Ovarian and endocrine changes during oestrus and early pregnancy in Arabian mares
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Citation

Abstract
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 progesterone and estradiol-17β 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 continuous significant increase in the follicular size starting from day –7 until reaching its largest size at 0-day of ovulation was accompanied by a continuous significant (P<0.05) decrease and increase in the profile of plasma progesterone (P4) and estradiol-17β (E1-17β), respectively. In addition, the minimum level of P4, and the maximum level of E1-17β were detected at 0-day of ovulation. Similarly, the faecal progesterone metabolites (20α-hydroxy-progesterone; i.e. 20α-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 E1-17β content was reaching its maximum value on day 1 after ovulation. Following ovulation, the plasma P4 and E1-17β content showed a continuous significant (P<0.05) increase, and the faecal levels of both 20α-G and E1-17β showed a continuous significant decrease. Meanwhile, the levels of P4 in plasma and 20α-G in faecal samples increased starting from the 3rd day post ovulation, and E1-17β decreased starting from the 2nd day post ovulation. The levels of P4 in plasma and 20α-G in faeces increased significantly (P<0.05) at days 14 up to 45 of gestation than those recorded during ovulation in non-pregnant mares. Moreover, the levels of E1-17β 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 non-pregnant mares. In conclusion, both ultrasonography and analysis of P4 and E1-17β in plasma, and 20α-G and E1-17β in faeces have a predictive value for assessment of the follicular sizes, ovulation time and early pregnancy in Arabian mares.

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 (Schwarzenberger et al., 1996). Several studies had been made to determine ovulation time in mares including the clinical and ultrasonographical examinations (Townson and Ginther, 1987; Sevinga et al., 1999; Abou El-Roos and El-Maghraby, 2000 and Watson et al., 2000). Ovulation was also predicated in oestrus mares by serial measurements of peripheral estrogen and progesterone concentrations (Bogh et al., 2000) in transitional mares. The ovarian endocrine activity in the mare can be evaluated through the use of faecal steroids or their metabolities (Barkhuff et al., 1993). Estrogens are end products of steroid metabolism and, therefore, the compounds in plasma and faeces are similar (Schwarzenberger et al., 1996). Meanwhile, the faecal estrogens in relation to reproductive status in mare were demonstrated by Bamberg et al. (1984); they were also demonstrated in cows (Mostle et al., 1984), in buffaloes (Ismail et al., 1987), and also in primates (Heisterman et al., 1993). The growth of the dominant follicle was associated with certain intra-follicular E1-17β and P4 levels in mares (Gerard et al., 1999). Meanwhile, ovarian activity of cyclic mares was monitored by measurement of P4 and E1-17β in plasma (Watson et al., 2000) and in follicular fluids (Bogh et al., 2000) in transitional mares. The ovarian endocrine activity in the mare can be evaluated through the use of faecal steroids or their metabolities (Barkhuff et al., 1993). Estrogens are end products of steroid metabolism and, therefore, the compounds in plasma and faeces are similar (Schwarzenberger et al., 1996). Meanwhile, the faecal estrogens in relation to reproductive status in mare were demonstrated by Bamberg et al. (1984); they were also demonstrated in cows (Mostle et al., 1984), in buffaloes (Ismail et al., 1987), and also in primates (Heisterman et al., 1993).

Determination of the preovulatory faecal estrogens peak proved to be less successful as compared to pregnancy
determination in mares (Sist et al., 1987 and Barkhuff et al., 1993). The faecal progesterone metabolites consist of several 5α-, 5β-pregnances and progestagens 20α-G (Schwarzenberger et al., 1992, 1993, 1996 and Messina et al., 1998). They reported also that the faecal progesterone metabolites in the mare belong to the 5β-pregnances progestagens. However, the faecal 20α-hydroxy-progesterone concentration can be used to evaluate cyclic activity in mares (Messina et al., 1998). 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 E1-17β) and faeces (20α-G and E1-17β) 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. All the mares at the beginning of the experiments were non-pregnant.

