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The Relationship Between Plasma Leptin and FSH Concentrations with Ovulation Rate in Iranian Native Sheep

[1]A. Towhidi, [1]R. Masoumi, [2]M.M. Moeini, [2]H. Solgi and [1]H. Moravej
[1]Department of Animal Science, Faculty of Agronomy and Animal Science,
University of Tehran, P.O. Box 31587-4111 Karaj, Iran
[2]Department of Animal Science, Faculty of Agriculture,
University of Razi, Kermanshah, Iran

Abstract: The aim of this study was to determine the relationship between plasma leptin and FSH concentration in Iranian sheep. Forty female Mehraban and Sanjabi sheep were used. All ewes were cyclic and synchronized with cloprestenol. The ewes were divided into two breed groups: Mehraban breed (n = 20) and Sanjabi breed (n = 20), feeding at maintenance level. On the first and second days of estrus cycle, blood samples were collected from the jugular vein. Ovulation number was determined by endoscopy 7 days after the second injection. Mean Plasma leptin concentrations on second day (4.74±0.15 and 4.68±0.10 ng mL^{-1}) were significantly higher than those on first day (2.64±0.11 and 2.56±0.04 ng mL^{-1}) for Mehraban and Sanjabi sheep, respectively ($p<0.01$). Mean plasma FSH concentrations on second day (2.75±0.17 and 2.74±015 ng mL^{-1}) were also significantly greater than those on first day (1.19±0.05 and 1.19±0.04 ng mL^{-1}) for Mehraban and Sanjabi ewes, respectively ($p<0.01$). In the present study, positive relationship has been shown between plasma Leptin and FSH concentrations ($p<0.01$) in Mehraban and Sanjabi sheep. Ovulation rate had a significant difference between Mehraban (1.20±0.33) and Sanjabi (1.07±0.1) ewes. Significant differences were not observed between concentrations of FSH and leptin with ovulation rate in both breeds ($p \leq 0.01$).

Key words: Leptin, FSH, Mehraban, Sanjabi, Ewe

INTRODUCTION

Leptin was discovered in 1994. It is a 16 kDa protein encoded by *obese* gene and produced by adipocytes (Kosior-Korzecka and Bobowiec, 2003). It affects food intake, energy expenditure and thermogenesis. Leptin is also a metabolic stimulant for reproduction (Barb, 1999). Mice that lack a functional leptin gene are obese and replacement of leptin in these animals resulted in a loss of appetite and a concomitant loss of body fat and normalization of body composition. In addition, leptin induces early puberty in rodents and has direct positive effects on many organs of the reproductive system (Hossner, 1998).

Several studies have been shown high correlation between FSH level and ovulation rate in ewes (Kosior-Korzecka and Bobowiec, 2003). In other side, during recent years it has been established that leptin acts as a metabolic signal for reproduction (Cioffi *et al*., 1996). It has been indicated that there is a high positive correlation between leptin and FSH with ovulation rate in Chal ewe (Towhidi *et al*., 2003). Consequently, it is possible that plasma leptin concentration is an index for ovulation rate and lambing rate in sheep but it has not yet been elucidated.

MATERIALS AND METHODS

Animals: Twenty cycling Mehraban ewes and twenty Sanjabi ewes, (3-5 years of age) with similar body weight and body condition score were used. During the course of experiment, ewes were feeding at maintenance level *ad libitum* and had free access to fresh water at all times. Animal were kept under control during 4 weeks from 16 Aug to 12 Sep. 2004. Mehraban ewes selected from Bu Ali Sina University farm in Hamedan and also Sanjabi ewes selected from Agriculture Research Center of Kermanshah.

Experimental design: Estrus was synchronized with two injections of 250 μg prostaglandin F2 α analogue (PG; Cloprostenol sodium, Nasr Ltd., Iran). The first $PGF_{2\alpha}$ was, injected on day -11 and the second one was injected on day 0. Estrus was detected by teaser ram after the second

Corresponding Author: A. Towhidi, Department of Animal Science, Faculty of Agronomy and Animal Science, University of Tehran, P.O. Box 31587-4111 Karaj, Iran
Tel/Fax: 0098 261 2248082

$PGF_{2\alpha}$ injection for 8 h. Two blood samples were collected from each sheep on day 1 and day 2 after the second injection. Mating occurred after second injection of pgf2 by 10 rams.

