Vet.Med.J.,Giza. Vol.48, No.4 (2000): 503-514.

EFFECT OF ESTROUS CYCLE AND FEMALE SEX STEROIDS ON PLASMA LEPTIN IN FEMALE RATS

G.S. ESSAWY

Department of Physiology, Faculty of Veterinary Medicine, Cairo University

Received: 5.7.2000.
Accepted: 17.8.2000.

SUMMARY

Leptin, a hormonal product of the obese (ob) gene plays an important role in the control of reproduction. Reproductive steroids, such as estrogen and androgens regulate fat distribution in the body. The present study was performed to evaluate the influence of estrous cycle and the sex steroids, particularly estradiol (E2) and progesterone on the leptin in female rats. Sixty-four adult female albino rats were used throughout the experiments. In the first experiment, the rats were segregated into the four phases of the estrous cycle by vaginal smears and blood samples were collected for measurement of plasma leptin, estrogen and progesterone. In the second experiment, the rats were divided into four groups, each of eight rats. The first to fourth groups were injected with vehicle (sesame oil), estradiol benzoate (5µg/kg b. wt), progesterone caproate (2mg/kg b. wt), and estradiol-progesterone subcutaneously daily for seven days, respectively and plasma leptin was measured. Leptin levels in female rats showed non-significant differences during the estrous cycle. A positive correlation existed between the plasma leptin level and the body weight among the phases of estrous cycle. There was no correlation between leptin level and both estrogen and progesterone levels during the estrous cycle. Estrogen treatment significantly increased the plasma leptin concentration, while progesterone or estrogen plus progesterone treatments had no effect. The present study demonstrated that 17β-estradiol can regulate leptin secretion in female rat and the physiological variations of circulating estrogen and progesterone have no significant effect on the leptin level during normal estrous cycle.

INTRODUCTION

Leptin, a 167-amino acid hormonal product of the obese (ob) gene (Zhang et al., 1994), is a signal of

the nutritional status. Leptin in mice not only causes weight loss, by suppressing food intake and increasing motor activity, energy metabolism, oxygen consumption, and body temperature (Campfield et al., 1995; Hwa et al., 1996; Hwa et al., 1997), and normalizes blood glucose and insulin levels (Schwartz et al., 1996), but also affect reproduction.

Mutant mice that are unable to produce leptin or its receptor (ob/ob and db/db mice, respectively) fail to undergo normal sexual maturation and remain infertile throughout life (Chehab et al., 1996). Administering leptin into ob/ob mice stimulates all aspect of their reproductive endocrine system and rescues their fertility (Ahima et al., 1996; Barash et al., 1996; Chehab et al., 1997).

Leptin plays an important role in control of reproduction by actions on the hypothalamus, pituitary gland and gonads. Leptin stimulates the luteinizing hormone-releasing hormone (LHRH) release from hypothalamus (Yu et al., 1997a; McCann et al., 1998). It stimulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from anterior pituitary gland (Yu et al., 1997b). Leptin plays a physiologically important role in the generation of steroid-induced LH and prolactin surges in female rats (Kohsaka et al., 1999; Watanobe et al., 1999). Leptin, at a physiological levels, can directly attenuate insulin (Spicer and Francisco, 1997) and insulin-like growth factor induced (Zachow and Magoffin, 1997; Agarwal et al., 1999) steroidogenesis of rat granulosa cells. Moreover, leptin suppresses steroidogenesis in bovine theca cells directly through its receptors (Spicer and Francisco, 1998; Spicer et al., 2000) and facilitates female sexual behavior in ad libitum fed female hamsters (Wade et al., 1997). Leptin administration, both in vivo and in vitro, inhibits ovulation in rat through a direct effect on the ovary (Duggal et al., 2000).

