Iranian Journal of Veterinary Research, Shiraz University, 2013, Vol. 14, No. 4, Pages 327-333

Leptin receptor mRNA expression in Taleshi ram gonads

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(Received 10 Oct 2012; revised version 15 Apr 2013; accepted 24 Apr 2013)

Summary

Leptin is secreted mainly by fat, which is involved in energy metabolism and reproduction. Leptin and its receptor (Ob-R) have been detected in human spermatozoa and testis, thus it can be concluded leptin involves in male physiology. The goal of this study was determination of the presence of leptin receptor mRNA in the ram testis, epididymis and spermatozoa by reverse transcription polymerase chain reaction (RT-PCR). Ejaculated spermatozoa from ten fertile Taleshi ram were collected by means of an artificial vagina. Testes, placental cotyledons and adrenal glands were obtained from abattoir. Placental cotyledons and adrenal glands were used as the positive control. Epididymal spermatozoa recovery was performed from epididymis. To purify spermatozoa, motile sperm were isolated by the swim-up procedure. Total RNA was isolated from epididymal spermatozoa, ejaculated spermatozoa, adrenal gland and placental cotyledon and then they were purified. The mRNA for the long form (Ob-Rb) and the short form (Ob-Ra) of leptin receptor was detected in testis. RT-PCR analysis of total RNA from epididymal spermatozoa and ejaculated spermatozoa revealed the presence of leptin mRNA in these cells. The mRNA for Ob-Rb was observed in epididymis and epididymal spermatozoa, but the Ob-Ra mRNA was absent. The presence of Ob-Ra mRNA was found in ejaculated spermatozoa, whereas Ob-Rb mRNA did not exist. It can be concluded that the mRNA for leptin receptor is present in ram gonads and spermatozoa.

Key words: Ram, Spermatozoa, mRNA, Ob-Rb, Ob-Ra

Introduction

Since the discovery of 16 kDa protein which has been called leptin by Zhang *et al*. (1994) many studies have been performed to determine the role of this hormone. Leptin, which is a hormone derived from the adipocyte, was originally considered to exert its satiety action in CNS, and it plays a key role in body weight homeostasis. Leptin modulates different systems via neuro-endocrine regulation, including adrenal, thyroid and gonadal axes. But additional functions of leptin have been suggested in peripheral organs in which leptin independently interacts with leptin receptor in peripheral tissue, as well as reproductive organs (Clarke and Henry, 1999).

To date, at least five Ob-R isoforms (Ob-Ra-e) have been cloned from rodents and exhibited widespread distribution in both central and peripheral tissues (Tartaglia *et al*., 1995). Alternative splicing of the Ob-R transcript results in the various products that vary in the length of the cytoplasmic region. Three Ob-R isoforms, the long form (Ob-Rb), the middle form (Ob-Ra) and the short form (Ob-Rc) were cloned and sequenced, respectively in bovine (Kawachi *et al*., 2007) and also the mRNA of Ob-Rb and Ob-Ra were reported in ovine (Thomas *et al*., 2001; Charles *et al*., 2006). Leptin receptor long isoform has been reported in the majority of farm animal tissues (Tartaglia, 1997).

It is known that animal and human models of leptin resistance and deficiency show a severe impairment of the

reproductive function (Caprio *et al*., 2003). Leptin receptor mRNA has been found in bovine, murine, human and porcine oocytes and human follicle (Cervero *et al*., 2006). Moreover, leptin receptors are present in several cell types in the human ovary (Clarke and Henry, 1999). Leptin interacts with the reproductive axis at multiple sites, with stimulatory effects at the hypothalamus and pituitary and inhibitory actions at the gonads (Moschos *et al*., 2002). The processes of interaction between leptin and reproductive system are different in male and female. Interestingly, whereas ob/ob females are invariably infertile, a limited number of ob/ob males have been reported to have normal reproductive development and to be fertile (Cunningham *et al*., 1999). It has been shown that leptin expresses differentially in men and women (Wauters and Van Gaal, 1999). After puberty onset, sex differences in both hypothalamic Ob-Rb expression (female>male) and plasma leptin levels (male>female) were observed in rat (Smith and Waddell, 2003).

