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A study of some hormones concentrations in horses: Influences of reproductive status and breed differences

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ABSTRACT

Objective: To learn more about reproductive physiology of adult female Arabian horses over a period of 24 months and to examine the effect of breed's difference between Arabian and European female horses over a period of 36 months on the circulatory levels of both metabolic hormones (IGF-1 and leptin). **Methods:** Thirty female Arabian mares and 22 European non pregnant brood mares exported from Swedish and Germany of ages from 3 to 7 years belonging to Mubarak Police Academy (Abaseia horse farm) was used. Rectal ultrasonography was conducted in Arabian horses to monitor ovarian activity which classified into cyclic ovarian activity, no ovarian activity, and ovarian tumor, pregnancy and postpartum mares were also included in this study. Blood samples from these mares were collected and analyzed for progesterone and Leptin, IGF-1 and Nitric oxide (NO). In the same time, blood samples were collected from Arabian and foreign breeds for IGF-1 and leptin analysis **Results:** There are significant increase in the IGF-1 (778.1±15.7 ng/mL and NO concentrations (39.83±9.15 μM/mL) in case of ovarian tumor. Significant decrease in leptin concentration was recorded (0.61±0.31 ng/mL) in case of postpartum cases. Inactive ovaries mare and pregnant one recorded significant increase in progesterone levels (10.1±1.46 and 22.6±2.0 ng/mL, respectively). On other hand Leptin recorded significant decrease in Arabian horses than European horses (0.86± 0.14 *vs*. 1.73±1.34), while IGF-1 have no significant change between two breeds. **Conclusion:** The knowledge of the normal and abnormal metabolic and sex hormones concentrations will help us to understand the role of these hormones in reproductive physiological and additionally, potential diagnostic and prognostic uses in both human and veterinary medicine, and will provide information for further research on this equine breeds as well as in human diseases.

1. Introduction

Recently, it was established that the horse is an animal model for human research[1]. Mares are considered seasonally polyestrous, where onset of the breeding season occurs in spring and is associated with increase in daylight, temperature, and availability of nutrients. Mares develop major ovulatory follicular waves and major and minor anovulatory waves during an estrous cycle[2, 3]. In major follicular waves, deviation occurs with development of a dominant follicle. The development of follicle dominance in monovular farm animals (cattle, mares) is highlighted by diameter deviation[4]. Deviation begins at the end of a common growth phase for the follicles of the wave and is characterized

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by continued growth of the developing dominant follicle and regression of the subordinate follicles. In mares, the end of the follicle has a mean diameter of 22.5 mm[4]. Leptin, IGF-1, progesterone and NO have assumed an important functional role in a variety of physiological reproductive conditions. Interest in the role of leptin in reproduction was initiated after demonstration that infertile ob/ob mutant mice, which lack the ability to produce leptin [5, 6]. Leptin has emerged as a neuroendocrine mediator in several systems, including the reproductive axis. Leptin provides information to the brain on energy status and may serve as a signal to the reproductive axis indicating a nutritional status adequate for the onset of cyclicity[7]. An apparent association between leptin and reproductive activity in horses was inferred from the observation that in mature mares, higher amounts of body fat were associated with high circulating concentrations of leptin during the summer and autumn months when mares were reproductively active[8]. At the same point, IGF-1 plays a role in ovarian follicular growth[9]. It has a direct regulatory effect on GnRH neurons. IGF- can also regulate the hypothalamic-pituitary - gonadal axis via action at pituitary and gonadal levels of this axis[10].

In last decade, nitric oxide (NO) was first recognized in the reproductive system by Igonarroo LJ[11] which has assumed an important functional role in a variety of physiological systems and different pathways. Therefore it is consider as a polyvalent molecule play a decisive role in the reproductive system[12]. The objective of this study was concern to understand the role of these hormones in reproductive physiological and pathological situations which will provide information for further research on this equine breeds as well as in humans.