Reproductive steroids, such as estrogen and androgens regulate fat distribution in the body. Androgens suppressed leptin production in human adipocytes (Ambrosius et al., 1998; Kiess et al., 1999; Hislop et al., 1999; Lagiou et al., 1999), in rat adipocytes (Watanobe and Suda, 1999; Machinal et al., 1999), whereas estrogen increased leptin expression in human (Mannucci et al., 1998; Elbers et al., 1999; Lavoie et al., 1999) and rats (Shimizu et al., 1997). Moreover, the gender influences leptin level in human and animal studies (Frederich et al., 1995; Ostlund et al., 1996; Isidori et al., 2000). Thus, the gonadal steroids correlated not only with fat synthesis but also with leptin axis.

This suggested that sex steroids could be involved in the regulation of leptin. Data from animal and human studies concerning the leptin level in the estrous or menstrual cycle are conflicting. The present study was performed to evaluate the influence of estrous cycle and the sex steroids, particularly estradiol (E2) and progesterone on the leptin

level in female rats.

MATERIALS AND METHODS

Animals:

Sixty-four adult female albino rats (125-150g) were used throughout the experiments. Food and water were available ad libitum.

Experimental Design:

All rats were subjected to vaginal smear examination and segregated into the four phases of the estrous cycle. In the first experiment, eight rats in each estrous phase were anaesthetized with ether anasthesia, weighed and blood samples were collected on heparin (20 IU/ml). Plasma were separated by centrifugation at 3000 rpm for 15 minutes and stored at -20°C until used for measurement of plasma leptin, estrogen and progesterone.

In the second experiment, the remaining thirty-two adult female rats were divided into four groups each of eight rats. The first group was kept as control and injected subcutaneously (s.c) by the vehicle (sesame oil). Rats in the second to fourth groups were injected with 5 µg/ kg b. wt estradiol benzoate, 2mg/kg b. wt progesterone caproate, and estradiol (5 µg/kg b. wt) plus progesterone (2mg/kg b. wt) subcutaneously daily for seven days, respectively. Rats were anaesthetized with ether anaesthesia, weighed and blood samples were collected at the end of the experiment on heparin (20 IU/ml). Plasma were separated by centrifugation at 3000 rpm for 15 minutes and stored at -20 °C until used for measurement of plasma leptin.

Hormonal assay:

Plasma leptin level was measured using active leptin immunoradiometric assay (IRMA) kit obtained from Diagnostic system laboratories (DSL, Webster, Texas, USA). The sensitivity of leptin assay was 0.10 ng/ml and the intraassay and interassay coefficient of variation were 3.7% and 5.2%, respectively.

Plasma estradiol (Xing et al., 1983) and progesterone (Kubasik et al., 1984) were measured using commercial RIA kits obtained from Diagnostic Products Corporation (Los Angeles, CA). The sensitivity of estrogen and progesterone assays was 1.4 pg/ml and 0.03 ng/ml, respectively. The intraassay and interassay coefficient of variation were 4.3% and 6.8% for estrogen and 3.6% and 3.9% for progesterone, respectively.

Statistical analysis:

The results are expressed as mean ± SE. One way analysis of variance (ANOVA) was used to determine whether there were statistically significant differences in hormonal levels (Snedecor and Cochran, 1990). Means were compared by the least

significant difference (LSD) at the 5 % level of probability. Correlation analyses were used to describe the relation between the levels of leptin, steroid hormones and body weight at the 5 % level of probability.

RESULTS

Leptin concentration in female rats showed non-significant differences among the estrous cycle (Table 1). Correlations between leptin levels and body weight were considered. A positive correlation ($r=0.82$, $r=0.91$, $r=0.80$, $r=0.86$, at $P<0.05$) existed between the leptin level and the body weight among the phases of estrous cycle, respectively. During the estrous cycle, estrogen was high in the proestrus while the progesterone remained high at diestrus and proestrus then declined. There was no correlation between the leptin level and the estrogen and progesterone levels during the proestrus ($r=0.22$ and $r=0.15$, at $P<0.05$), estrus ($r=0.20$ and $r=0.12$, at $P<0.05$), metestrus ($r=0.17$ and $r=0.16$, at $P<0.05$), and diestrus ($r=0.18$ and $r=0.14$, at $P<0.05$) phases, respectively.