To understand fully the peripheral effects of leptin as a local factor, it is necessary to study the structure and the peripheral tissue distribution of its receptor (Ob-R). The role of leptin receptor to the proper functioning of the male gonad has been less clear. It was suggested that there was species difference for the expression of leptin receptor in spermatozoa and leptin receptor long isoform may not be present on human sperm (Hatami-Baroogh *et al*., 2010). There is little information about the expression of leptin receptor in the ram gonads although the presence of leptin receptor mRNA have been reported in some farm animals testis (Rago *et al*., 2009). Thus, the goal of this study was determination of the presence of leptin receptor mRNA in the ram testis, epididymis and spermatozoa by RT-PCR.

Materials and Methods

Samples collection and preparation

Ejaculated spermatozoa from ten fertile Taleshi ram were obtained by means of an artificial vagina during the breeding season (autumn to early winter). Semen was diluted 1:1 (v/v) with tris glucose diluents (300 mM tris (hydroxymethyl) aminomethane, 95 mM citric acid monohydrate, 28 mM D-(+)-Glucose, 2000 IU/mL penicillin G and 0.4 mg/mL streptomycin pH = 7.0) and transported to the laboratory in an insulated Styrofoam box. A complete semen analysis was performed on each sample: sperm concentration was $>2.5 \times 10^9$/mL with motility >65% and viability >70%. To purify spermatozoa, motile sperm were isolated by the swim-up procedure; 250 µL of semen were layered under 1 mL of tris glucose diluents in each of the 15-mL centrifuge tubes. After incubation for 1 h at 37°C in an atmosphere with 5% CO_2, 750 µL was removed from the top of each tube. Sperm were concentrated by centrifugation at 700 × g for 10 min and the top of suspension was removed.

Testes of ten Taleshi rams, placental cotyledons and adrenal glands of 10 ewes were obtained from abattoir. Placental cotyledons and adrenal glands were frozen in liquid nitrogen and stored but testes were placed in sterile plastic containers, including warm (37°C) normal saline (0.9% NaCl), and their lids were sealed tightly. They were transported in an insulated Styrofoam box, which was heated by water bottles with a temperature of 37°C. In the laboratory, the caudal epididymis was isolated from testis. The tissue of testes and half of epididymises were frozen and stored by liquid nitrogen. Epididymal spermatozoa were obtained by slicing and suspending of the remaining caudal epididymal tissue in Petri dish containing 5 mL tris glucose diluents. The samples were agitated and incubated at room temperature for 10 min; liquid phase containing epididymal spermatozoa was collected. Recovered epididymal spermatozoa were centrifuged for 10 min at 700 × g at room temperature; the supernatant was removed. The swim-up procedure was performed to obtain purified epididymal spermatozoa the same as ejaculated spermatozoa and purity of both samples was microscopically checked.

RNA isolation, purity and concentration

Homogenized ewe placental cotyledon

tissue was used as a source of cells expressing leptin receptor. Total RNA from cotyledon, adrenal glands, testes, epididymal tissue, ejaculated and epididymal spermatozoa were extracted according to Chomczynski and Sacchi (1987). Briefly, 0.5 mL denaturing solution containing 4 M guanidinium thiocyanate was added to 2 × 10^9 cells or 50-100 mg tissue. The homogenate was mixed sequentially with 50 µL sodium acetate (2 M, pH = 4), equal volume of water saturated phenol, and 0.2 volume of chloroform/isoamylalcohol (49:1). The resulting mixture was centrifuged, yielding an upper aqueous phase containing total RNA. After a 100% isopropanol precipitation, RNA pellet was redissolved in 300 µL denaturing solution, reprecipitated with isopropanol, and washed with 75% ethanol. RNA samples were resuspended in 30 µL diethylpyrocarbonate (DEPC) - treated water and stored at -80°C.

