciji. Po ovulaciji se je od tretjega dne dalje vrednost plazemskega P4 in fekalnega 20∝-G konstantno statistično značilno (P<0.05) poviševala, medtem ko se je vrednost estrogena v plazmi ali blatu konstatno zniževala. Med brejostjo sta v blatu statistično značilno narasli vrednosti P4 20∝-G od 14. do 45. dneva v primerjavi z dnevom 0 pri nebrejih kobilah. Raven E1-17ß je značilno narasla (P<0.05) v blatu in krvni plazmi med 21. in 25. dnem brejosti, če jo primerjamo z vrednostmi v prvih dveh tednih brejosti pri brejih oz. pri nebrejih kobilah. Ugotovimo lahko, da so vse uporabljene metode, kot so ultrazvočna preiskava, merjenje P4 in E1-17ß v plazmi ter 20∝-G in E1-17ß v blatu, uporabne za napovedovanje velikosti jajčnega folikla, čas ovulacije in ugotavljanje zgodnje brejosti pri arabskih kobilah.

Ključne besede: arabske kobile; estradiol-17ß; gestageni; ultrazvočna preiskava; estrus; brejost