Iranian Journal of Reproductive Medicine Vol.8. No.3. pp: 95-100, Summer 2010

The Leptin concentrations in seminal plasma of men and its relationship to semen parameters

Seyed Gholam Ali Jorsaraei[1] Ph.D., Hiroaki Shibahara[2] M.D., Ph.D., Ayustawati[2] M.D., Ph.D., Yuki Hirano[2] M.D., Ph.D., Tatsuya Suzuki[2] M.D., Eisa Tahmasbpour Marzony[3] M.Sc., Mahtab Zainalzadeh[1] M.D., Mitsuaki Suzuki[2] M.D., Ph.D.

1 Fatemeh Zahra Infertility and Health Reproductive Research Center, Babol University of Medical Sciences, Babol, Iran.
2 Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, Tochigi, Japan.
3 Department of Biology, Faculty of Basic Science, Azad University of Sari, Sari, Iran.

Received: 16 September 2009; accepted: 25 April 2010

Abstract

Background: Leptin is a polypeptide hormone secreted by white adipose tissue in proportion to body energy. Although the participation of leptin in female reproduction is well established, any role in male reproductive function is at best tenuous.

Objective: The objective of this study was to compare the leptin concentration in human seminal plasma and then the relationships between seminal leptin and semen parameters were evaluated.

Materials and Methods: Semen samples were provided from 71 men; normozoospermic (n=22), asthenozoospermic (n=31) and oligoasthenozoospermic (n=18) referring to Jichi Medical University Hospital for in vitro fertilization-embryo transfer (IVF-ET) treatment. After liquefaction, all sperm specimens were evaluated for sperm parameters and motility characteristics by computer-assisted semen analysis (CASA) system. After semen analysis, concentrations of leptin in seminal plasma of all groups were measured by ELISA.

Results: The mean concentrations of leptin in seminal plasma of normozoospermic, asthenozoospermic and oligoasthenozoospermic men were 0.75+/-0.09 ng/ml, 0.8+/-0.14 ng/ml and 0.8+/-0.15 ng/ml, respectively. A trend was observed for a lower leptin concentration in seminal plasma of normozoospermic men compared with asthenozoospermic and oligoasthenozoospermic men. There was a significant negative correlation between seminal plasma leptin concentration with sperm motility ($p<0.05$) and Curvilinear Velocity of the sperm kinetic parameter ($p<0.01$).

Conclusion: It was demonstrated that there was a significant correlation between seminal leptin with the sperm motility.

Key words: *Leptin, Sperm quality, Male infertility, Seminal plasma, Fertilization rate.*

Introduction

Leptin is a 167 - amino acid polypeptide hormone, identified in 1994 by positional cloning in the mouse and human (1).

Corresponding Author:
Seyed Gholam Ali Jorsaraei, Fertility and Infertility Research Center (Fatemeh Zahra), Babol University of Medical Sciences, Babol, Iran.
Email: alijorsara@yahoo.com

This hormone is secreted by the white adipose tissue in proportion to body energy (fat) stored. Leptin functions as a satiety factor in the regulation of body weight (2-5). Importantly, in addition to its well-known role in energy balance, leptin was soon identified as a permissive regulator of human reproductive maturity and serve as mediator in a wide range of neuroendocrine systems, including the reproductive axis (2, 5-9).

So far, many studies have pointed to a direct role of leptin in the control of male reproductive

function (10-11). However, in contrast to its well proven effects in female fertility (14, 15), the actual role of the hormone in the regulatory network controlling male reproductive function has been a matter of debate (11). Some studies support the role of serum leptin in the regulation of gonadal functions in men indirectly via the central neuroendocrine system (16) and directly via peripheral tissue membrane receptors (17). New reports suggest that leptin plays an important role in relaying energetic status to reproduction (11). It may be hypothesized that leptin in uncapacitated sperm is involved in the accumulation of energy substrates, which would be spent during capacitation (11, 18).

