INTERRELATIONSHIP OF SOME HORMONES BELONGING TO HPG AND ENERGY AXES IN HORSES DURING VARIOUS REPRODUCTIVE STATES

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ABSTRACT

In order to reveal interrelationships of hormones during pregnancy and estrus in animals, some reproductive and metabolic hormones including FSH, inhibin B, prolactin and insulin were measured in blood serum of Mongolian horses with respective immunoassays. Concentrations of blood serum FSH, inhibin B and prolactin in mares during their estrus, early, mid and late pregnancy periods were compared. The comparisons demonstrate concentrations of FSH and inhibin B were inversely proportional especially during estrous cycle of mares. As well, these hormones were also measured in blood serum of stallions with the purpose of assessing their reproductive activity. Concentrations of both hormones were lower in blood serum of stallions, which have harems of less conceived mares or it means higher inhibin concentration results in less reproductive activity. Prolactin was minimal during estrus and early pregnancy, while it increases during late pregnancy, but there was no correlations with above two hormones. Measurements of insulin was helpful to learn whether the animal is suffering from metabolic disorder such as insulin resistance, because last half of pregnancy of mares coincides with the period of fattening of pastured animals. However, main outcome of the present study is that all above hormones might be acting in closer relationships during various reproductive states, despite they belong to different axes of endocrine system, including hypothalamic-pituitary-gonadal (FSH and inhibin) and energy (insulin and prolactin) axes.

KEY WORDS: Mongolian horse, reproductive state, hormones, HPG, energy axis

INTRODUCTION

Various hormones such as FSH, LH, inhibin, prolactin, insulin and many others are associated with various states of reproduction in both humans and animals. Hormones are divided into several endocrine axes according to their modes of actions. For example, there are hypothalamic-pituitary-gonadal axis (HPG), hypothalamic-pituitary-adrenal axis (HPA) and hypothalamic-pituitary-thyroid axis (HPG) according to traditional understanding of hypothalamic-pituitary release of hormones. As well, a novel concept in endocrinology that relates to energy regulation is the gut-brain axis. this axis is involved in the regulation of satiety, food intake, glucose and fat homeostasis, insulin secretion and bone metabolism. A number of hormones such as insulin, glucagon, cortisol, epinephrine, leptin, adiponectin, GH and prolactin, which are involved in energy metabolism, are included in so-called "energy" axis by some authors [3, 11, 13, 15, 27, 31].

A hormone inhibin was studied relatively later and it has been revealed that the hormone is glycoprotein consisting of alfa and beta dimers, which is produced in ovarian granulose cells in females and testicle Sertoli cells in males, its synthesis is stimulated with the increase of FSH in the blood and the hormone inhibits FSH release from pituitary gland [10, 25, 28, 32]. Bergfelt et al [1] measured circulating inihibin during estrus cycle of mares and reported the hormone increases enormously, when ovulation takes place. Hadley [10] mentioned inhibin is synthesized in seminiferous tubule and sertoli cells in male animals and Sertoli cells alone don not decrease FSH concentrations unless spermatogenesis occurs. Also Goudet et al [11] determined concentrations of gonadotropic and inhibin hormones during various stages of follicular development.

Production and secretion of prolactin is elevated during pregnancy, and this hormone has been recognized as a potent growth factor for pancreatic beta-cells [2, 18]. Prolactin metabolism appears to differ between species. For example, studies with human subjects have shown a higher prolactin release after eating in women than in men [4]. In addition, the time at which prolactin peaks after eating appears to differ among species. For instance, prolactin peaks approximately 1 h post-eating in humans [24, 4] but at 6 h in cattle. As well, prolactin concentration is high in stallions during breeding season [8]. Insulin might be partially responsible for this post-prandial hyperprolactinemia [35].

The present study aims to reveal relationships between some circulating hormones, which belong to HPG and energy axes, during various states of reproduction of Mongolian horses.

MATERIALS AND METHODS

Animals and blood sampling

In the study, a total of 38 native Mongolian horses (5-9 years old), kept on open pastures in the areas about 50 km far from Ulaanbaatar city, were selected and mares were classified into estrus, diestrus, early, mid and late pregnant ones according to their reproductive states. The blood samples were collected from jugular vein into vacutainer tubes. Serum samples were harvested and stored at -20^{0}C until assayed.

