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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