Stress and its effects on horses reproduction

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Abstract

A total of 90 mares and horses were subjected to blood sampling for determining the effect of management (farm), reproductive condition, sex, age, breed and month of the year during breeding on circulating levels of cortisol and sex hormones. Blood samples were collected from December to the following June from four farms. Blood sera underwent testosterone, estradiol, progesterone and cortisol assay using ELISA kits. Cortisol levels were significantly low in lactating mares during their foal heat but significantly high levels were recorded in both repeat breeder mares and horses used for racing. High and significant testosterone and estradiol levels were recorded in both stallions used for breeding especially after semen collection and early pregnant mares. Similar testosterone levels were recorded in both early pregnant mares and racing horses but high levels were recorded in stallions. Estradiol was high in both early pregnant and mares with endometritis but the highest levels were observed in stallions. Horses held in private farms had high cortisol levels compared to those of governmental farms. In contrast to mares, horses had low cortisol and high estradiol levels. Cortisol levels were high from April to June (Spring and early summer) compared to its levels from December to March (Winter). Arab horses had low cortisol compared to native and imported foreign breeds. In conclusion, environmental condition, exercise, breed, management and the purpose of raising horses all are affecting its cortisol levels.

Introduction

Interactions between the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axis are numerous, however, glucocorticoids have frequently been indicated as one major factor mediating the suppressive effects of stress on reproductive functions. In the horse, the dominant glucocorticoid in plasma is cortisol.1 Not only, glucocorticoids suppress reproductive functions at the hypothalamic, pituitary, ovarian and also uterine level in mares2 but also in women,3 rodents,4 sheep5 and cattle.6 Therefore, stressful events may contribute to low reproductive efficiency due to glucocorticoid mediated inhibition of reproduction in a variety of species.

Stress has been defined as any event resulting in increased activity of the hypothalamic-pituitary-adrenal (HPA) axis.7 The end result of HPA axis activation is a rise in plasma corticosteroid concentrations. Accordingly, plasma corticosteroids are often monitored experimentally to gauge an animal’s perception of the stressfulness of various situations and clinically to evaluate the function of the axis. Stress and increased body temperature induced by racing may have negative effects on fertility. On the other hand, high quality nutrition and management of racing horses may have positive effects.8

In equine breeding, the mare is not only exposed to repeated restraint and gynaecological examinations, but also to other stressful events such as transportation to the breeding farm and loss of the normal environment and social companions; all together these events may cause increased secretion of glucocorticoids and cortisol. Exercise,9-11 transportation,12 pain,13 social stress,14 acute exercise,15 and sexual activity16,17 increased cortisol in horses. A potential indicator of animal welfare is the absence of stress, but to date there is no standard definition of stress and no single biochemical assay system to measure stress.18 Repeated administration of dexamethasone to mares in oestrus is associated with decreased uterine oedema, suppression of LH and a high rate of ovulation failure.19

Egypt holds different horse breeds pure Arabian, several European breeds and native breeds. Arabian horses are used for leisure, dancing and breeding. Most European and other imported breeds are used for riding, sports, training and few could be used for breeding. Native horses are mainly used for work, training and breeding. Horses are held in separate paddocks on private and governmental farms but management differs from farm to another and also according to the value of the horse or mare and purpose of raising it. Studies on effects of stress on reproduction are deficient in Egypt. To create a database for steroid reference values in horses and to assist in physiological characterization and diagnosis of endocrine disorders in horses, this study aims at investigating the circulating levels of cortisol as a marker of stress and sex hormones in relation to management (farm), age, gender, breed, month and reproductive status in horses.

Materials and Methods

Animals

A total of 90 horses (1-12 years old) were used in the present study. They were divided into groups according to sex, purpose of rearing and reproductive condition into females (n=63) and males (n=27). Females included fillies (n=16) ≤2 years old, normal cycling mares (n=16), sub-clinical endometritis (repeat breeders, n=21), early pregnant (n=5) ≤45 days pregnant and lactating during the foal heat (n=5). Males included colts<2 years old (n=4), stallions of age 5-12 years old (n=16) and castrated horses used for racing (n=7). Mares with repeat breeder history (n=21) were subjected to ordinary microbial examination to identify the causative microorganisms. Others were apparently healthy. Four horse-holding farms were used for collecting blood samples. The first two farms are governmental and the other two are private.