Hormonal assay

Horse blood serum inhibin B and prolactin concentrations were measured by Inhibin B and Prolactin ELISA test kits (DSL, Webster, Texas, USA) according to the manufacturer's instruction. Intra and inter-assay CVs were 9.3% and 10.6% for inhibin B and 7.9% and 6.8% for prolactin respectively.

Blood serum FSH and insulin concentrations in both stallions and mares were measured by solid phase Equine FSH and Equine Insulin ELISA test kits (Endocrine Technologies, INC, Newark, USA) according to the manufacturers protocol. Intra and inter-assay CVs were 8.6% and 9.3% for FSH and 10.1% and 9.8% for insulin respectively. Pregnancy of mares were checked by solid phase sandwich PMSG-ELISA test kit (DRG Instruments GmbH, Germany). Intra and inter assay CVs for PMSG were 7.9% and 8.6% respectively.

Statistical analysis

Pearson's correlation coefficient was used to examine the relationship between concentrations of the hormones. All data of measurements were calculated by using Student's test. Results with a P value of <0.05 were considered to be statistically significant.

RESULTS

Concentrations of FSH and inhibin B of HPG or reproductive axis, and prolactin and insulin of energy axis were measured in blood serum of Mongolian horses with respective immunoassays. As well, these hormones were also measured in blood serum of stallions with the purpose of assessing their reproductive activity. Concentrations of blood serum FSH, inhibin B and prolactin in mares during various reproductive states such as estrus, diestrus, early, mid and late pregnancy periods were compared.

Table 1

Comparison of blood serum concentrations of FSH, inhibin B and prolactin in mares during reproductive states

Reproductive states	FSH, ng/ml	Inhibin B, pg/ml	Prolactin, ng/ml	Insulin,µIU/ml
Estrus	19.6±1.1	148.05±12.4	6.3±0.4	3.89±1.03
Diestrus	4.45±0.5	486.1±35.3	5.16±0.3	3.63±1.26
Early pregnancy	6.45±0.5	354.1±37.7	4.25±0.17	5.75±1.42
Mid pregnancy	10.2±1.5	234.9±38.9	4.3±0.2	25.16±2.23
Late pregnancy	13.6±2.1	364.9±38.9	9.3±0.5	12.36±1.59

Table 1 shows higher FSH and lower inhibin B concentration were found during estrus of mares, while FSH decreases and inhibin B increases after more than 40 days after covering by stallion. Concentrations of FSH and inhibin B in blood serum of horses have strong and negative correlation, while there were none or very weak correlation between concentrations of prolactin and inhibin B in blood serum of horses. Blood serum insulin concentrations in mares during estrust, diestrus and early pregnancy were lower, while it is higher during mid and late pregnancy periods.

Stallions were divided into 2 groups in accordance with the conceptions rates of mares in the respective harems and circulating hormones were measured and compared (Table 2).

Table 2

Comparison of blood serum concentrations of FSH, inhibin B and prolactin in stallions.

Groups	FSH, ng/ml	Inhibin B, pg/ml	Prolactin, ng/ml	Insulin, µIU/ml
Average	8.86±2.8	2959.58±396.4	6.14±0.9	12.63±1.0
Group 1	2.4±1.0	3901.0±251.8	5.75±0.15	9.98±1.2
Group 2	13.17±1.5	2331.9±101.4	6.4±1.6	11.59±1.3
P	>0.010	<0.010	>0.0500	>0.0500

Above table shows for stallions of group 1, concentration of FSH is low, and inhibin B is high, while for group 2 stallions they are vice versa and prolactin concentrations in groups were not significantly different (P>0.500).

Comparisons of conception rates of mares in harems of both group stallions demonstrated that conception rate of mares sired by those stallions with greater inhibin B and lower FSH concentrations accounted for 66.7±9.1%, whereas the rate of mares of another group accounted for 85.7±5.9% (P<0.100).

In order to measure concentrations of blood serum hormones in mares, the animals were divided into 2 groups with that whether they are conceived or in estrus, and both groups were compared with each others.