Leptin is expressed in the seminiferous tubules and in seminal plasma and also directly acts on testis (4, 19), but its cellular origin in these contexts is not exactly defined. The most likely source has been shown either seminal vesicle or prostate tissue (9). On the contrary, the leptin receptors have been identified in the Leydig cells (8, 20). Besides, recent study found that human ejaculated spermatozoa secrete leptin that can affect some events tightly related to this process (11).

Leptin secretion by sperm suggested that the sperm has ability to modulate its metabolism, according to its energy needs, independently by systemic leptin expression. This may represent a protective mechanism in male reproduction to guarantee the accumulation of energy substrates to maintain the gamete fertilizing capability (11, 12). Therefore, it was suggested that testes may contribute to leptin secretion and low levels of seminal leptin may be a risk factor for idiopathic male infertility. Nevertheless, the role of leptin in male reproductive function and sperm quality or capacitation has not been completely assessed and it was considered that more studies are required to clarify this issue.

This study focused primarily on leptin concentration in the seminal plasma of normozoospermic, asthenozoospermic and oligoasthenozoospermic men. The associations of seminal plasma leptin concentration with sperm quality were evaluated.

Materials and methods

Semen analysis

Semen specimens were provided by 71 men (age between 22-40 years old) that referred to Jichi Medical University Hospital for *in vitro* fertilization-embryo transfer (IVF-ET) treatment. All semen samples were collected by masturbation after 2-3 days of sexual abstinence in a sterile plastic jar. After liquefaction, semen specimens were evaluated for sperm concentration, motility and motility characteristics: VCL (curvilinear velocity), ALH (amplitude of lateral head displacement), BFC (beat cross frequency) and LIN (linearity), according to the guidelines of the World Health Organization (20). Morphology smears were scored using the Kruger's strict criteria (21). All sperm specimens were evaluated with computer-assisted semen analysis (CASA) system (Hamilton Thorne Research, Beverly, USA), as described previously (23). After analysis of sperm parameters, patients were divided into three groups: normozoospermic (n=22), asthenozoospermic (n=31) and oligoasthenozoo-spermic (n=18) men.

Asthenozoospermia was indicated by a sperm concentration of $>20\times10^6$/ml, normal morphology of >4% and motility of <50% and oligoasthenozoospermia was indicated by a sperm concentration of $<20\times10^6$/ml, normal morphology of >4% and motility of <50%. Normozoospermia was indicated by a sperm concentration of $>20\times10^6$/ml, normal morphology of >4% and motility of >50%.

Measurement of seminal plasma leptin

After semen analysis, the seminal fluids were separated by centrifugation (8000 rpm, for 15 min) and were stored at -20^0 C until use. Leptin concentration in seminal plasma was measured by ELISA (active human leptin; Diagnostic system laboratories, Texas, USA) by using an automatic immunodiagnostic analyzer (Personal lab analyzer, Azwell, Tokyo, Japan) that previously described by Ayustawati *et al* (24).

Statistical analysis

An independent t-test was considered to compare the scores of each of the measures and some of the parameters data between groups. The ANOVA model was utilized for statistical analyses of leptin concentration between all groups. The Pearson correlation test and linear regression was used to analysis and examines the relation between the seminal leptin with semen parameters. $p<0.05$ was considered statistically significant.

Results

The results of the classic semen analysis are shown in table I. A trend was observed for a lower leptin concentration in seminal plasma of

normozoospermic compared with asthenozoospermic and oligoasthenozoospermic men. The mean concentrations of leptin in seminal plasma of 22 normozoospermic men were 0.75+/-0.09 ng/ml, while in 31 asthenozoospermic and 18 oligoasthenozoospermic men were 0.8+/-0.14 ng/ml and 0.8+/-0.15 ng/ml, respectively (Table I). A significant correlation was observed between seminal plasma leptin concentrations with sperm motility (Figure 1A; $p<0.05$) and the VCL of sperm kinetic parameter (Figure 1B; $p<0.01$).

Table I. Comparison of semen characteristics and leptin concentration between normozoospermic, asthenozoospermic and oligoasthenozoospermic, men.