Concentration and purity of RNA were determined by Biophotometer (Eppendorf, Germany) (OD 260/280 and 260/230 ratio) and visualized by electrophoresis after DNase I treatment on a 2% agarose gel, stained with 0.5 µg/mL ethidium bromide. RNA samples with no DNA bands after DNaseI treatment were used in RT-PCR reactions.

cDNA synthesis and RT-PCR

RNA samples were treated by DNase I at 37°C for 30 min and then the enzyme was inactivated at 75°C for 15 min. Treated RNA was reverse transcribed to cDNA in a 20 µL final volume containing 1 µg of extracted RNA, 200 ng random hexamer and 0.5 mM Deoxyribonucleotide triphosphate (dNTP) Mix. The mixture was heated at 65°C for 5 min then 4 U (in 1 µL) RNase inhibitor, RT buffer (50 mM Tris-HCL, 75 mM KCL, 3 mM $MgCL_2$), 10 mM DTT and 200 units M-MuLV Reverse transcriptase (Fermentas, Germany) were added. This mixture was incubated for 60 min at 37°C. The prepared cDNA was incubated at 75°C for 15 min to denature the M-MuLV-RT and then stored at -20°C.

Aliquots of 3 µL of the first standard cDNA reaction were amplified in a 50 µL reaction volume containing a final concentration of 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 2 U of recombinant Taq DNA polymerase (Fermentas) and 0.4 mM of primers. The cDNA primers to the leptin receptor sequence which recognizes all known splice variants of the leptin receptor (Ob-R) were 5′-TGCCACCAAATACAACATATGACT-3′ (99 to 122) and 5′-CTTAGTTTCAACAA CTGCCTCAGA-3′ (176 to 199, Genbank U63719), 101 bp product (Thomas *et al*., 2001). The cDNA primers to the long form of the ovine leptin receptor, Ob-Rb, were 5′-GATGAGATGGTGCCAACAACTA-3′ (121 to 142) and 5′-TGGGTTTCTATTTCC CATGATC-3′ (407 to 428, Genbank OAU62124), 308 bp product (Thomas *et al*., 2001). The cDNA primers to the short form of the ovine leptin gene, Ob-Ra, were 5′-TTGAGAAGTACCAGTTCAGTC-3′ (2480 to 2500) and 5′-CAAAGAATGTCCGTTCT CTTCT-3′ (2735 to 2756, Genbank AY278244) which generated a 277 bp product (Charles *et al*., 2006). The cDNA primers to β-actin were 5′-CCAACCGTGA GAAGATGA-3′ (413 to 430) and 5′-GAAG GAAGGCTGGAAGAG-3′ (853 to 870, Genbank HM067830), 458 bp product.

PCR was performed on a Eppendorf thermal cycler (Mastercycler® personal, Eppendorf, Germany) using the following conditions: 94°C (4 min) 1 cycle; 94°C (1 min), 55°C (1 min), 72°C (1 min) 45 cycles; 72°C (10 min) 1 cycle. 15 µL of each PCR product were subjected to electrophoresis in 1.5% agarose gel and stained with 0.5 µg/mL ethidium bromide.

Results

RT-PCR results are shown in Fig. 1. RNA isolated from cotyledon and adrenal cells were used as positive control. RT-PCR with specific primers for Ob-R, Ob-Rb, Ob-Ra and β-actin revealed a predicted RT-PCR product of 98, 308, 277 and 458 bp in length, respectively (Fig. 1, lanes 17-24).

RT-PCR analysis of total RNA from epididymal spermatozoa and ejaculated spermatozoa revealed the presence of leptin mRNA in these cells. The presence of Ob-Ra mRNA was observed in ejaculated spermatozoa, whereas Ob-Rb mRNA did not