Blood sampling: Blood samples (10 mL) were taken by heparinized venepuncture on day 1 and day 2 to detect of plasma leptin and FSH concentrations. Blood tubes were centrifuged (15 min at 4°C and 3000×g) and plasma was stored at -20°C.

Leptin and FSH assay: Plasma Leptin concentration were measured using commercially radioimmunoassay Kit for mutlispecies leptin (Linco research Ltd., USA) and plasma FSH were measured by ELISA kit (Tabeshyar noor. Iran). Sensitivity of leptin assay was 0.04 ng mL^{-1} and intra-assay coefficient of variation were 5%. Sensitivity of FSH assay was 1% and intra-assay coefficient of variation was 11.1%, respectively.

Endoscopy: Ovulation number was determined by endoscopy. Laparoscopy was carried out 7 days after the second $PGF_{2\alpha}$ injection in all ewes under the local anesthesia. After the endoscopy, animals were treated by streptomycin for 3 days.

Data and statistical analysis: Statistical differences in plasma leptin and FSH concentration were assessed by repeated measures ANOVA with proc MIXED in SAS. The relationship between measured variables were determined by linear regression. Means were compared by LSMeans procedure. Difference of ovulation numbers was compared by χ^2 test. Statistical model was: treatment (α), time (β), ($\alpha\beta$) treatment×time interaction.

$$Y_{ij} = \mu+\alpha_i + \beta_i + (\alpha\beta)_{ij}+e_{ij}$$

RESULTS

Leptin: The analysis of variance for plasma leptin concentration in samples of 1st and 2nd day of Mehraban and Sanjabi sheep has shown a significant increase on 2nd day ($p<0.01$). No significant difference in plasma leptin concentration between Mehraban and Sanjabi ewes was found (Table 1).

FSH: The analysis of variance for plasma FSH concentration in sample of 1st and 2nd day of Mehraban and Sanjabi ewes has been indicated a significant increase on 2nd day ($p<0.01$). There were not any significant differences in plasma FSH concentration between Mehraban and Sanjabi sheep (Table 1).

Estrus and ovulation rate: All ewes showed estrus signs during 2nd day. Ovulation rate had a significant difference between Mehraban (1.20±0.33) and Sanjabi (1.07±0.1) ewes (Table 1).

The correlation of the leptin, FSH and ovulation rate: There was a positive correlation between plasma leptin and FSH concentrations in both breeds ($p<0.01$). There was no correlation between plasma leptin and FSH concentration with ovulation rate in both breeds.

DISCUSSION

This study is the first report of the circulating concentration of leptin in Mehraban and Sanjabi sheep and indicating changes in plasma leptin and FSH concentration during follicular phase in these breeds. The plasma leptin concentrations in these sheep are within the ranges that previously reported. The plasma leptin concentration increased significantly during the heat estrus in both ewes. The other reports confirmed this result. They are referred to discuss leptin's role in estrus (Rogol, 1983; Keisler *et al*., 1999; Kiess *et al*., 1999). Leptin is a regulator of estrus in female mice (Ahima *et al*., 1997; Chehab *et al*., 1997) and rats (Cheung *et al*., 1997; Gruaz *et al*., 1998; Plant and Durrant, 1997). Numerous reports have characterized temporal changes in systemic leptin levels during estrus in humans (Blum *et al*., 1997; Horlick *et al*., 2000) and explain the well documented gender differences in leptin secretion (Kennedy *et al*., 1997). As valid leptin immunoassays were developed for domestic animals, changes in systemic leptin concentrations during puberty in domestic animals will be forthcoming.