Variable	Normozoospermic (n=22)	Asthenozoospermic (n=31)	Oligoasthenozoospermic (n=18)
Age (years)	28.68 ± 4.7	29.96 ± 5.15	29.33 ± 5.65
Volume (ml)	3.5 ± 1.3	3.6 ± 0.94	3.5 ± 1.8
Sperm count ($\times 10^6$/ml)	134.6 ± 74.3	41.75 ± 21.49**	15.11 ± 9.66**
Total sperm count ($\times 10^6$)	426.97 ± 223.81	149.19 ± 79.94**	41.88 ± 27.42**
Normal morphology (%)*	23.27 ± 5.8	8.9 ± 5.7**	8.6 ± 3.9**
Motility (%)	61.5 ± 6.7	21.93 ± 9.7**	20.3 ± 10.81**
VCL (µm/s)	78.58 ± 13.7	72 ± 12.48	77.08 ± 13.81
LIN (%)	62.18 ± 8.5	56.09 ± 5.36***	51.83 ± 8.07**
ALH (µm/s)	3.04 ± 1.12	2.78 ± 1.27	2.59 ± 1.08
BCF (HZ)	25.7 ± 3.8	24.4 ± 4.1	26.6 ± 3.4
Leptin (ng/ml)	0.75 ± 0.09	0.8 ± 0.14	0.8 ± 0.15

Results are presented as mean ± S.D; *According to Kruger's *et al* (1986).
**p<0.001; ***p<0.01, by using 1-way ANOVA followed by post hoc Newman-Keuls test when values of asthenozoospermic or oligoasthenozoospermic are compared with normozoospermic.

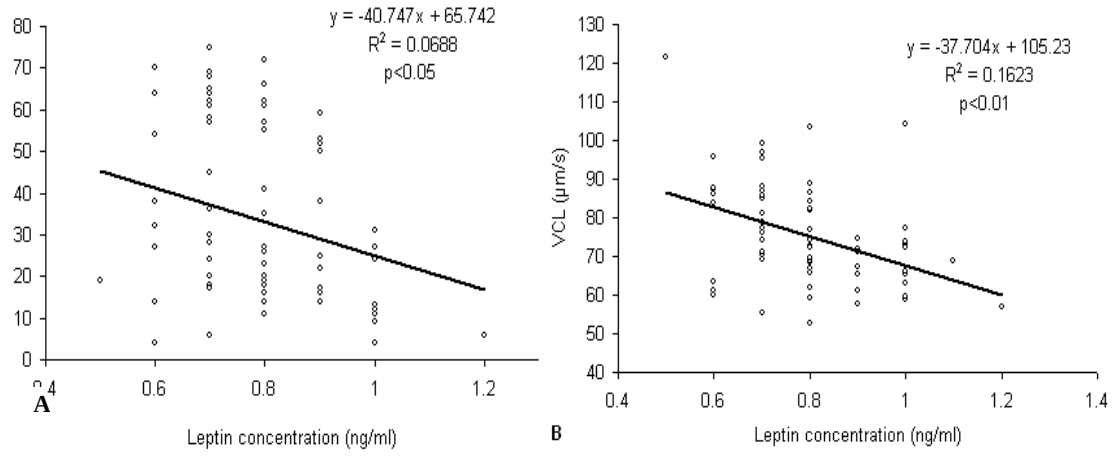

Figure 1. Correlation between the seminal plasma leptin concentration with (A) sperm motility and (B VCL (curvilinear velocity) of the sperm kinetic parameters in all groups. Seminal plasma leptin was negatively correlated with sperm motility (r2 = 0.0688; p< 0.05) and VCL (r2 = 0.1623; p< 0.01). The Pearson correlation test was used to analyze and examine the relationship between Leptin with sperm motility and VCL.

Discussion

In the last few years, several reports have shown that leptin is present in unexpected organs, such as the stomach, muscle and placenta or in fluids such as milk (25-27), and in the field of reproduction (20). Leptin acts at the hypothalamic level and the entrance to puberty (26, 29). Normal leptin secretion is necessary for reproductive function to proceed and may be a signal allowing for the point of initiation of the end progression toward puberty (30-32).