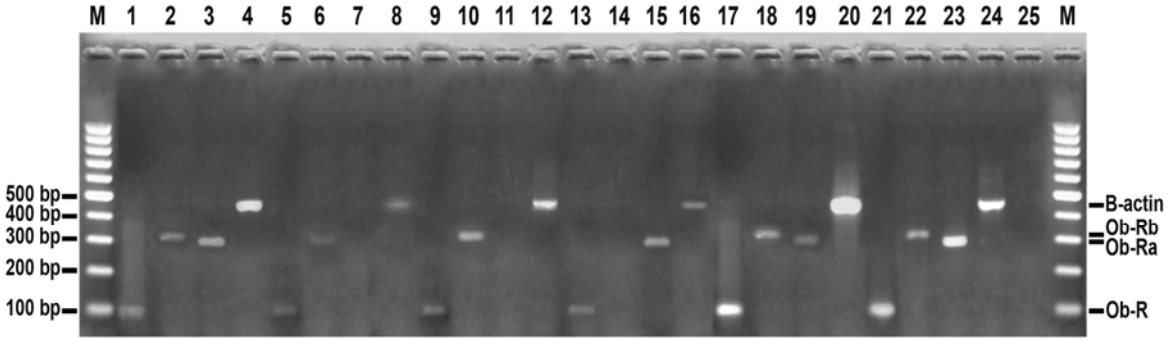

Fig. 1: Representative Ob-R, Ob-Rb, Ob-Ra and β-actin transcripts in testis, tail of epididymis, epididymal sperm, ejaculated sperm, cotyledon and adrenal. M = Molecular weight standards (1 Kb DNA ladder, Fermentas). The numbers in the Figure indicate: (1-4) RT-PCR result of Ob-R, Ob-Rb, Ob-Ra and β-actin of testis; (5-8) RT-PCR result of Ob-R, Ob-Rb, Ob-Ra and β-actin of epididymal sperm; (9-12) RT-PCR result of Ob-R, Ob-Rb, Ob-Ra and β-actin of epididymis; (13-16) RT-PCR result of Ob-R, Ob-Rb, Ob-Ra and β-actin of ejaculated sperm, (17-24) PCR positive samples; (25) blank. Cotyledon cells (17-20) and adrenal cells (21-24) were used as positive control for Ob-R, Ob-Rb and Ob-Ra

exist (Fig. 1, lanes 13-16). Amplification of cDNA prepared with RNA isolated from both epididymis tissue and epididymal spermatozoa predicted RT-PCR products of 98, 308 and 458 bp for OB-R, Ob-Rb and β-actin, respectively, but it lacked the Ob-Ra mRNA (Fig. 1, lanes 5-12). The mRNA for Ob-R, Ob-Rb, Ob-Ra and β-actin were detected in all samples of testis tissue (Fig. 1, lanes 1-4).

Discussion

Leptin, as a nutritional signal, does participate in the functional regulation of the male reproductive system. The mechanisms of leptin are multifaceted and likely involve actions at different levels of the hypo-thalamic-pituitary-gonadal axis (Tena-Sempere and Barreiro, 2002). In *Ovis aries*, the express of Ob-R mRNA was reported in hypothalamus and pituitary (Dyer *et al*., 1997) but it was not evaluated in ram testis, epididymis and ejaculated spermatozoa until now.

We observed that Ob-R mRNA was expressed in male gonads where spermatozoa were produced (testis), stored and made acquisition of motility and potential fertility (epididymis). Ram testis and epididymis expressed long form of leptin receptor (Ob-Rb mRNA), whereas short form of leptin receptor (Ob-Ra mRNA) was detected only in testis. In comparison to previous studies on mammals, our data reveal a species-specificity of Ob-R expression in ram epididymis. Ob-Rb and Ob-Ra have been reported in human (Ishikawa *et al*., 2007), mouse (El-Hefnawy *et al*., 2000), rat (Caprio *et al*., 2003) bovine (Kawachi *et al*., 2007) and swine testis (Rago *et al*., 2009). Furthermore, the existence of leptin sensors in white adipose tissue of rat epididymis was illustrated (Niijima, 1998) and also the gene expression of leptin and leptin receptors, Ob-Ra and Ob-Rb, were shown in the rat (Gombar *et al*., 2012) and swine epididymis (Rago *et al*., 2009). In the current experiment, there is a difference between ram testis and ram epididymis in the isoforms of leptin receptor mRNA, thus, it could be speculated that the possible control mechanism of leptin was not the same in both tissues.