Table 3

Blood serum insulin and prolactin concentrations their correlations in Mongolian horse

	Variables	No. of samples	Prolactin, ng/ml	Insulin, µIU/ml	Correlation, r	Notes
1	Stallions	5	6.74±0.55 (5.6-8.4)	12.63±1.0 (10.4-13.0)	-0.81	Negative
2	Mares	33	6.28±0.17 (4.3-8.3)	9.75±1.42 (5.7-39.4)	0.1	Weak positive
	P		>0.500	<0.001		
3	Total	38	6.3 (4.3-8.4)	10.1 (5.7-39.4)	0.07	Very weak positive
	p		<0.001	<0.001		

From table 3 it is demonstrated that insulin and prolactin concentrations of stallions have negative correlation, whereas in mares they have weak positive correlation.

DISCUSSION

Reproductive hormones in the present study were FSH and inhibin B, which belong to HPG axis, and two metabolic hormones insulin and prolactin, which are involved in energy axis according to some authors [31], while others noted they are involved in adipoinsular or growth hormone axis [3, 13, 16]. In our view, a hormone prolactin can be included in the main components of this axis, despite Toribo et al [31] mentioned the hormone is associated with this energy axis.

Watson et al [34] measured mares blood plasma FSH, inihibin A, and inhibin isoforms by using ELISA test during estrus in spring season. As a result it was found there is inverse relationship between FSH and inhibin iduring estrus of mares. According to study by Donadeu et al [7] no fluctuations of inhibin B concentrations in fluid of ovarian follicles of various sizes for mares were observed. Nagaoka at al [21] found measurement of inihibin B concentrations may allow precise determination of ovulation period in mares. Above studies demonstrated that concentrations of both inhibin B and FSH were inversely related, decreasing in heat and increasing during early pregnancy, and associated with reproductive activity and semen quality of stallion are consistent with the results of our study. Roser et al [7] measured concentrations of FSH, estradiole and inhibin in mares in heat and stallions during late spring and autumn, when their reproductive activities differ. However, there was positive correlation between concentrations of FHS and inhibin B for whole period of reproductive states including estrus, diestrus and gestation [26]. Results of our study are aslo in agreement with those informed by other authors. Negative correlation between concentrations of circulating FSH and inhibin during estrus according to their study ($r=-0.7359$, $p<0.01$) is consistent with the results of the present study ($r=-0.77412$). Studies of blood serum concentrations of inhibin B in horses in comparison with prolactin [12] found no significant relationships between concentrations of both hormones. However, Vick et al [33] reported obese mares exhibited a significantly longer duration of the estrous cycle, significant increases in circulating concentrations of leptin and insulin.

Prolactin concentrations in pituitary and serum of mares were affected by season and could be increased by artificially long photoperiods in winter [29, 30], despite it is lowest some in mammalians in winter by some authors [30]. De Pew et al [6] found that stallions exhibited a prolactin response that was approximately twice that observed in mares. Prolactin secretion in horses may be stimulated by aspects of eating other than the feed-stuff itself [24]. Total feed deprivation had little effect on the subsequent prolactin response to a meal or to other known secretagogues [20]. According to results of our study, prolactin concentration was higher in stallions than mares, but it remains unclear whether prolactin response after grazing. According to Thompson et al [30] mares had higher prolactin concentrations in serum than stallions during summer. For periods of estrus, diestrus and pregnacy, serum prolactin concnetrations in mare ranged between 4.3±0.2 (mid pregnancy) and 9.3±0.5 ng/ml (late pregnancy). Heidler et al [12] analyzed growth hormone (GH), insulin-like growth factor 1 (IGF-1), leptin, luteinising hormone (LH) and prolactin in mares from late pregnancy throughout lactation, and noted prolactin concentrations reached a maximum in the week of foaling and decreased rapidly thereafter. As reported by Billestrup and Nielsen [2] prolactin concentration increases in pregnant woman and the hormone is seen to be a potential growth factor for beta cells of the pancreas. According to Worthy et al [36] plasma prolactin concentrations increased significantly in the last week of pregnancy and remained high although variable in early lactation. Also in our study serum prolactin rose in late pregnancy period. It means besides of relations between these hormones and various reproductive states, both prolactin and insulin concentrations can be interrelated. Therefore such change can also occur in other mammals, including horses. In our study, there was slight correlation between these hormones in pregnant mares. Some authors noted pregnancy is associated with alterations in the regulation of glucose metabolism caused by the actions of human placental lactogen (prolactin) and progesterone; these hormones antagonize the actions of insulin, leading to a state of relative insulin resistance as pregnancy progresses [17].