In the present study, the plasma leptin concentrations during estrus were significant greater than those during follicular phase. These changes do not appear to be due to variations in circulating estrogen and/or progesterone concentrations since sex steroids

Table 1: Mean (±SEM) BW, BCS, PLC, PFL and OR in ewes

Trait / Breed	No.	BW (kg)	BCS	PLC (ng mL^{-1}) Day 1	PLC (ng mL^{-1}) Day 2	PFL (ng mL^{-1}) Day 1	PFL (ng mL^{-1}) Day 2	OR
Mehraban	20	40±2.5	2.24±0.2	2.60±0.01^a	4.74±0.15^b	1.19±0.05^a	2.75±0.17^b	1.20±0.33^a
Sanjabi	20	39±3.1	2.15±0.3	2.56±0.04^a	4.68±0.10^b	1.19±0.04^a	2.74±0.15^b	1.07±0.1^b

Values with different superscripts in each trait are significantly different ($p<0.01$), BW: Body Weight; BCS: Body Condition Score; PLC: Plasma Leptin Concentration; PFL: Plasma FSH Concentration, OR: Ovulation Rate

have no effect on circulating leptin in young women (Teirmaa *et al*., 1998) postmenopausal women (Castracane *et al*., 1998). However, some studies have indicated that estrogens may increase leptin secretion in rats (Chu *et al*., 1998). Further research is required to determine the reason of changes in circulating concentration of leptin during the estrus cycle of domestic animals. The reason of increased leptin level ($p<0.01$) during estrus was apparently related to energy balance in ewes because effects of energy balance on reproduction mediated by leptin.

Leptin is secreted predominantly by white adipose tissue cells (Zhang *et al*., 1994); is found in circulation; and might be synthesized, secreted, or sequestered within cells in liver, skeletal muscle, gut, placenta and Central Nervous System (CNS) (Masuzaki *et al*., 1997). Plasma leptin concentrations are positively correlated with adiposity in human beings and rodents allowed access to food *ad libitum*. Exogenous leptin treatment in animals reverses the effects of food restriction or food deprivation on aspects of reproduction, including the onset of puberty (Chehab *et al*., 1997; Ahima *et al*., 1997; Barash *et al*., 1996), the length of the estrous cycle (Chehab *et al*., 1996), the length of lactational diestrus, gonadotropin and gonadal steroid levels (Ahima *et al*., 1997; Yu *et al*., 1997) and pulsatile LH secretion (Finn *et al*., 1998;) in mice, rats, hamsters, nonhuman primates and ruminants. In another study, subcutaneous infusions of mouse leptin that maintain plasma levels of total leptin within the physiological range of rats prevent fasting-induced changes in neuroendocrine function and in NPY, POMC and CART mRNA levels in the arcuate nucleus in rats (Ahima *et al*., 1997). Articles in this issue examine the role of leptin in estrous behavior, puberty, the length of lactational diestrus and offspring development.

The increase in plasma leptin concentration during estrus would be more acutely responsive to changes in the availability of oxidizable metabolic fuels than to overall levels of body adiposity. Plasma leptin concentrations and *ob* gene expression alter more rapidly than do body fat levels in response to a variety of energetic challenges such as fasting and refeeding and cold exposure in rats, mice, hamsters and human beings. Plasma leptin levels, like reproductive processes, appear to be sensitive to fuel availability rather than to energy reserves. Changes in metabolism might affect the synthesis and secretion of hormonal modulators such as leptin, which in turn might influence reproductive processes. The idea that hormone secretion is controlled by changes in metabolic fuel oxidation rather than by ligand-receptor interaction is not without precedent. Leptin synthesis and secretion might be influenced by the oxidative substrates that are part of the hexosamine biosynthetic pathway in tissue such as white adipose tissue and skeletal muscle. In support of this idea, *ob* gene expression is increased by infusion with UDP-*N*-acetylglucosamine, glucose, lipid and uridine, all of which would be expected to increase the concentrations of the end-products of the hexosamine biosynthetic pathway. Furthermore, treatment with inhibitors of glucose oxidation significantly decreases plasma leptin concentrations in Syrian hamsters and adipocytes in culture (Blum and Schneider, unpublished data). These data are consistent with the idea that fluctuations in plasma leptin might act as a reporter of fuel oxidation and thus might have the potential to act as a mediator of the metabolic signal. Hence elevation of leptin level during estrus apparently is a stimulant for hypothalamic-hypophysial-gonads axis because reproduction is a energy-consumed process.