To our knowledge, the presence of Ob-R mRNA in the ram gonads has not been demonstrated and there has been no information on the possible actions of leptin on ram gonads yet. The present study illustrated the expression of the two isoforms Ob-R mRNA (Ob-Ra and Ob-Rb) in the ram genital duct. Epididymis and testis contain both spermatozoa and somatic cells; therefore, there may be leptin receptor mRNA expression in somatic cells or gametes in these sites. Leptin has been suggested to regulate the proliferation and differentiation of mouse testicular germ cells by the activation of STAT3 (El-Hefnawy *et*

al., 2000) and the dysfunction of spermatogenesis appears to be associated with an altered leptin and leptin receptor expression in human testis (Ishikawa *et al*., 2007). Moreover, it was reported that leptin receptor expression on leydig cells was inversely correlated with serum testosterone concentration in azoospermia infertile men (Ishikawa *et al*., 2007). After spermatogenesis and spermiation, changes in the sperm membrane and cytoplasm, by addition and deletion of several proteins and molecules, occur during the epididymal transit (Moore, 1990). In most species, sperm attain progressive motility and fertilizing capacity during their transit through various regions of the epididymis. The biological actions of leptin on target tissues are carried out through interaction with its specific membrane receptor (Tartaglia *et al*., 1995). Therefore, the presence of Ob-R mRNA in testis and epididymis may suggest a role for leptin in ram gonadal function, as shown in human (Ishikawa *et al*., 2007) and rat (Caprio *et al*., 2003).

It is our belief that this is the first time that the presence of Ob-Rb mRNA in ejaculated spermatozoa and Ob-Ra mRNA epididymal spermatozoa have been reported in ram; the size of the band for Ob-Rb and Ob-Ra detected after gel electrophoresis is similar to that found in other ovine tissues (Thomas *et al*., 2001; Charles *et al*., 2006). The results obtained from the ejaculated spermatozoa were in agreement with the data in bovine (Nikbakht *et al*., 2010), human (Jope *et al*., 2003) and swine (De Ambrogi *et al*., 2007).

It is noteworthy to mention, the Ob-R subtypes show different functional capacities. The full length isoform Ob-Rb contains intracellular motifs required for activation of the JAK/STAT pathway and it is considered to be a fully functional receptor (Frühbeck, 2006); whereas short isoform (Ob-Ra), having box 1 domain, activates only JAKs (Margetic *et al*., 2002). On the other hand, there are differences between epididymal spermatozoa, as the cell for storage in the gonads, and ejaculated spermatozoa in structure and function (Gloria *et al*., 2011; Hori *et al*., 2011). Therefore, it is likely different type of leptin receptor in these cells may relate to their function.

Spermatozoa are highly differentiated and specialized cells which are responsible for transporting paternal genome to oocyte. It seems the component of seminal plasma and female fluid tract regulate the function of spermatozoa. Many hormone receptors have been reported such as receptors of leptin (Jope *et al*., 2003; De Ambrogi *et al*., 2007; Nikbakht *et al*., 2010), GnRH (Lee *et al*., 2000), LH/hCG (Eblen *et al*., 2001), estrogen α (Solakidi *et al*., 2005), estrogen β (Solakidi *et al*., 2005), androgen (Solakidi *et al*., 2005), progesterone (Wu *et al*., 2005) and calcitonin (Adeoya-Osiguwa and Fraser, 2003) in ejaculated spermatozoa. It is generally believed that the functions of spermatozoa are regulated by the transduction of these receptors for successful fertilization (Naz and Sellamuthu, 2006). The activation of leptin receptor in human spermatozoa has been demonstrated to cause both glycogen synthesis kinase phosphorylation and the inhibition of acetyl-CoA carboxilase expression through PI3K/AKt pathway; as a result, it is involved in controlling spermatozoa energy expenditure (Andò and Aquila, 2005). Furthermore through PI3K/AKt pathway, the leptin receptor control apoptosis in human spermatozoa (Jope *et al*., 2003); these roles should also be studied in ram spermatozoa.