Table (1): Leptin concentration during the different phases of estrous cycle of female albino rats (n=8).

Parameters	Proestrus	Estrus	Metestrus	Diestrus
Body weight (g)	133.875 ±3.88	136.625 ±4.03	130.000 ±3.78	135.625 ±3.59
Leptin (ng/ml)	1.03 ±0.058	0.95 ±0.055	0.99 ±0.063	1.09 ±0.058
17β-Estradiol (pg/ml)	22.14^{abc} ±0.72	16.25^{a} ±0.75	15.33^{b} ±0.76	16.21^{c} ±0.75
Progesterone (ng/ml)	44.26^{abc} ±1.03	39.12^{ad} ±0.97	41.08^{bc} ±0.98	52.13^{cde} ±1.09

* Values are expressed as mean ±SE.
* Values having the same letter in the same row are significantly different from each other at $P<0.05$.
* LSD was 2.16 for 17β-estradiol and 2.95 for progesterone.

Table (2): Plasma leptin level after estrogen and progesterone treatment in female albino rats (n=8).

Parameters	control	Estrogen	Progesterone	Estrogen-progesterone
Body weight (g)	139.875 ±3.19	138.250 ±3.76	139.000 ±3.47	134.875 ±3.45
Leptin (ng/ml)	1.04^{a} ±0.060	2.30^{abc} ±0.080	1.03^{b} ±0.059	1.08^{c} ±0.056

* Values are expressed as mean ±SE.
* Values having the same letter in the same row are significantly different from each other at $P<0.05$.
* LSD of leptin was 0.19.

Estrogen treatment for 7 days significantly increased the plasma leptin concentration, while the progesterone or estrogen-progesterone treatments had no effect (Table 2). A positive correlation ($r=0.92$, $r=0.91$, $r=0.95$, $r=0.90$, at $P<0.05$) existed between the leptin level and the body weight among the control, estrogen, progesterone, and estrogen-progesterone treatments, respectively.

DISCUSSION

Leptin, at physiological concentrations, directly affects steroidogenesis of theca cells and normally fluctuating concentrations of leptin in blood may play an important role in communicating the metabolic status of the animal to the reproductive system (Spicer and Francisco, 1998).

Leptin levels showed no significant changes throughout the estrous cycle and there was no correlation between the leptin level and the estrogen and progesterone levels during the different phases of estrous cycle in female rats in the current study. These findings are consistent with that of Amico et al. (1998), Brann et al. (1999), Pinilla et al. (1999) and Watanobe and Suda (1999) in cycling rats. Furthermore, leptin levels were not affected either by ovariectomy alone or by the administration of physiological doses of estradiol, progesterone, or both after ovariectomy (Watanobe and Suda, 1999).

In the present study the plasma level of estradiol was high during proestrus while progesterone level was high at diestrus and proestrus . These data are consistent with that of Smith et al. (1975), Morissette et al. (1992), and Mora et al., (1994). They found that the estrogen began to increase at

diestrus and reach its peak on proestrus then declined to the base line values by estrus and metestrus, while progesterone was increased at diestrus and proestrus. Proestrus or preovulatory increase of progesterone are ovarian and adrenal in origin (Nequin and Schwartz, 1971), and it is believed that this progesterone co-acts with estrogens in the positive feedback for release of ovulatory LH (Lee et al., 1990), and facilitates the release of the secondary surge of FSH that initiates the ovarian follicular recruitment leading to a new ovulatory estrous cycle (Knox et al., 1993).

In human studies, leptin levels during the menstrual cycle are conflicting. leptin levels showed no significant changes throughout the menstrual cycle in women (Mills et al., 1998; Lin, 1999; Yamada et al., 2000), and there was no positive correlation between leptin, estrogen and progesterone levels during the menstrual cycle (Teirmaa et al., 1998; Mills et al., 1998; Lin, 1999; Hadjiet al., 2000). These data clearly indicate that physiological alterations of circulating estrogen and progesterone have no significant effect on the leptin during normal menstrual cycle. On the contrary, the serum leptin levels were significantly higher in the luteal phase than in follicular phase in pre-. menopausal women (Riad-Gabriel et al., 1998; Quinton et al., 1999; Cella et al., 2000; Ludwig et al., 2000).