METHODS

Heat detection was done by day after day teasing with fertil stallion. The ovarian changes during oestrus period were detected by rectal palpation and transrectal ultrasonographic examination. All mares were naturally bred every second day from the detection of growing follicles (>20 mm in diameter) until ovulation during oestrus (Camillo et al., 1997). Pregnancy was diagnosed using transrectal ultrasound scanning of the uterus at day 14–15 post ovulation (Rigby et al., 1999), and confirmed at 21st up to 45th day later (Ginther, 1995). The ovarian and uterine scanning were done using Pie-Medical Vet-200 ultrasound with transducer 5 and 7.5 MHz (Mitsubishi 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 (n=2), day –6 (n=3), day –5 (n=4), day –4 (n=5), day –3 (n=6), day –2 (n=8), day –1 (n=8) until ovulation that represented by day 0 (n=8). All the samples were collected daily for 7 days later (post ovulation) from all mares (each group=8 mares). Samples were collected from all mares on days 14 (n=8), 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, then blood samples were centrifuged at 3000 rpm20 minutes. The harvested plasma were stored in portions at -20°C until hormonal analysis. The faecal samples (20 gm) were collected from all mares and extracted according to Wasser et al. (1988) and Schwarzenberger et al. (1991), 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 according to Dobson (1974) and Naber et al. (1999), and faecal progestagens was assayed according to Schwarzenberger et al. (1996) and Messina et al. (1998). 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 (range=0.03 to 0.16 ng/ml). The intra- and inter-assay coefficients of variation were 9.0 and 9.3% respectively. While, estradiol-17β in plasma was assayed according to Abraham (1977) and Xing et al. (1983). Estradiol-17β 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 E1-17β was performed according to Heisterman et al. (1993) and Schwarzenberger et al. (1996).

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 E1-17β, also the faecal 20α-G and E1-17β levels during the pre and post ovulatory period are shown in Table 1 and Figure 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 E1-17β, starting from day –7 (0.98±0.19 ng/ml and 24.00±2.00
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pg/ml, respectively). The minimum level of P4 (0.18±0.05 ng/ml), and the maximum level of E1-17β (78.75±4.20 pg/ml) were detected at 0-day of ovulation. A similar trend was observed for the faecal 20α-G content that show decrease in its value starting from day –7 (196.85±16.67 ng/gm) reaching its minimum level at second day post ovulation (82.67±7.29 ng/gm). Meanwhile, the faecal E1-17β 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 E1-17β showed a continuous increase, while the faecal 20α-G and E1-17β showed a continuous decrease in their profiles. Meanwhile, the concentrations in plasma P4 and faecal 20α-G increased starting from the 3rd day post ovulation, while plasma and faecal E1-17β decreased starting from the 2nd day post ovulation.

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

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

<table>
<thead>
<tr>
<th>Day estimated before ovulation</th>
<th>Full cycle size (min)</th>
<th>Plasma P4 (ng/ml)</th>
<th>Faecal 20α-G (ng/gm)</th>
<th>Plasma E1-17β (pg/ml)</th>
<th>Faecal E1-17β (pg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -7</td>
<td>16:00±0:01 f</td>
<td>0.98±0.19 bc</td>
<td>196.04±4.61 dcd</td>
<td>16.04±3.50 f</td>
<td>120.03±6.05 dcd</td>
</tr>
<tr>
<td>Day -6</td>
<td>10:13±0:13 c</td>
<td>0.61±0.35 cde</td>
<td>150.78±4.74 cde</td>
<td>27.66±4.30 f</td>
<td>130.96±5.34 d</td>
</tr>
<tr>
<td>Day -5</td>
<td>21:31±0:08 de</td>
<td>0.76±0.15 cde</td>
<td>169.46±4.64 dcd</td>
<td>32.54±4.79 f</td>
<td>138.75±6.66 d</td>
</tr>
<tr>
<td>Day -4</td>
<td>22:04±0:9 c</td>
<td>0.62±0.33 dcd</td>
<td>152.14±4.57 d</td>
<td>27.06±4.21 cd</td>
<td>142.56±5.20 de</td>
</tr>
<tr>
<td>Day -3</td>
<td>15:30±0:33 b</td>
<td>0.48±0.29 cde</td>
<td>130.04±8.76 fgh</td>
<td>37.64±4.05 cde</td>
<td>135.87±7.82 d</td>
</tr>
<tr>
<td>Day -2</td>
<td>13:45±0:48 a</td>
<td>0.54±0.30 d</td>
<td>166.62±4.67 f</td>
<td>44.74±4.17 c</td>
<td>166.78±6.95 b</td>
</tr>
<tr>
<td>Day -1</td>
<td>11:00±0:13</td>
<td>0.63±0.30 g</td>
<td>132.56±7.22 h</td>
<td>52.56±3.69 b</td>
<td>155.64±6.22 c</td>
</tr>
<tr>
<td>Day 0</td>
<td>10:12±0:04 a</td>
<td>0.50±0.25 d</td>
<td>132.34±7.18 f</td>
<td>79.15±6.20 a</td>
<td>181.07±6.39 a</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.53±0.14 d</td>
<td>119.56±7.34 f</td>
<td>40.26±5.45 c</td>
<td>197.30±7.27 a</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>1.16±0.55 h</td>
<td>87.67±27.93 f</td>
<td>39.52±6.29 d</td>
<td>142.58±7.83 b</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>1.62±0.44 h</td>
<td>136.14±3.0 f</td>
<td>35.37±4.13 c</td>
<td>122.54±3.99 g</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>2.17±0.59 h</td>
<td>199.21±6.05 c</td>
<td>32.02±4.74 d</td>
<td>108.92±5.93 b</td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>2.56±0.60 h</td>
<td>237.45±4.65 b</td>
<td>31.14±4.33 c</td>
<td>105.96±4.09 m</td>
<td></td>
</tr>
<tr>
<td>Day 6</td>
<td>2.95±0.73 h</td>
<td>265.39±4.39 m</td>
<td>39.14±6.37 c</td>
<td>96.03±3.87 j</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>3.48±0.88 h</td>
<td>240.47±5.32 d</td>
<td>56.37±4.86 g</td>
<td>93.97±5.03 l</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The hormonal profile is a reliable clinical investigation method of oestrus and pregnancy detection using analysis of progesterone and estradiol-17β in mares (Schwarzenberger et al., 1992). Meanwhile, the analysis of steroid hormones in plasma and faecal samples offer the potential of addressing...
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many timely, integrative problems in reproduction and conservation biology (Wasser et al., 1991). 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 (Schwarzenberger et al., 1992, 1996). 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 E1-17β, starting from day –7. The minimum and maximum levels of P4 and E1-17β reached at day of ovulation, respectively. Similarly, faecal 20α-G content showed a decrease in its value starting from day –7 reaching its minimum level at 2nd day post ovulation, meanwhile, the faecal E1-17β content was reaching its maximum value on day 1 after ovulation. Following ovulation, the plasma P4 and faecal 20α-G levels showed a continuous increase (starting from the 3rd day post ovulation), and decrease in the profiles of plasma and faecal E1-17β (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 E1-17β and decrease in the concentration of plasma P4 (Illera et al., 1993). However, production of estrogen by the large follicles is consistent with the oestrous-like uterine echotexture which seemed approximately related to the growing phase of large follicle (Koskinen et al., 1990). A high relation between the ultrasonography findings and hormonal concentration, showing the increase of E1-17β and the decrease of P4 concentration, corresponding to the days of the oestrous cycle at which the experiments were performed (Pieron and Ginther, 1985 and Townson and Ginther, 1987). In addition, the incidence of diestrous ovulations in mares is considerably higher (Stabenfeldt et al., 1972), 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 E1-17β extended up to the second day post ovulation. Results from faecal hormone analysis indicated a useful characterizing and retrospectively predicting oestrous cyclicity and the occurrence of ovulation. Furthermore, cyclicity and ovulation were also confirmed by the rise and fall of the progestagens and E1-17β excretion during the pre- and post-ovulatory periods (Wasser et al., 1991).