In the present study, positive relationship has been shown between plasma leptin and FSH concentrations ($p<0.01$). Furthermore, a significant relationship between plasma concentrations of leptin and FSH have been observed in other studies (Towhidi *et al*., 2003; Kosior-Korzecka and Bobowies, 2003).

This may not be an example of a simple function between nutrition and reproduction that why, at the estrous time when sheep were in ovulation time, they were not at nutritional restriction and they were feeding at maintenance level. A positive correlation has been indicated between Leptin and FSH in Mehraban and Sanjabi sheep ($p<0.01$). In one study, it was shown that the plasma leptin and FSH concentrations in fat and thin sheep were not significantly different after maintaining on either *ad libitum* or restricted diets for 16 months. But, we have reported that the plasma leptin and FSH concentrations were significantly different after maintaining on restricted diets (Towhidi *et al*., 2003). Gonadotrophin secretion is reduced in animals and human that to be nutritionally restricted (Barb, 1999).

The relationship between feeding and leptin and FSH production is directly pertinent to the above issues. FSH stimulates the secretion of leptin by adipocytes but this response is so slow.

We have shown that ovulation rate have a positive correlation with plasma leptin concentration in Iranian Chal ewes, but it was not observed that leptin directly affects ovulation rate (Towhidi *et al*., 2003). Most studies have indicated negative effects of leptin on gonadal function. A wide range of factors (for example, species,

season, metabolic status and steroidal status) need to be considered in conducting such experiments. There is little information to show that leptin acts in an acute manner to regulate reproduction in the short term.

The results of this experiment showed that changes in plasma leptin is necessary and/or sufficient for changes in reproduction and also endogenous changes do in leptin precede changes in reproduction during fluctuating energetic conditions. We concluded that leptin may be a factor with respect to the onset of estrous cycle.

Implication: It has been shown that there is a significant correlation betweenplasma leptin and FSH concentrations during estrus of sheep. Differences between and within species particular breed of sheep in this study indicated that level of leptin with ovulation rate from breed to breed is different but it wasn't significant. Perhaps these results are due to the multifarious effects on others hormones.

REFERENCES

Ahima, R.S., J. Dushay, S.N. Flier, D. Parbakaran and J.S. Flier, 1997. Leptin accelerates the onset of puberty in normal female mice. J. Clin. Invest., 99: 391-395.

Barash, I.A., C.C. Cheung, D.S. Weigle and H. Ren *et al*., 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology, 137: 3144-31447.

Barb, C.R., 1999. The brain-pituitary-adipocyte axis: Role of leptin in modulating neuroendocrine function. J. Anim. Sci., 77: 1249-1257.

Blum, W.F., P. Englaro, S. Hanitsch, A. Juul and N.T. Hertel *et al*., 1997. Plasma leptin levels in healthy children and adolescents: Dependence on body mass index, body fat mass, gender, pubertal state and testosterone. J. Clin. Endocrin. Metab. 82: 2904-29010.

Castracane, V.D., R.R. Kraemer, M.A. Franken, G.R. Kraemer and T. Gimpel, 1998. Serum leptin concentration in women: Effect of age, obesity and estrogen administration. Fertil. Steril., 70: 472-477.

Chehab, F.F., M.E. Lim and R. Lu, 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat. Genet., 12: 318-320.

Chehab, F.F., K. Mounzih, R. Lu and M.E. Lim, 1997. Early onset of reproductive function in normal female mice treated with leptin. Science, 275: 88-90.

Cheung, C.C., J.E. Thornton, J.L. Kuijper, D.S. Weigle, D.K. Clifton and R.A. Steine, 1997. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology, 138: 855-858.