Despite there were some findings in regard to interrelationships between several hormones including blood serum FSH, inhibin B, prolactin and insulin in our study, more detailed studies will be necessary in relation to a whole set of hormones belonging to both reproductive and energy axis during various states of reproduction in animals.

CONCLUSION

Results of the present study demonstrate that each hormones measured in this study have their own characteristics depending on states of both reproduction and metabolism. Based on above mentioned results, it has been concluded that FSH and inhibin B concentrations in blood of horses change with inverse relationships during estrus of mare and it is possible to determine reproductive activity of stallions with concentration of inhibin B in blood serum. As well, there is a tendency of having relationship between FHS and inhibin B of reproductive axis and metabolic hormones such as insulin and prolactin. Reproductive states including estrus and pregnancy are closely associated with hormones of energy exis, because any physiological processes, especially reproductive functions, which are regulated by hormones such as FSH and inhibin B require energy in the animal body, which is regulated by various factors, including hormones of energy axis.

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REFERENCE

1. Bergfelt D.R., et al. (1991) Curculating concentrations of immunoreactive inhibin and FSH during the estrous cycle of mares. J.Equine Vet.Sci., V 11: p 319-321
2. Billestrup N., Nielsen J.H (1991) The stimulatory effect of growth hormone, prolactin and placental lactogen on beta-cell proliferation is not mediated by insulin like growth factor-1, Endocrinology, 129; p 883-888
3. Breton C. (2013) The hypothalamus-adipose axis is a key target of developmental programming by maternal nutritional manipulation, Journal of Endocrinology; 216, R19-R31,
4. Carlson H.E., et al (1983) Prolactin stimulation by meals related to protein content, J.Clin.Endocrinol.Metab., 57; p 334-338
5. Cavinder C.A., et al, (2012) Late gestational nutrition of the mare and potential effects on endocrine profiles and adrenal function of the offspring, The Professional Animal Scientist 28: p 344-350
6. De Pew C.L., et al (1994) Plasma concentration of prolactin, glucose, insulin, urea nitrogen, and total amino acids in stallions after ingestion of feed or gastric administration of feed components, J.Anim.Sci., 72; p 2345-2353
7. Donadeu, F.X. et al, (2001) Changes in concentrations of follicular fluid factors during follicle selection in mares, J.Biol.Reprod., p 1111-1118,
8. Gerlach T., et al, (2000) Regulation of seasonal reproductive activity in the stallion, ram and hamster, Animal Reproduction Science; p 197-213
9. Goudet, G. et al., (1999) Intrafollicular content of LH receptor, alfa inhibin, and aromatase in relation to follicular growth, estrous cycle stage and oocyte competence for in vitro matuartion in the mare, J.Biol Reprod, Vol 60, p 1120-1127
10. Hadley, M.C. (1992), Endocrinology, Prentice Hall, p 459-460
11. Hart K.A., (2014) “Review of endocrine function during health and illness of neonatal foals” AAEP Focus on The First Year of Life Proceedings
12. Heidler B., et al, (2003) Effects of lactation on metabolic and reproductive hormones in Lipizzaner mares. Domestic Animal Endocrinology. Jul;25(1) :p 47-59
13. Hurcombe S.D.A., (2011), Hypothalamic-pituitary gland axis function and dysfunction in horses, Vet Clin Equine 27 p 1-17