Recently, it was hypothesized that the net effect of leptin upon male reproductive function may depend on the circulating level of the molecule (33). Besides, the available evidence is suggestive of a tightly regulated, complex mode of leptin action in different levels in the male gonadal axis that involves not only stimulatory but also inhibitory effects (34-37).

Predominant stimulatory effects are observed at leptin levels above a minimal threshold; in contrast, direct inhibitory actions may take place in the presence of a significantly elevated leptin concentration (11). The presence of leptin in the genital tract, including the seminiferous tubules or seminal plasma, may influence the mechanisms involved in the sperm maturation (37), capacitation (11, 39) or mobility development of spermatozoa (4).

However, in this study, there was a negative signification between seminal leptin and sperm motility. So far, most studies indicated both positive and negative effects of leptin in gonadal functions (38). Some authors indicated that the concentration of leptin in seminal plasma of men with normal semen sample is significantly lower than pathological groups (4) and has a negative correlation with the motility of human spermatozoa (3).

Steiman *et al* (16) measured leptin in the serum of normozoospermic, oligoasthenoteratozoospermic (OAT) and azoospermic men. The mean concentrations of serum leptin in these groups were 5.0+/-0.5 µg/l, 7.1+/-4.6 µg/l and 7.6+/-0.8 µg/l, respectively. This data suggested that azoospermic men have significantly higher levels of leptin in their serum than fertile men, but the mean of serum leptin in OAT group were not significantly different in comparison with both fertile and azoospermic groups. In our study, a trend was observed for a lower leptin concentration in seminal plasma of normozoospermic compared with asthenozoospermic and oligoasthenozoospermic men.

Therefore, it is suggested, that asthenozoospermic or oligoasthenozoospermic men have enough leptin concentration in their seminal plasma for sperm capacitation or any other processes of fertilization. These results suggested that spermatozoa can not be the only main source for seminal leptin. Accomplished to recent studies, the source of leptin in seminal plasma is likely either seminal vesicle or prostate tissue in addition to spermatozoa (9, 40). On the other hand, it is possible that testes tissue contributes to seminal leptin production or secretion (9).

In this study the presence of leptin in human seminal plasma was observed, of course we couldn't find which organs is able to secret leptin, but it is obvious that leptin plays roles in spermatogenesis or sperm capacitation, and facilitate ovarian cycle (27, 28). Therefore it can be a marker of the quality of the follicle and viability of the embryo (39).

Several reports indicated that leptin concentration in seminal plasma did not show any relationship with the spermiogram parameters, such as concentration, motility, vitality, morphology, head alteration and volume (9). But other studies reported a negative correlation with the motility of human spermatozoa (4) which is in agreement with our study. In the present study, there was a significant negative correlation between seminal leptin and sperm motility. Therefore, it appears that, the role of seminal leptin in male reproductive function is less well known and the relationship between seminal leptin and gonadal function is still unresolved.

In conclusion, our data showed that seminal plasma leptin concentration was negatively correlated with sperm motility and VCL of the sperm kinetic parameter.

References

1. Zhang Y, Proneca R, Maffei M, Barone M, Leopold L, Friedman JM. Position cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425-432.
2. Tena-Sempere M, Barreiro ML, Lage M, Dieguez C, Casanueva FF. Role of leptin and ghrelin in the regulation of gonadal function. *Expert Rev Endocrinol Metab* 2007; 2: 239-249.

3. Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin regulation neuroendocrine systems. *Front Neuroendocrinol* 2000; 21:263-307.
4. Glander HJ, Lammert A, Paasch U, Glasow A, Kratzsch J. Lepin exits in tubuli seminiferi and in seminal plasma. *Andrologia* 2002; 34:227-233.
5. Henson MC, Castracane VD. Leptin in pregnancy. *Biolo Reprod* 2000; 63: 1219–1228.
6. Wauters M, Considin RV, Van Gaal LF. Human leptin: from as adipocyte hormone to an endocrine mediator. *Eur J Endocrinol* 2000; 143: 293-311.
7. Kawamura K, Sato N, Fukuda J, Kodama H, Kumagai J, Tanikawa H, et al. Leptin promotes the development of mouse preimplanation embryos in vitro. *Endocrinol* 2002; 143: 1922-1931.
8. Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: a review. *Fertil Steril* 2002; 3: 433-444.
9. Camina JP, Lage M, Menendez C, Grana M, Garcia-Devesa J, Dieguez C, et al. Evidence of free leptin in human seminal plasma. *Endocrine* 2002; 17: 169-174.
10. Tena-Sempere M, Barreiro ML. Leptin in male reproduction: the testis paradigm. *Mol Cell Endocrinol* 2002; 188: 9–13.
11. Aquila S, Gentile M, Middea M, Catalano S, Morelli C, Pezzi V, et al. Leptin secretion by human ejaculated spermatozoa. *J Clin Endocrinol Metab* 2005; 90: 4753–4761.
12. Lange Consiglio A, Dell'Aquila ME, Fiandanese N, Ambruosi B, Cho YS, Bosi G, et al. Effects of leptin on in vitro maturation, fertilization and embryonic cleavage after ICSI and early developmental expression of leptin (Ob) and leptin receptor (ObR) proteins in the horse. *Reprod Biol Endocrinol* 2009; 7:113.
13. Magni P, Martini L, Motta M. Leptin actions on the reproductive axis. *J Clin Endocrinol Metab* 2001; 86: 946–947.
14. Brannian JD, Schmidt SM, Kreger DO, Hansen KA. Bseline non-fasting serum leptin concentration to body mass index ratio is predictive of IVF outcomes. *Hum Reprod* 2001; 16: 1819-1826.
15. Spicer LJ, Francisco CC. The adipose gene product leptin: evidence of direct inhibitory role in ovarian function. *Endocrinol* 1997; 138: 3374-3379.
16. Steiman N, Gamzu R, Yogev L, Botchan A, Schreiber L, Yavetz H. Serum leptin concentrations are higher in azoospermic than in normozoospermic men. *Fertil Steril* 2001; 75: 821–822.
17. Tena-Sempere M, Pinilla L, Gonzalez LC, Dieguez C, Casanueva FF, Aguilar E. Leptin inhibits testosterone secretion from adult rat testis in vitro. *J Endocrinol* 1999; 161: 211–218.
18. Wabitsch M, Ballauff A, Holl R, Blum W, Heinze E, Remschmidt H, et al. Serum leptin, gonadotropin and testosterone concentrations in male patients with anorexia nervosa weight gain. *J Clin Endocrinol Metab* 2001; 86: 2982-2988.
19. Jope T, Lammert A, Kratzsch J, Paasch U, Glander HJ. Leptin and leptin receptor in human seminal plasma and in human spermatozoa. *Int J Androl* 2003; 26: 335–341.
20. Caprioa M, Fabbrinic E, Riccid G, Bascianic S, Gnessic L, Arizzic M, et al. Ontogenesis of leptin receptor in rat leydig cells. *Biol Reprod* 2003; 68: 1199-1207.
21. World Health Organization WHO. Laboratory Manual for the Examination of Human Semen and Semen-cervical Mucus Interaction, 4th ed. Cambridge, UK7 Cambridge University Press; 1999. p: 4 – 23.
22. Kruger TF, Menkveld R, Stander FSH, Lombard CJ, Van der Merwe JP, Van Zyl JA. Sperm morphologic features as a prognostic factor in in-vitro fertilization. *Fertil Steril* 1986; 46: 1118–1123.
23. Hirano Y, Shibahara H, Obara H, Suzuki T, Takamizawa S, Yamaguchi C, et al. Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in vitro. *J Assist Reprod Genet* 2001; 18: 213–218.