Chu, S.C., Y.C. Chou, J.Y. Liu, C.H. Chen, J.C. Shyu and F.P. Chou, 1999. Fluctuation of serum leptin level in rats after ovariectomy and the influence of estrogen supplement. Life Sci., 64: 2299-2306.

Cioffi, J.A., A.W. Shafer, T.A. Zupancic, J. Smith-Gbur, A. Mikhail, D. Platika and H.R. Snodgrass, 1996. Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. Nat Med., 2: 585-859.

Finn, P.D., M.J. Cunningham, K.Y. Pau, H.G. Spies, D.K. Clifton and R.A. Steiner, 1998. The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrine, 139: 4652-4662.

Gruaz, N.M., M. Lalaoui, D.D. Pierroz, P. Englaro, P.C. Sizonenko, W.F. Blum and M.L. Aubert, 1998. Chronic administration of leptin into the lateral ventricle induces sexual maturation in severely food-restricted female rats. J. Neuroendocrinol., 10: 627-633.

Horlick, M.B., M. Rosenbaum, M. Nicolson, L.S. Levine, B. Fedun, J. Wang, R.N. Pierson Jr and R.L. Leibel, 2000. Effect of puberty on the relationship between circulating leptin and body composition. J. Clin. Endocrinol. Metab., 85: 2509-2518.

Hossner, K.L., 1998. Cellular, molecular and physiological aspects of leptin: Potential application in animal production. Can. J. Anim. Sci., 78: 463-472.

Keisler, D.H., J.A. Daniel and C.D. Morrison, 1999.The role of leptin in nutritional status and reproductive function. J. Reprod. Fertil. Suppl., 54: 425-435.

Kennedy, A., T.W. Gettys, P. Watson, P. Wallace, E. Ganaway, Q. Pan and W.T. Garvey, 1997. The metabolic significance of leptin in humans: Gender-based differences in relationship to adiposity, insulin sensitivity and energy expenditure. J. Clin. Endocrinol. Metab., 82: 1293-1300.

Kiess, W., A. Reich, K. Meyer, A. Glasow, J. Deutscher, J. Klammt, Y. Yang, G. Muller and J. Kratzsch, 1999. A role for leptin in sexual maturation and puberty? Horm. Res., 51(Suppl 3): 55-63.

Kosior-Korzecka, U. and R. Bobowiec, 2003. Changes in the level of endogenous leptin, FSH, 17α-oestradiol and metabolites during lupin-induced increase in ovulation rate in ewes. J. Vet. Med. Series, A. 50: 343.

Masuzaki, H., Y. Ogawa, N. Sagawa, K. Hosoda, T. Matsumoto, H. Mise, H. Nishimura, Y. Yoshimasa, I. Tanaka, T. Mori and K. Nakao, 1997. Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. Nat. Med., 3: 1029-1033.

Plant, T.M. and A.R. Durrant, 1997. Circulating leptin does not appear to provide a signal for triggering the initiation of puberty in the male rhesus monkey (Macaca mulatta). Endocrinology, 138: 4505-4508.

Rogol, A., 1983. Editorial: Leptin and puberty. J. Clin. Endocrinol. Metab., 83: 1089-1091.

Teirmaa, T., V. Luukkaa, J. Rouru, M. Koulu and R. Huupponen, 1998. Correlation between circulating leptin and luteinizing hormone during the menstral cycle in normal-weight women. Eur. J. Endocrinol., 139: 406-411.

Towhidi, A., H. Khazali, F. Rostami and A.H. Ahadi, 2003. The effects of energy intake level, body condition score and leptin on ovulation rate in fat-laited ewes. Proc. Br. Soc. Anim. Sci., pp: 80.

Yu, W.H., M. Kimura, A. Walczewska, S. Karanth and S.M. McCann, 1997. Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci. USA., 94: 1023-1028.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold and J.M. Friedman, 1994. Positional cloning of the mouse obese gene and its human homologue. Nature, 372: 425-432.