Leptin receptor (leptin-R) expression is regulated during the estrous cycle. The unchanged serum leptin levels during the estrous cycle together with the correlation between the expression of leptin-R and neuropeptide Y provide circumstantial evidence that regulation of leptin receptor abundance in the hypothalamus governs the biological actions of leptin (Bennett et al., 1999). Total leptin receptor transcript levels were lowest in proestrus in the choroid plexus, these changes correspond inversely with levels of circulating estradiol in the rat 4-day estrous cycle (Bennett et al., 1999).

17β-estradiol increased leptin secretion in male and female rats adipose tissue in vitro (Kristensen et al., 1999). Also, administration of 17β-estradiol significantly elevated leptin mRNA levels in adipose tissue of intact (Brann et al., 1999), or ovariectomized female rat in vivo (Shimizu et al., 1997) and in vitro (Murakami et al., 1995). Estrogen elevated the decreased leptin serum level after ovariectomy in rats (Chu et al., 1999). The present results concerning the effect of estrogen treatment (5μg/kg b. wt s.c for 7 days) are compatible with the previous observations in vivo and in vitro studies and demonstrate the importance of estrogen on leptin secretion in vivo in female rat. Moreover, estrogen replacement therapy in postmenopausal women significantly increases total serum leptin levels (Elbers et al., 1999; Lavoie et

al., 1999). In addition, high level of estrogen treatment stimulates the increase of plasma leptin in normal cyclic women (Lin, 2000).

On the other hand, administration of estrogen did not affect either leptin mRNA expression or the circulating concentration of leptin in rats (Wu-Peng et al., 1999). Moreover, studies on estrogen replacement therapy have failed to demonstrate an association between estrogen intake and leptin levels in postmenopausal women (Kohrt et al., 1996; Castracane et al., 1998).

The ob gene has a consensus sequence of the estrogen responsive element in its promoter region (Savouret et al., 1994). High affinity estrogen specific macromolecular binding of 17β-[^{3}H] estradiol takes place in the cytoplasmic fraction of various white adipose tissues (Wade and Gray, 1978). In female rats, ovariectomy induced a 25% decrease in ob gene mRNA expression in perirenal adipocytes and estrogens modulate ob gene expression at the mRNA level through sex steroid receptor-dependent transcriptional mechanisms (Machinal et al., 1999).

Progesterone treatment s.c daily for 7 days had no effect on plasma leptin levels in female rats. This finding is consistent with that of Kristensen et al. (1999), who found that progesterone did not affect the leptin secretion in male and female rats adipose tissue in vitro. Moreover, physiological progesterone replacement therapy did not influence leptin levels in young cycling women (Stock et al., 1999), and postmenopausal women (Lavoie et al., 1999).

Estrogen-progesterone treatment s.c had no effect on plasma leptin levels in female rats in the current study. Administration of progesterone (1mg/rat) after estradiol injection did not enhance the elevated leptin mRNA levels in adipose tissue (Brann et al., 1999). In addition, Estrogen-progestin oral contraceptives in young women did not affect serum leptin concentrations (Castracane et al., 1998; Teirmar et al., 1998).

In conclusion, Leptin concentration in female rats showed non-significant differences among the estrous cycle. Estradiol benzoate increased the plasma leptin concentration, while the progesterone caproate or estrogen plus progesterone treatments had no effect. The present study demonstrated that 17β-estradiol can regulate leptin secretion in female rat and the physiological alterations of circulating estrogen and progesterone have no significant effect on the leptin during normal estrous cycle.

REFERENCES

Agarwal, S. K.; Vogel, K.; Weitsman, S. R. and Magoffin, D. A. (1999): Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa

and theca cells of the human ovary. J. Clin. Endocrinol. Metab., 84(3):1072-1076.