Sampling protocol

Blood samples were drawn from the jugular vein from all groups between 10:00 and 11:00 a.m. to minimize the effect of circadian rhythm on hormone measurements. Additional samples of the racer group were collected shortly after race to exclude doping in fresh plain vial for sera harvesting. Sampling was performed in mares after gynecological examination for detection of mature follicle or early pregnancy and in stallions after semen collection.
Hormone assaying

Serum cortisol,20 progesterone,21 estradiol22 and total testosterone23 concentrations were analyzed using a commercially available Enzyme immunoassay kit supplied by Medical Biological Service S.r.l. (Milano, Italy). Sensitivity of the assay was 0.4 μg/dL, intra- and inter-assay CV were 2.9% and 3.8%, respectively for cortisol; 10 pg/mL, 9.1% and 9.8% for estradiol; 0.1 ng/mL, 10.6% and 12.6% for progesterone; 0.022 ng/mL, 6.6% and 7.3% for testosterone.

Statistical analysis

The effect of reproductive condition, gender, age and management (farm) in addition to month of sampling was studied using simple one way ANOVA and the statistical significance between means was compared using Duncan multiple range test; (P<0.05) was considered significant. Student t-test was also performed to study the effect of gender on cortisol and sex hormones. All data are presented as means±standard error (SE) of the means. Pearson correlation coefficients were also performed between the assayed hormones in both sexes. All tests were performed using computer package of the statistical analysis system SPSS.24

Results

Gender effect

In contrast to the insignificantly high cortisol in females compared to males, the reverse is true in estradiol levels. Testosterone levels are significantly (P=0.038) low in females (Table 1) compared to males.

In females, correlation made between cortisol and testosterone is positive and significant (r=0.3; P=0.039) but that with estradiol is highly significant strong and positive (r=0.42; P=0.009). The correlation between progesterone and estradiol is more strong and highly significant (r=0.55; P=0.001) than that with testosterone (r=0.36; P=0.011). Similarly, strong positive and highly significant correlation is found between estradiol and testosterone (r=0.47; P=0.008). In males, cortisol has a high negative but not significant correlation with estradiol (r=-0.47; P=0.064). Estradiol is positively correlated with testosterone but correlation even high is not significant (r=0.38; P=0.28).

Breed effect

The Arabian horses have significantly low cortisol, testosterone and progesterone levels (Table 1) compared to both Foreign and native. Native horses and mares have significant low estradiol compared to Arabians and foreign ones.

Farm effect

Mares in farm 1 have significant low cortisol levels compared to the other three farms (Table 1).

Reproductive condition

Cortisol levels are significantly low in lactating mares during early postpartum (foal heat) but its levels are high in stallions (Table 2) and early pregnant mares follow by colts, fillies and cyclic mature mares. Significant high levels of cortisol are found in only repeat breeder mares due to sub-clinical endometritis and also race horses. Testosterone is significantly low in fillies and high in cyclic and early pregnant mares. Similar testosterone levels are recorded in repeat breeder and early pregnant mares. Estradiol levels are significantly in both early pregnant mares within the female group and stallions within the male group. Low estradiol levels are recorded in both colts and fillies (Table 2).

Month

Cortisol increases in its level from December (winter) to June (late spring) and this increase is accompanied by an increase in both testosterone and estradiol (Table 3).

Discussion

The effect of racing on males’ cortisol levels just after race is obvious in the present work. Although this increase was not correlated with any sharp alteration in both testosterone and estradiol levels compared to stallions used for breeding only. Similarly, twice cortisol levels were found after the country round compared to its levels before the country round.25 On the other hand, during competitions, experienced jumpers have significantly lower concentration of plasma cortisol than inexperienced

Table 1. Effect of sex, breed and farm management on levels of cortisol, testosterone, estradiol and progesterone.

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>Cortisol</th>
<th>Testosterone</th>
<th>Estradiol</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arab</td>
<td>45</td>
<td>14.81±1.22</td>
<td>2.68±32</td>
<td>370.18±2.04</td>
<td>3.55±0.34</td>
</tr>
<tr>
<td>Foreign</td>
<td>20</td>
<td>23.67±1.37</td>
<td>4.79±50</td>
<td>322.62±21.57</td>
<td>5.16±1.05</td>
</tr>
<tr>
<td>Native</td>
<td>25</td>
<td>22.93±1.49</td>
<td>3.77±40</td>
<td>267.93±23.59</td>
<td>4.54±0.57</td>
</tr>
<tr>
<td>Farm</td>
<td></td>
<td>0.001</td>
<td>0.002</td>
<td>0.13</td>
<td>0.0001</td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>16.93±1.73</td>
<td>4.69±42</td>
<td>302.59±29.35</td>
<td>5.6450.86</td>
</tr>
<tr>
<td>Female</td>
<td>63</td>
<td>18.96±1.05</td>
<td>2.98±34</td>
<td>313.8±16.65</td>
<td>5.6450.86</td>
</tr>
</tbody>
</table>

Table 2. Effect of reproductive condition, gender and age on mean levels of cortisol, testosterone, progesterone and estradiol.