2. Materials and methods

2.1. Experimental design

A total of 30 female Arabian mares and 22 European non pregnant brood mares exported from Swedish and Germany of ages from 3 to 7 years belonging to Mubarak Police Academy (Abaseia horse farm) were selected for performing this experiment over a period of 36 months. These animals were always kept under regular veterinary observation and fed Egyptian clover barseem (Trifolium alexandrinum) hay beside concentrated ration that was formulated to meet energy requirement. Water was provided ad. libitum.

Arabian mares sub were classified according to reproductive conditions during two reproductive seasons (during 24 months) into cyclic (n=17), inactive ovary (n=15), pregnant (n= 20), postpartum (n= 5) and repeated cyclic suffer from ovarian tumor (n= 3). This classification was performed after gynecological examination using ultrasonography (US) with a NOVEK scanner (Germany) equipped with an endo-rectal linear array B-mode real time multi-frequency 2.6- 7.5 MHz transducer. On the other hand, during non breeding season (3 years) both Arabian and European female horses examined for the effect of breed differences on both IGF-1 and Leptin metabolic hormones.

2.2. Blood sample collection

Blood samples were collected from Arabian horses during breeding season for 2 years after reproductive sub classification. In the same time, monthly and during non-breeding season for 3 years, blood samples were drawn from all European and Arabian animals via jugular vein after aseptic preparation and desensitization (lidocaine 2%) using venipuncture and were placed on ice until centrifugation (3 000 × g for 10 min at 4 ℃). Serum samples were harvested and frozen at -2 ℃ until hormonal assay.

2.3. Hormonal assay

Leptin was analyzed by use of a previously validated multi-species leptin radioimmunoassay (Linco Research Inc., St. Charles, MO) and insulin like growth factor-1 (IGF-1; BioSource Europe S. A. Belgium) were estimated by radioimmunoassay (RIA). The limit sensitivity, intra- and inter-assay coefficients of variation (CV) were 3.4 ng/mL, 1.9% and 4.1% for IGF-1 and were 1.0 ng/mL, 2.8% and 8% for leptin. Progesterone was determined by RIA procedure using commercial kits supplied by Diagnostic Laboratories USA[13]. Intra- and inter-assay coefficients of variation were 4.9 and 6.1%.Sensitivity of the assay was 0.02 ng/mL. All analyses were performed in duplicate.

2.4. Nitric oxide estimation

Nitric oxide (NO) was also assayed using ELISA. For measuring serum nitrite according to Rajaraman[14], 100 μL of serum samples were mixed with an equal volume of freshly prepared Greiss reagent, incubated for 10 min at room temperature and absorbency measured at 570 nm using a micro titer plate reader. The nitrite level in serum samples was calculated by comparing the optical density against the nitrite standard curve of sodium nitrite in distilled water.

2.5. Statistical analysis

All data were studied using simple one way ANOVA and the statically significance between means were compared using Duncan multiple range test. Levels of significance P<0.05 and P> 0.01 were chosen to identify the significance. All data are presented as mean± standard error (SE).The statistical analysis was performed using software computer programme of Statistical Package for Social Science (SPSS) version 16.0[15].

3. Results

3.1. Hormonal analysis in different reproductive conditions in Arabian horse

By detection of different hormonal analysis and nitric oxide in different reproductive conditions of Arabian horses during breeding season, we noticed that there are significant increase of IGF-1 and NO in ovarian tumor horses comparing with different other reproductive conditions. On the same time, Leptin recorded significant decreasing in postpartum condition comparing with other reproductive conditions. Progesterone significantly increased in both inactive ovary mares and those are pregnant in other reproductive conditions.

Table 1

Influence of different reproductive status on some hormonal and nitric oxide levels in female Arabian horses.

Reproductive phases	Analyzed metabolites			
	IGF-1 (ng/mL)	Leptin (ng/mL)	Nitric oxide (μ M/mL)	Progesterone (ng/mL)
Cyclic(n=17)	430.8±38.6[a]	2.19±0.81[a]	14.10±2.83[a]	4.50±1.10[a]
Inactive (n=15)	312.3±17.7[a]	1.23±0.51[a]	20.72±5.72[a]	10.10±1.46[b]
Pregnant (n=20)	304.1±24.1[a]	1.94±0.62[a]	19.79±2.91[a]	22.60±2.00[b]
Postpartum(n=5)	476.3±67.0[a]	0.61±0.31[b]	14.77±5.05[a]	2.10±0.90[a]
Ovarian tumour (n=3)	778.1±15.7[b]	1.65±0.23[a]	39.83±9.15[b]	4.40±3.79[a]

Values are mean ± SE, values within a column with different small superscripts are significantly different (P< 0.01).