Ahima, R. S.; Prabakaran, D.; Mantzoros, C.; Qu, D.; Lowell, B.; Maratos-Flier, E. and Flier, J. S. (1996): Role of leptin in the neuroendocrine response to fasting. Nature, 382(6588):250-252.

Ambrosius, W. T.; Compton, J. A.; Bowsher, R. R. and Pratt, J. H. (1998): Relation of race, age, and sex hormone differences to serum leptin concentrations in children and adolescents. Horm. Res., 49(5):240-246.

Amico, J. A.; Thomas, A.; Crowley, R. S. and Burmeister, L. A. (1998): Concentrations of leptin in the serum of pregnant, lactating, and cycling rats and of leptin messenger ribonucleic acid in rat placental tissue. Life Sci., 63 (16):1387-1395.

Barash, I. A.; Cheung, C. C.; Wigle, D.S.; Ren, H.; Kabitting, E. B.; Kuijer, J. L.; Clifton, D. K. and Steiner, R. A. (1996): Leptin is a metabolic signal to the reproductive system. Endocrinology, 133:3144-3147.

Bennett, P. A.; Lindell, K.; Wilson, C., Carlsson, L. M., Carlsson, B. and Robinson, I. C. (1999): Cyclical variations in the abundance of leptin receptors, but not in circulating leptin, correlate with NPY expression during the oestrous cycle. Neuroendocrinology, 69(6): 417-423.

Blum, W. F.; Englaro, P.; Heiman, M.; Attanasio, A. M.; Kiess, W. and Rascher, W. (1997): Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage and testosterone. J. Clin. Endocrinol. Metab., 82:2904-2910.

Brann, D. W.; De Sevilla, L.; Zamorano, P. L. and Mahesh, V. B. (1999): Regulation of leptin gene expression and secretion by steroid hormones. Steroids, 64(9): 659-663.

Campfield, L. A.; Smith, F. J.; Guisez, Y.; Devos, R. and Burn, P. (1995): Recombinant mouse ob protein: evidence for a peripheral signal linking adiposity and central neural networks. Science, 269:546-549.

Castracane, V. D.; Kraemer, R. R.; Franken, M. A.; Kraemer, G. R. and Gimpel, T. (1998): Serum leptin concentration in women: effect of age, obesity, and estrogen administration. Fertil. Steril., 70(3): 472-477.

Cella, F.; Giordano, G. and Cordera, R. (2000): Serum leptin concentrations during the menstrual cycle in normal-weight women: effects of an oral triphasic estrogen-progestin medication. Eur. J. Endocrinol., 142(2):174-178.

Chehab, F. F.; Lim, M. E. and Lu, R. (1996): Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nature Genetics, 12(3):318-320.

Chehab, F. F.; Mounzih, K.; Lu, R. and Lim, M. E. (1997): Early onset of reproductive function in normal female mice treated with leptin. Science, 275(5296):88-90.

Chu, S. C.; Chou, Y. C.; Liu, J. Y.; Chen, C. H.; Shyu, J. C. and Chou, F. P. (1999): Fluctuation of serum leptin level in rats after ovariectomy and the influence of estrogen supplement. Life Sci., 64(24):2299-306.

Duggal, P. S.; Van Der Hoek, K. H.; Milner, C. R.; Ryan, N. K.; Armstrong, D. T.; Magoffin, D. A. and Norman, R. J. (2000): The in vivo and in vitro effects of exogenous leptin on ovulation in the rat. Endocrinology, 141 (6):1971-1976.

Elbers, J. M.; De Roo, G. W.; Popp-Snijders, C.; Nicolaas-Merkus, A.; Westerveen, E.; Joenje, B. W. and Netelenbos, J. C. (1999): Effects of administration of 17beta-

oestradiol on serum leptin levels in healthy postmenopausal women. Clin. Endocrinol., 51(4):449-454.

Frederich, R.; C.; Hamann, A.; Anderson, S.; Lollman, B. and Fleir, J. S. (1995): Leptin levels reflect body lipid content in mice. Nat. Med., 1: 1311-1314.