<table>
<thead>
<tr>
<th>Age /year</th>
<th>Fillies</th>
<th>Cyclic</th>
<th>Early pregnant</th>
<th>Subclinical endomet</th>
<th>Lactating</th>
<th>Colts</th>
<th>Stallions</th>
<th>Racers</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2.5</td>
<td>16</td>
<td>16</td>
<td>5</td>
<td>21</td>
<td>5</td>
<td>4</td>
<td>16</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol</td>
<td>21.12±1.40</td>
<td>14.32±1.61</td>
<td>17.97±1.99</td>
<td>26.64±1.06</td>
<td>6.56±1.68</td>
<td>18.49±3.07</td>
<td>13.21±1.94</td>
<td>24.65±3.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>Testosterone</td>
<td>1.38±0.29</td>
<td>2.91±0.65</td>
<td>4.58±2.09</td>
<td>4.55±0.89</td>
<td>2.87±1.12</td>
<td>3.28±1.37</td>
<td>5.66±0.74</td>
<td>4.72±1.31</td>
<td>0.0001</td>
</tr>
<tr>
<td>Estradiol</td>
<td>241.22±15.03</td>
<td>305.76±26.24</td>
<td>433.19±71.68</td>
<td>410.79±28.10</td>
<td>318.84±16.65</td>
<td>271.72±69.95</td>
<td>473.73±7.76</td>
<td>302.59±33.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>Progesterone</td>
<td>2.4±0.58</td>
<td>4.64±1.03</td>
<td>5.89±1.07</td>
<td>6.3±0.84</td>
<td>1.62±0.33</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
jumper horses, suggesting that horses become conditioned to the psychological stress of the show environment. So stallions’ fertility did not suffer from racing during the mating year. In stallions of the present investigation, cortisol levels was similar to that of normal cyclic mares but was lower than those horses used for racing and even colts, so sexual excitement in stallions had low effect on cortisol levels compared to racing or age. In contrast, cortisol is secreted in response to sexual stimulation, exercise, and twitching but in geldings, cortisol is released in response to exercise, and twitching.

Although race increasing cortisol levels compared to stallions after semen collection or natural breeding but testosterone levels were higher in stallions than in race horses. This may referred to that race horses are subjected to multiple stressors such as transport to the race area, the race itself and the presence of large numbers of people around horses before and after race for checking, grooming and dressing. The decrease in testosterone levels in race horses compared to stallions may referred to the use of castrated horses for racing to facilitate controlling them and this testosterone originated from their adrenals. Moreover, chronic stress decreases testosterone production but acute stressors have a much less predictable influence on testosterone concentrations, with authors reporting no changes, decreases or even increases in testosterone. The manner in which an organism reacts to stress is not only influenced by the type, duration and intensity of the stressor; it also depends on the presence of circulating sexual steroid hormones.

The higher cortisol concentrations in both fillies and colts (younger horses) of the present study compared to mature normal cycling mares and stallions agree with Fazio et al., who reported that growth affected cortisol values and cortisol concentrations is positively correlated with body weight in both colts (r=0.80; P=0.001) and fillies (r=-0.82; P=0.001) during growth from birth to weaning. Growth and weaning could modulate the ovarian system and pituitary-adrenocortical axis of Thoroughbred foals. High cortisol levels have been observed just before birth and in the first 2 h after birth of the term foals.

In agreement with Escrivanho et al., gender has no significant effect on cortisol levels either at rest or in response to exercise. In contrast to Fazio et al., females had insignificant high cortisol than males whatever they were fillies or mature cycling mares or both and that most fillies and colts of the present work are older than Fazio’s study.