For the study of ovarian activity in mares, several investigators have measured the concentration of P4 in blood (Allen and Porter, 1987, Eckersall and Harvey, 1987, Elmore et al., 1988). There is agreement that concentrations below 1 ng/ml plasma (Ginther, 1979) 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 E1-17β concentrations were similar to those found by others in the late luteal and follicular phases of the oestrous cycle of the mare (Plotka et al., 1972, Eckersall and Harvey, 1987, Elmore et al., 1988). 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 (Adlercreutz et al., 1979). 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 (Schwarzenberger et al., 1996), but they have also shown that a small proportion of the circulating steroids is secreted through the mucosa of the large intestine (Shill et al., 1990). Furthermore, steroids might be unevenly distributed in the faecal balls of horses (Palme et al., 1996).

The levels of P4 in plasma and 20α-G in faeces was significantly increased at days 14 up to 45 of gestation than those recorded during at ovulation in non-pregnant mares. Meanwhile, the levels of E1-17β in both plasma and faeces was increased at days 21 up to 45 of gestation than those estimated during 14th day of gestation as well as in non-pregnant mares. 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 (Schwarzenberger et al., 1996). 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 (Sevinga et al., 1999). During the oestrous cycle and pregnancy, P4 is produced by corpus luteum and its
metabolites circulated in the peripheral plasma and may be excreted via faeces (Desaulniers, 1989), 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 (Schwarzenberger et al., 1993). 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 (Irvine et al., 1990). Similar to our findings, faecal progestagen analysis has been successfully used for monitoring corpus luteum function and pregnancy (Schwarzenberger et al., 1991, 1992, Heistermann et al., 1993, Wasser et al., 1994). 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 (Sist et al., 1987, Barkhoff et al., 1993). 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 (Ginther et al., 1989).

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 (Schwarzenberger et al., 1992, 1993). Subsequently, the differences between the concentrations of both P4 and E1-17β 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 fundamental reproductive endocrinology and provide information regarding the oestrus cycle and early pregnancy. Meanwhile, the E1-17β 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.

References


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