Hadji, P.; Hars, O.; Bock, K.; Sturm, G.; Bauer, T.; Emons, G. and Schulz, K. D. (2000): The influence of menopause and body mass index on serum leptin concentrations. Eur. J. Endocrinol., 143(1):55-60.

Hislop, M. S.; Ratanjee, B. D.; Soule, S. G. and Marais, A. D. (1999): Effects of anabolic-androgenic steroid use or gonadal testosterone suppression on serum leptin concentration in men. Eur. J. Endocrinol., 141(1):40-46.

Hwa, J. J.; Ghibaudi, L.; Compton, D.; Fawzi, A. B. and Strader, C. D. (1996): Intracerebroventricular injection of leptin increases thermogenesis and mobilizes fat metabolism in ob/ob mice. Horm. Metab. Res., 128(12): 659-663.

Hwa, J. J.; Fawzi, A. B.; Graziano, M. P.; Ghibaudi, L.; Williams, P.; Van Heek, M.; Davis, H.; Rudinski, M.; Sybertz, E. and Strader, C. D. (1997): Leptin increases energy expenditure and selectively promotes fat metabolism in ob/ob mice. Am. J. Physiol., 272(4 Pt 2): R1204-1209.

Isidori, A. M.; Strollo, F.; More, M.; Caprio, M.; Aversa, A.; Moretti, C.; Frajese, G.; Riondino, G. and Fabbri, A. (2000): Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. J. Clin. Endocrinol. Metab., 85 (5):1954-1962.

Kiess, W.; Reich, A.; Meyer, K.; Glasow, A.; Deutscher, J.; Klammt, J.; Yang, Y.; Muller, G. and Kratzsch, J. (1999): A Role for Leptin in Sexual Maturation and Puberty?. Horm. Res., 51 Suppl S3: 55-63.

Knox, K. L.; Bingatrom, S. J, Schwartz, N. B. (1993): RU486 blocks the effects of inhibin antiserum or luteinizing hormone on the secondary follicle-stimulating hormone surge. Endocrinology, 133:277-283.

Kohrt, W. M.; Landt, M. and Birge, S. J. Jr. (1996): Serum leptin levels are reduced in response to exercise training, but not hormone replacement therapy, in older women. J. Clin. Endocrinol. Metab., 81(11):3980-3985.

Kohsaka, A.; Watanobe, H.; Kakizaki, Y.; Habu, S. and Suda, T. (1999): A significant role of leptin in the generation of steroid-induced luteinizing hormone and prolactin surges in female rats. Biochem. Biophys. Res. Commun., 254(3): 578-581.

Kristensen, K.; Pedersen, S. B. and Richelsen, B. (1999): Regulation of leptin by steroid hormones in rat adipose tissue. Biochem. Biophys. Res. Commun., 259(3):624-630.

Kubasik, N. P.; Hallauer, G. D. and Brodows, R. G. (1984): Evaluation of a direct solid-phase radioimmunoassay for progesterone, useful for monitoring luteal function. Clin. Chem., 30(2):284-286.

Lagiou, P.; Signorello, L. B.; Mantzoros, C. S.; Trichopoulos, D.; Hsieh, C. C. and Trichopoulou, A. (1999): Hormonal, lifestyle, and dietary factors in relation to leptin among elderly men. Ann. Nutr. Metab., 43(1):23-29.

Lavoie, H. B.; Taylor, A. E.; Sharpless, J. L.; Anderson, E. J.; Strauss, C. C. and Hall J. E. (1999): Effects of short-term hormone replacement on serum leptin levels in postmenopausal women. Clin. Endocrinol. (Oxf), 51(4): 415-422.

Lee, W. S.; Smith, M. S. and Hotlinan, G. E. (1990): Progesterone enhances the surge of luteinizing hormone by increasing the activation of luteinizing hormone-releasing hormone neurons. Endocrinology, 127:2604-2606.

Lin, K. C. (1999): Changes of circulating leptin levels during normal menstrual cycle: relationship to estradiol and progesterone. Kao Hsiung I Hsueh Ko Hsueh Tsa Chih, 15(10): 597-602.