In this study, repeat breeder mares underwent several gynecological examinations before breeding to access size of mature follicles and after natural breeding to detect early pregnancy using ultrasound and sometimes twitching is used in addition to moving the animal to the examination spot and even detection of infection all these factors may increase cortisol levels to values near to those of horses after race. Although gynaecological examinations in the mare seem to act as stressors and increase cortisol secretion but this was not negatively influence fertility and in animals familiar with that procedure concentrations are not elevated. However, long-term exposure as in teaching or research mares result in acclimatization. In contrast to cattle and sheep, no effects of cortisol on fertility parameters (oestrus duration, pregnancy rates) could be found in the horse.

Season of breeding mares in Egypt starts from October till the end of the following May in most governmental farm and private farms to avoid the adverse environmental conditions and the absence of the green palatable Egyptian clover Barsem (Trifolium Alexandrinum) during hot summer. Whatever the animals are adapted to the hot summer temperature and increased humidity, the increase in cortisol levels during May and June may be attributed to the increase in heat stress where environmental temperature exceeds 30ºC. As well as ambient temperatures higher than 30ºC increase cortisol levels.

Early pregnant mares in this study were having high testosterone and estradiol compared to normal cyclic and lactating mares during their foal heat but such increase has been ascribed to equine chorionic gonadotropin (eCG) stimulation of luteal testosterone synthesis. Moreover, testosterone levels were high at estrus in mares peripheral plasma and 11-13 days before the next estrus either before or after the fall in progesterone levels. Testosterone was also found to increase from the very beginning of pregnancy, reaching peak values 10 times higher than the basal values at the seventh month and then return to basal values by the week after parturition. Testosterone binding by plasma proteins was investigated in non-pregnant and pregnant mares throughout gestation. Maternal gonads would be responsible for the testosterone increase in early pregnancy, during the period of pregnant mare serum gonadotropin production, and the fetal-placental unit for the subsequent increase.

The presence of testosterone in peripheral blood of cyclic normal and repeat breeder mares due to sub-clinical endometritis of the present study is also recorded in mare follicular fluid at a concentration, which is double higher than that in peripheral plasma, suggesting that the follicle may contribute to the production of circulating testosterone. A biosynthetic pathway for estradiol-17 beta which involves testosterone is therefore likely to occur in the mare ovary. In domestic mongolian horses, cortisol (98.70%) is the predominant steroid, followed by aldosterone (0.37%), androstenedione (0.35%), 17-OH-progesterone (0.21%), estradiol (0.17%), progesterone (0.14%), and testosterone (0.06%).

In conclusion, the change in horse management from farm to different breed, month, infection and racing increases stress and in turn the most common physiological response to stress is an increase in circulating cortisol levels.

### Table 3. Effect of month during the breeding on mean levels of cortisol, testosterone, progesterone and estradiol.

<table>
<thead>
<tr>
<th>Month</th>
<th>Cortisol (µg/dL)</th>
<th>Testosterone (ng/dL)</th>
<th>E2 (ng/ml)</th>
<th>P4 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>December</td>
<td>9.52±4.63ab</td>
<td>4.51±0.375ab</td>
<td>287.56±15.65ab</td>
<td>3.51±0.19ab</td>
</tr>
<tr>
<td>January</td>
<td>15.04±5.05ab</td>
<td>3.20±0.48a</td>
<td>217.73±51.14a</td>
<td>5.47±2.72a</td>
</tr>
<tr>
<td>February</td>
<td>16.57±2.28ab</td>
<td>2.05±0.41a</td>
<td>285.25±33.81a</td>
<td>4.01±0.90a</td>
</tr>
<tr>
<td>March</td>
<td>17.99±1.12ab</td>
<td>3.23±0.50b</td>
<td>327.37±17.06a</td>
<td>5.12±0.83a</td>
</tr>
<tr>
<td>April</td>
<td>25.88±2.15a</td>
<td>4.97±0.54ab</td>
<td>431.58±43.56a</td>
<td>9.15±1.30a</td>
</tr>
<tr>
<td>May</td>
<td>26.79±1.66b</td>
<td>5.46±1.04b</td>
<td>328.00±14.23b</td>
<td>4.66±0.92a</td>
</tr>
<tr>
<td>June</td>
<td>27.05±4.95b</td>
<td>5.52±0.53b</td>
<td>484.73±21.14b</td>
<td>6.39±0.67ab</td>
</tr>
</tbody>
</table>

*P* Value 0.001 0.051 0.029 0.015

*Means with different superscripts are significantly different at *P*<0.05.

References