3.2. Influence of horse breed differences on IGF-1 and leptin concentrations

The Arabian horses have significantly lower leptin concentration comparing with that of European horses, while no significant result was recorded in case of IGF-1.

Table 2

Influence of breed differences on serum IGF-1 and leptin concentrations (ng/mL).

Horse breeds	Metabolic hormones	
	IGF-1	Leptin
Arabian	382.50±19.6[a]	0.86±0.14[b]
Foreign	347.56±27.5[a]	1.73±1.34[a]

Values are mean ± SE, Values within a column with different small superscripts are significantly different (P< 0.05).

4. Discussion

4.1. Influence of different reproductive conditions on some hormones and NO levels in female Arabian horses

By estimation of hormonal analysis in different reproductive conditions in Arabian horses, we noticed that there are significant increase in both IGF-1 and NO levels in animals suffering from ovarian tumour in comparison with different other reproductive conditions. Insulin-like growth factor-I (IGF-I) is known to be involved in the development and progression of several types of solid tumors including ovarian cancer. IGF-I levels in local tissue is subject to both endocrine and paracrine/autocrine regulation[16]. IGF-I has well-documented mitogenic and antiapototic activity[17, 18] believed to mediate its effect on neoplastic development. Elevated prediagnostic circulating IGF-I has been associated with increased risks of several cancer types, including cancers of the breast, prostate, and colon[19, 20]. In the ovary, the IGF-I signaling system has a key role in the regulation of the normal function of the organ, including follicular growth, development, steroidogenesis, and atresia [21, 22]. IGF peptides, receptors, binding proteins (BPs), and BP proteases are all expressed in both normal and malignant ovarian epithelial cells[23]. Studies *in vitro* have shown that the overexpression of IGF-I receptor can induce malignant transformation of ovarian epithelial cells[24]. Furthermore, initial evidence suggests that IGF-I receptor could be a potential new molecular target in ovarian cancer treatment[25]. Besides direct effects as a growth factor, it has also been hypothesized that IGF-I could promote ovarian tumor formation by modulating ovarian steroidogenesis[23, 26]. In the same time nitric oxide (NO) was involved in an autocrine/paracrine regulation of the developing follicle and have direct effect on granulosa cells, theca cells or on the developing oocytes. So No must be considered as an important general regulator of the ovulatory process. For that many studies supported that NO may play a role in several disorders associated with the reproductive system[12]. NO is both cytotoxic and cytostatic against microorganisms and malignant cells[27, 28] with synthesis of NO by malignant cells causing NO-mediated apoptosis. As a free radical, NO can react to produce proxy nitrites which can directly and indirectly cause DNA damage[29]. If produced for a long period of time, excess NO production can lead to mutations and ultimately to cancer[30, 31]. In a study by Taveres-Murta *et al*.[32] increased levels of NO metabolites in the tumor microenvironment were found in cases of ovarian cancer. Similarly, supernatants of cell cultures obtained from well-differentiated, malignant ovarian tumors were found to contain higher levels of NO metabolites compared to cell cultures from patients with poorly differentiated tumors[33].

The serum leptin concentration profiles for the mares during postpartum in our study were similar to those reported by others [34, 35]. Serum leptin concentrations were less in the postpartum period. This postpartum reduction in blood serum leptin may be due to a loss of placental leptin because placental leptin mRNA expression has been reported in humans[36, 37] and sheep[38]. However, a postpartum reduction in blood serum leptin concentrations has been reported in humans[36, 37] and Japanese monkeys [39] but not in sheep[38]. This decrease in blood serum leptin may serve as a means to protect mares against negative energy balance by encouraging feed intake in mares during early lactation. Erlanson-Albertson and Zetterstro[40] argue that leptin has more of a permissive role in hunger signaling (low leptin concentrations = stimulus for feed intake) rather than a role as a satiety signal per se (high leptin concentrations = stimulus to decrease feed intake). It is logical that energy signaling during early lactation should be directed toward feed intake in order for mares to maintain good body condition and subsequently an adequate milk supply.