14. Irvine C.H., et al, (2000) Reproductive hormone profiles in mares during the autumn transition as determined by collection of jugular blood at 6 h intervals throughout ovulatory and anovulatory cycles, Journal of Reproduction and Fertility, 118, p 101-109
15. Kawai M., et al, (1999) Adaptation of pancreatic islet B-cell during the last third of pregnancy: regulation of B-cell function and proliferation by lactogenic hormones in rats, European Journal of Endocrinology 141, p 419-425
16. Kieffer T.J. and J.F. Habener (2000) The adipoinsular axis: effects of leptin on pancreatic β-cells, Am. J.Physiol. Endocrinol. Metab. 278: E1-E14
17. Krentz A. J., et al, (2002) Insulin Resistance: A Clinical Handbook; Blackwell Ltd; UK
18. Labriola L., et al (2007) Beneficial effects of prolactin and laminin on human pancreatic islet-cell cultures. Mol Cell Endocrinol. 263(1-2):p 120-133
19. Li-Yuan Yu-Lee., (2002) Prolactin modulation of immune and inflammatory responses, Recent Prog.Horm.Res., 57: p 435-455
20. Nadal M.R., et al (1997) Effect of feeding and feed deprivation on plasma concentrations of prolactin, insulin, growth hormone, and metabolites in horses. J Anim Sci. 75(3): p 736-44
21. Nagaoka, K. et al., (1999) A selective increase in circulating inhibin and inhibin pro-αC at the time of ovulation in the mare, Am.J.Physiol Endocrinol Metab Vol. 277; E870-E875
22. Nambo Y., et al, (1996) High concentrations of immunoreactive inhibin in the plasma of mares and fetal gonads during the second half of pregnancy, Reproduction, Fertility and Development 8(8) p 1137-1145
23. Nambo Y., et al, (2002) Inhibin as a possible indicator of follicular development during perinatal period in mares, Journal of Reproduction and Development, 48: p 265-270
24. Quigley M.E., et al (1981) Acute prolactin release triggered by feeding, J.Clin.Endocrinol.Metab., 52; p 1043-1046
25. Rivier C., et al. (1986) Inhibin-mediated feedback control of FSH secretion in the female rat. Science, 234, p 205-208
26. Roser, J.F., et al, (1994) Inhibin activity in the mare and stallion, J. Domestic Animal Endocrinology, Vol.11, p 87-100
27. Salazar-Ortiz J., et al, (2011) Effects of nutritional cues on the duration of the winter anovulatory phase and on associated hormone levels in adult female Welsh pony horses (Equus caballus), Reproduction Biology and Endocrinology, p 9:130
28. Stewart B.L., and J.F.Roser, (1998) Effects of age, season, and fertility status on plasma and intratesticular immunoreactive (IR) inhibin concentrations in stallions, p 129-139
29. Thompson D.L., et al, (1997) Prolactin administration to seasonally anestrous mares: Reproductive, metabolic, and hair-shedding responses, Journal of Animal Science, 75: p 1092-1099
30. Thompson D.L., et al, (1986) Concentrations of prolactin, luteinizing hormone and follicle stimulating hormone in pituitary and serum of horses: effect of sex, season and reproductive state, Journal of animal science,63: p 854-860
31. Toribio R.E., et al, (2001) Endocrine dysregulation in critically ill foals and horses, Vet Clin Equine 27, p 35-47;
32. Ueno, N., et al. (1987) Isolation and partial characterisation of follistatin: a single-chain Mr 35000 monomeric protein that inhibits the release of FSH, 1: Proc Natl Acad Sci USA, Dec; 84 (23) : p 8282-8286
33. Vick M.M., et al, (2006) Obesity is associated with altered metabolic and reproductive activity in the mare: effects of metformin on insulin sensitivity and reproductive cyclicity" Reproduction, Fertility and Development18(6) p 609-617
34. Watson, E.D. et al., (2002) Plasma FSH, inhibin A and inhibin isoforms containing pro- and αC during winter anoestrus, spring transition and the breeding season in mares. J.Reproduction, 123, p 535-542
35. Weiss J.M., et al (2003) Effects of insulin on LH and prolactin secretion and calcium signaling in female rat pituitary cells, Archives of Gynecology and Obstetrics, 299(1) p 45-50
36. Worthy K., et al, (1986) Plasma prolactin concentrations and cyclic activity in pony mares during parturition and early lactation, Journals of Reproduction & Fertility Ltd, U.K. 77, p 569-57