At present, there is no information on the possible actions of leptin on ram spermatozoa; yet, it can be speculated that the presence of a receptor mRNA may indicate that leptin exerts metabolic effects even in ram, as already demonstrated in humans (Aquila *et al*., 2005).

Acknowledgement

Author would like to thank the vice chancellor's office for research of the University of Guilan for supporting this research.

References

Adeoya-Osiguwa, S and Fraser, L (2003). Calcitonin acts as a first messenger to regulate adenylyl cyclase/cAMP and mammalian sperm function. Mol. Reprod.

Dev., 65: 228-236.

Andò, S and Aquila, S (2005). Arguments raised by the recent discovery that insulin and leptin are expressed in and secreted by human ejaculated spermatozoa. Mol. Cell. Endocrinol., 245: 1-6.

Aquila, S; Gentile, M; Middea, E; Catalano, S and Andò, S (2005). Autocrine regulation of insulin secretion in human ejaculated spermatozoa. Endocrinology. 146: 552-557.

Caprio, M; Fabbrini, E; Ricci, G; Basciani, S; Gnessi, L; Arizzi, M; Carta, AR; De Martino, MU; Isidori, AM; Frajese, GV and Fabbri, A (2003). Ontogenesis of leptin receptor in rat leydig cells. Biol. Reprod., 68: 1199-1207.

Cervero, A; Domínguez, F; Horcajadas, JA; Quiñonero, A; Pellicer, A and Simón, C (2006). The role of the leptin in reproduction. Curr. Opin. Obstet. Gynecol., 18: 297-303.

Charles, AD; Hyatt, K; Mlynarczyk, M; Kaushal, KM and Myers, DA (2006). Long-term hypoxia increases leptin receptors and plasma leptin concentrations in the late-gestation ovine fetus. Am. J. Physiol. Regul. Integr. Comp. Physiol., 291: 1406-1413.

Chomczynski, P and Sacchi, N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 162: 156-159.

Clarke, IJ and Henry, BA (1999). Leptin and reproduction. Rev. Reprod., 4: 48-55.

Cunningham, MJ; Clifton, DK and Steiner, RA (1999). Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol. Reprod., 60: 216-222.

De Ambrogi, M; Spinaci, M; Galeati, G and Tamanini, C (2007). Leptin receptor in boar spermatozoa. Int. J. Androl., 30: 458-461.

Dyer, CJ; Simmons, JM; Matteri, RL and Keisler, DH (1997). Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. Domest. Anim. Endocrinol., 14: 119-128.

Eblen, A; Bao, S; Lei, Z; Nakajima, S and Rao, C (2001). The presence of functional luteinizing hormone/chorionic gonadotropin receptors in human sperm. J. Clin. Endocrinol. Metab., 86: 2643-2648.

El-Hefnawy, T; Ioffe, S and Dym, M (2000). Expression of the leptin receptor during germ cell development in the mouse testis. Endocrinology. 141: 2624-2630.

Frühbeck, G (2006). Intracellular signalling pathways activated by leptin. Biochem. J., 393: 7-20.

Gloria, A; Contri, A; De Amicis, I; Robbe, D and Carluccio, A (2011). Differences between epididymal and ejaculated sperm characteristics in donkey. Anim. Reprod. Sci., 128: 117-122.

Gombar, F; Sampaio, F and Ramos, C (2012). Maternal malnutrition alters the leptin expression in the rats epididymis. Presented at Society for Endocrinology IECE May 2012, Florence, Italy. Endocrine Abstracts. 29: 1043.

Hatami-Baroogh, L; Razavi, S; Zarkesh-Esfahani, H; Tavalaee, M; Tanhaei, S; Ghaedi, K; Deemeh, MR; Rabiee, F and Nasr-Esfahani, MH (2010). Evaluation of the leptin receptor in human spermatozoa. Reprod. Biol. Endocrinol., 8: 17.

Hori, T; Matsuda, Y; Kobayashi, M; Kawakami, E and Tsutsui, T (2011). Comparison of fertility on intrauterine insemination between cryopreserved ejaculated and cauda epididymal sperm in dogs. J. Vet. Med. Sci., 73: 1685-1688.