Serum progesterone was significantly increased in both inactive ovaries and during pregnancy. This result is in agreement with that reported by Roth *et al*. [41] and AboEl-maaty[42]. In pregnant mares, chorionic girdle cells invade the maternal endometrium 35-40 days after ovulation and form equine chorionic gonadotrophin (eCG)-secreting endometrial cups[44]. Serum concentrations of eCG reach peak concentrations about 60 days after ovulation. In the same time, progesterone has an important role in maintaining uterine quiescence during pregnancy. It reduces uterine contractility by hyperpolarizing the myometrium and by reducing the number of gap junctions and receptors for contractile agents in the myometrium[44]. In the meantime, other studies recorded the female had small inactive ovaries, increased LH concentrations and baseline progesterone concentrations, which are all characteristics of reproductive senescence [45].

4.2. Influence of breed differences on serum IGF-1 and leptin concentrations

In our study we attempt to clarify some metabolic hormones differences between Arabian and European horses . In horses, few studies have investigated the relationship between serum leptin and breed. Potential breed differences suggest a possible genetic link with leptin. It is a common belief in equine community that certain breeds considered to be "easy keepers" [46]. Previous studies in horses recorded that serum leptin are more likely in combination with the influence of diet, body fat mass, physical exercise, genetic, gender insulin sensitivity and other factors [46,47]. However, it still remains unclear as to whether this difference in leptin concentrations between Arabian and European horses is due direct effect of breed or due to these other factors. In other species as cows Delavaud *et al*. [48] recorded that, the effect of breed tended to have a small effect on plasma leptin level, which was depend on difference in body weight which decreased by 70% in lean compared to fat Holstein cows. So the differences in leptin concentration between breeds depend mainly on their differences in body weight. This finding supported our finding as Arabian horses have lighter body weight than that of heavy European horses. In all we are believing that, further research is needed to identify mechanisms behind the relation between leptin and different horse breeds. On other hand, IGF-1 concentration had non-significant change between Arabian and European breeds in our study. Serum IGF-1 concentrations in horses depend on many factors, such as breed[49], age [50], nutritional status, particularly dietary protein supply and feed deprivation and refeeding[51], exercise, training and fitness level[52] and body weight. To our knowledge, the studies concerning comparative interbreeds are more limited as we referenced before. Ozawa *et al*. [49] compared IGF-1 concentrations in heavy horses (Percheron and Breton breeds), light horses (Thoroughbreds) and ponies (Shetland and Falabella). Despite the positive correlations between body weight and plasma IGF-1 concentrations, the means in heavy horses did not exceed that of light horses or ponies with smaller body weights. These findings are inconsistent with what we find in our study. Arabian horse have lighter body weight than that of European but there is no significant differences between two breeds in IGF-1 concentrations. In the same time, serum IGF-1 concentrations in the Arabian horses in our study were lower than that reported for other breeds, such as Standardbreds[53] and Thoroughbreds [52, 54] and higher than Andalusian horses [50]. For instance, they reported mean IGF-1 concentrations are between 60 and 120 ng/mL for Andalusian horses aged between six and fourteen years. These values were lower than those observed in Arabian horses of the same age (serum IGF-1 concentrations 382.50 ± 19.60 ng/mL) and in European horses (serum IGF-1 concentrations 374.56 ± 27.50 ng/mL). We cannot say with confidence whether these results are down to the use of a different laboratorial method used to measure IGF-1 and/or from the different equine breeds. In all the studies previously mentioned, IGF-1 was separated from its binding proteins, although the procedure varied and therefore, these results should be interpreted with caution.

Conflict of interest statement

We declare that we have no conflict of interest.

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