Lin, K. C. (2000): Increase of leptin levels following exogenous administration of estrogen in women with normal menstruation. Kao Hsiung I Hsueh Ko Hsueh Tsa Chih, 16(1):13-19.

Ludwig, M.; Klein, H. H.; Diedrich, K. and Ortmann, O. (2000): Serum leptin concentrations throughout the menstrual cycle. Arch. Gynecol. Obstet., 263(3):99-101.

Machinal, F.; Dieudonne, M. N.; Leneveu, M. C.; Pecquery, R. and Giudicelli, Y. (1999): In vivo and in vitro ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. Endocrinology, 140(4):1567-1574.

Mannucci, E.; Ognibene, A.; Becorpi, A.; Cremasco, F.; Pellegrini, S.; Ottanelli, S.; Rizzello, S. M.; Massi, G.; Messeri, G. and Rotella, C. M. (1998): Relationship between leptin and oestrogens in healthy women. Eur. J. Endocrinol., 139(2):198-201.

McCann, S. M.; Kimura, M.; Walczewska, A.; Karanth, S.; Rettori, V. and Yu, W. H. (1998): Hypothalamic control of gonadotropin secretion by LHRH, FSHRF, NO, cytokines, and leptin. Domest. Anim. Endocrinol., 15 (5):333-344.

Mills, P. J.; Ziegler, M. G. and Morrison, T. A. (1998): Leptin is related to epinephrine levels but not reproductive hormone levels in cycling African-American and Caucasian women. Life Sci., 63(8): 617-623.

Mora, O. A.; Cabrera, M. M. and Sanchez-Criado, J. E. (1994): Hormonal pattern of the pheromonal restoration of cyclic activity in aging irregularly cycling and persistent-estrus female rats. Biol. Reprod., 51:920-925.

Morissette M.; Garcia-Segura, L. M.; Belanger, A. and Di Paolo, T. (1992): Changes of rat striatal neuronal memberane morphology and steroid content during the estrous cycle. Neuroscience, 49(4):893-902.

Murakami, T.; Lida, M. and Shima, K. (1995): Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem. Biophys. Res. Commun., 214: 1260-1267.

Nequin, L. G. and Schwartz, N. B. (1971): Adrenal participation in the timing of mating and LH release in the cyclic rat. Endocrinology, 88:325-331.

Ostlund, R. E. Jr.; Yang, J.W.; Klein, S. and Gingerich, R. (1996): Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J. Clin. Endocrinol. Metab., 81(11): 3909-3913.

Pinilla, L.; Seoane. L. M.; Gonzalez, L.; Carro, E.; Aguilar, E.; Casanueva, F. F. and Dieguez, C. (1999): Regulation of serum leptin levels by gonadal function in rats. Eur. J. Endocrinol., 140(5): 468-473.

Quinton, N. D.; Laird, S. M.; Okon, M. A.; Li, T. C.; Smith, R. F.; Ross, R. J. and Blakemore, A. I. (1999): Serum leptin levels during the menstrual cycle of healthy fertile women. Br. J. Biomed. Sci., 56(1):16-19.

Riad-Gabriel, M. G.; Jinagouda, S. D.; Sharma, A.; Boyadjian, R. and Saad, M. F. (1998): Changes in plasma leptin during the menstrual cycle. Eur. J. Endocrinol., 139

(5):528-531.

Savouret, J. F.; Rauch, M.; Redeuilh, G.; Sar, S.; Chauchereau, A.; Woddruff, K.; Parker, M. G. and Milgrom, E. (1994): Interplay between estrogens, progestins, retinoic acid and AP-1 on a single regulatory site in the progesterone receptor gene. J. Biol. Chemist., 269:28955-28962.

Schwartz, M. W.; Baskin, D. G.; Bukowski, T. R.; Kujiper, J. L.; Foster, D.; Lasser, G.; Prunkard, D. E.; Porte, D.; Woods, S. C.; Seeley, R. J. and Weigle, D. S. (1996): Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes, 45: 531-535.