Ishikawa, T; Fujioka, H; Ishimura, T; Takenaka, A and Fujisawa, M (2007). Expression of leptin and leptin receptor in the testis of fertile and infertile patients. Andrologia. 39: 22-27.

Jope, T; Lammert, A; Kratzsch, J; Paasch, U and Glander, HJ (2003). Leptin and leptin receptor in human seminal plasma and in human spermatozoa. Int. J. Androl., 26: 335-341.

Kawachi, H; Yang, SH; Hamano, A; Matsui, T; Smith, SB and Yano, H (2007). Molecular cloning and expression of bovine (Bos taurus) leptin receptor isoform mRNAs. Comp. Biochem. Physiol. B. Biochem. Mol. Biol., 148: 167-173.

Lee, C; Ho, J; Chow, S; Yasojima, K; Schwab, C and McGeer, P (2000). Immunoidentification of gonadotropin releasing hormone receptor in human sperm, pituitary and cancer cells. Am. J. Reprod. Immunol., 44: 170-177.

Margetic, S; Gazzola, C; Pegg, GG and Hill, RA (2002). Leptin: a review of its peripheral actions and interactions. Int. J. Obes. Relat. Metab. Disord., 26: 1407-1433.

Moore, H (1990). Development of sperm-egg recognition processes in mammals. J. Reprod. Fertil. Suppl., 42: 71-78.

Moschos, S; Chan, JL and Mantzoros, CS (2002). Leptin and reproduction. Fertil. Steri., 77: 433-444.

Naz, RK and Sellamuthu, R (2006). Receptors in spermatozoa: are they real? J. Androl., 27: 627-636.

Niijima, A (1998). Afferent signals from leptin sensors in the white adipose tissue of the epididymis, and their reflex effect in the rat. J. Auton. Nerv. Syst., 73: 19-25.

Nikbakht, G; Roostaei Ali Mehri, M; Baghbanzadeh, A; Tajik, P; Tamanini, C and Emam, M (2010). Leptin receptor mRNA in bull ejaculated spermatozoa. Reprod. Domest. Anim., 45: 237-242.

Rago, V; Aquila, S; Guido, C and Carpino, A (2009). Leptin and its receptor are expressed in the testis and in the epididymis of young and adult pigs. Anat. Rec. (Hoboken). 292: 736-745.

Smith, JT and Waddell, BJ (2003). Developmental changes in plasma leptin and hypothalamic leptin receptor expression in the rat: peripubertal changes and the emergence of sex differences. J. Endocrinol., 176: 313-319.

Solakidi, S; Psarra, AM; Nikolaropoulos, S and Sekeris, CE (2005). Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. Hum. Reprod., 20: 3481-3487.

Tartaglia, LA (1997). The leptin receptor. J. Biol. Chem., 272: 6093-6096.

Tartaglia, LA; Dembski, M; Weng, X; Deng, N; Culpepper, J; Devos, R; Richards, GJ; Campfield, LA; Clark, FT; Deeds, J; Muir, C; Sanker, S; Moriarty, A; Moore, KJ; Smutko, JS; Mays, GG; Wool, EA; Monroe, CA and Tepper, RI (1995). Identification and expression cloning of a leptin receptor, OB-R. Cell. 83: 1263-1271.

Tena-Sempere, M and Barreiro, ML (2002). Leptin in male reproduction: the testis paradigm. Mol. Cell. Endocrinol., 188: 9-13.

Thomas, L; Wallace, JM; Aitken, RP; Mercer, JG; Trayhurn, P and Hoggard, N (2001). Circulating leptin during ovine pregnancy in relation to maternal nutrition, body composition and pregnancy outcome. J. Endocrinol., 169: 465-476.

Wauters, M and Van Gaal, L (1999). Gender differences in leptin levels and physiology: a role for leptin in human reproduction. J. Gend. Specif. Med., 2: 46-51.

Wu, J; Tsai, P; Lee, S and Cheng, F (2005). Characterisation of the progesterone receptor on canine spermatozoa. Reprod. Fertil. Dev., 17: 733-741.

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