24. Ayustawati, Shibahara H, Hirano Y, Suzuki T, Takamizawa S, Suzuki M. Serum leptin concentrations in patients with severe ovarian hyperstimulation syndrome during in vitro fertilization-embryo transfer treatment. *Fertil Steril* 2004; 82: 579-585.
25. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. Anutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* 1998; 18: 684-688.
26. Senaris R, Garcia-Caballero T, Casabiell X, Gallego R, Castro R. Synthesis of leptin in human placenta. *Endocrinology* 1997; 138: 4501-4504.
27. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, et al. The stomach is a source of leptin. *Nature* 1998; 20: 790-793.
28. Garcia-Mayor RV, Andrade MA, Rios M, Lage M, Dieguez C, Casanueva FF. Serum leptin levels in normal children: relationship to age, gender, body mass index, pituitary-gonadal hormones and pubertal stage. *J Clin Endocrinol Metab* 1997; 82: 4144-4148.
29. Mantzoros CS, Filer JS, Lesem MD, Brewerton TD, Jimerson DC. Cerebrospinal fluid leptin in anorexia nervosa: Correlation with nutritional status and potential role in resistance to weight gain. *J Clin Endocrinol Metab* 1997; 82: 1845-1851.
30. Kiess W, Muller G, Galler A, Reich A, Deutscher J, Klammt J, et al. Body fat mass leptin and puberty. *J Pediatr Endocrinol Metan* 2000; 1: 717-722.
31. Cervero A, Dominguez F, Horcajadas JA, Quinonero A, Pellicer A, Simon C. The role of leptin in reproduction. *Curr Opin Obstet Gynecol* 2006; 18: 297-303.
32. Kratzsch J, Hockel M, Kiess W. Lptin and pregnancy outcome. *Curr Opin Obstet Gynecol* 2000; 12: 501-505.
33. Jin Y-X, Cui X-S, Han Y-J, Kim N-H. Leptin accelerates pronuclear formation following intracytoplasmic sperm injection of porcine oocytes: Possible role for MAP kinase inactivation. *Animal Reproduction Science* 2009; 115:137–148.
34. Tena-Sempere M, Manna PR, Zhang FP, Pinilla L, Gonzalez LC, Dieguez C, et al. Molecular mechanisms of leptin action in adult rat testis: Potential targets for leptin-induced inhibition of steriodogenesis and pattern of leptin receptor messenger ribonucleic acid expression. *J Clin Enocrinol Metab* 2001; 86: 2982-2988.
35. Tena-Sempere M, Huhtraniemi IT. Gonadotropins and gonadotropin receptors. In: reproductive medicine. Molecular, Cellular and Genetic Fundamentals. Fauser. BCJM (Ed). *Parthenon Publishing, NY, USA* 2003; 225-244.
36. Sahu A. Ahypothalamic role in energy balance with special emphasis on leptin. *Endocrinology* 2004; 14: 2613-1620.
37. Haron MN, D'Souza UJ, Jaafar H, Zakaria R, Jeet Singh H. Exogenous leptin administration decreases sperm count and increases the fraction of abnormal sperm in adult rats. *Fertil Steril* 2009; 93: 322-324.
38. Smith GD, Jackson LM, Foster DL. Leptin regulation of reproductive function and fertility. *Theriogenology* 2002; 57: 73-86.

39. Li HW, Chiu PC, Cheung MP, Yeung WS, O WS. Effect of leptin on motility, capacitation and acrosome reaction of human spermatozoa. *Int J Androl* 2008; 32: 687- 694.
40. Caprio M, Fabbrini E, Isiodori AM, Aversa A, Fabbri A. Leptin in reproduction. *Trends Endocrinol Metab* 2001; 12: 65-72.
41. Nikbakht G, Ali Mehr MR, Baghbanzadeh A, Tajik P, Tamanini C, Emam M. Leptin Receptor mRNA in Bull Ejaculated Spermatozoa. *Reprod Domest Anim* 2010; 45: 237-242.
42. Mantzoros CS, Cramer DW, Liberman RF, Barbieri, RL. Predictive value of serum and follicular fluid leptin concentration during assisted reproductive cycles in normal women with the polycystic ovarian syndrome. *Hum Reprod* 2000; 15: 539-551.