Shimizu, H.; Shimomura, Y.; Nakanishi, Y.; Futawatari, T.; Ohtani, K.; Sato, N. and Mori, M. (1997): Estrogen increases in vivo leptin production in rats and human subjects. J. Endocrinol., 154(2): 285-292.

Smith M. S.; Freeman , M. E. and Neill, J. D. (1975): The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotrophin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology, 96(1):219-226.

Snedecor, G.W. and Cochran, W.G. (1990): Statistical Methods. 9th Ed. Oxford and J.B.H. Publishing Co.

Spicer, L. J. and Francisco, C.C. (1997): The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. Endocrinology, 138(8):3374-3379.

Spicer, L. J. and Francisco, C. C. (1998): Adipose obese gene product, leptin, inhibits bovine ovarian thecal cell steroidogenesis. Biol. Reprod., 58(1): 207-212.

Spicer, L. J.; Chamberlain, C. S. and Francisco, C. C. (2000): Ovarian action of leptin: effects on insulin-like growth factor-I-stimulated function of granulosa and thecal cells. Endocrine, 12(1):53-59.

Stock, S. M.; Sande, E. M. and Bremme, K. A. (1999): Leptin levels vary significantly during the menstrual cycle, pregnancy, and in vitro fertilization treatment: possible relation to estradiol. Fertil. Steril., 72(4):657-662.

Teirmaa, T.; Luukkaa, V.; Rouru, J.; Koulu, M. and Huupponen, R. (1998): Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. Eur. J. Endocrinol., 139(2):190-194.

Wade, G. N. and Gray, J. M. (1978): cytoplasmic 17β-{3_H}estradiol binding I rat adipose tissues. Endocrinology, 103:1695-1701.

Wade, G. N.; Lempicki, R. L.; Panicker, A. K.; Frisbee, R. M. and Blaustein, J. D. (1997): Leptin facilitates and inhibits sexual behavior in female hamsters. Am. J. Physiol., 272(4 Pt 2): R1354-1358.

Watanobe, H. and Suda, T. (1999): A detailed study on the role of sex steroid milieu in determining plasma leptin concentrations in adult male and female rats. Biochem. Biophys. Res. Commun., 259(1):56-59.

Watanobe, H.; Suda, T.; Wikberg, J. E. and Schioth, H. B. (1999): Evidence that physiological levels of circulating leptin exert a stimulatory effect on luteinizing hormone and prolactin surges in rats. Biochem. Biophys. Res. Commun., 16;263(1):162-165.

Wu-Peng, S.; Rosenbaum, M.; Nicolson, M.; Chua, S. C. and Leibel, R. L. (1999): Effects of exogenous gonadal steroids on leptin homeostasis in rats. Obes. Res., 7(6): 586-592.

Xing, S.; Chkan, S. Z. and Disczfalusy, U. (1983): Validation of radioimmunoassay for estradiol-17β by isotope

dilution-Mass spectrometry and by a test of radiochemical purity. Clinica Chemica Acta, 135:189-201.

Yamada, M.; Irahara, M.; Tezuka, M.; Murakami, T.; Shima, K. and Aono, T. (2000): Serum leptin profiles in the normal menstrual cycles and gonadotropin treatment cycles. Gynecol. Obstet. Invest., 49(2):119-123.

Yu, W. H.; Walczewska, A.; Karanth, S. and McCann, S. M. (1997a): Nitric oxide mediates leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. Endocrinology, 138(11):5055-5058.

Yu, W. H.; Kimura, M.; Walczewska, A.; Karanth, S. and McCann, S. M. (1997b): Role of leptin in hypothalamic-pituitary function. Proc. Natl. Acad. Sci., 94(3): 1023-1028.

Zachow, R. J. and Magoffin, D. A. (1997): Direct intraovarian effects of leptin: impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17 beta production by rat ovarian granulosa cells. Endocrinology, 138(2): 847-850.

Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L. and Friedman, J. M. (1994): Positional cloning of the mouse obese gene and its human homologue. Nature. 372: 425-4