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Correlation between leptin content and sperm retrieval in cases of functional azoospermia

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KEYWORDS

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Abstract *Introduction:* Testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) became the preferable techniques for solving the problem of azoospermic men. Non-invasive techniques are needed to predict sperm retrieval chance before TESE to avoid the psychological and physiological problems that may be developed.

Aim: To investigate the correlation between serum, seminal and testicular leptin levels and sperm retrieval in functional azoospermic men.

Methods: The study included 61 men classified into 4 groups; normozoospermia (NOR), obstructive azoospermia (OA), positive non-obstructive azoospermia (NOA (+)) and negative non-obstructive azoospermia (NOA (−)). Blood FSH, LH, Prolactin, Free and Total testosterone levels plus serum and seminal leptin levels were measured for all groups. For azoospermic groups, TESE and testicular leptin level were applied.

Main outcome measures: Both OA and NOR groups were used as control groups. The prediction accuracy for FSH and serum, seminal and testicular leptin was compared by receiver operating characteristic (ROC) curve analysis.

Results: There were no significant differences in serum leptin levels among the four groups. Azoospermic groups showed higher seminal leptin levels than the NOR group. Seminal and Testicular leptin levels of NOA (−) men were significantly increased in comparison with OA and NOA (+) men. There was a significant negative correlation between serum leptin and total testosterone concentrations, and a significant positive correlation between testicular and seminal leptin concentrations. In ROC curve; for differentiation between positive and negative NOA, areas under

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the curve (AUC) of testicular and seminal leptin were greater than that of serum leptin. The combination of seminal leptin with FSH gave AUC greater than that of FSH alone.

Conclusion: There is a role for leptin in spermatogenesis, and seminal leptin can be used as a good assistant marker to increase the prediction accuracy for sperm retrieval in NOA men especially in combination with FSH.

Introduction

Azoospermia is a medical condition of males having total absence of spermatozoa from the ejaculate. It is found in 1–3% of the male population and approximately 10% of infertile males (Jarow et al., 1989). Azoospermia is classified according to etiology into OA and NOA. Obstructive azoospermia is failure of sperm transport due to the presence of obstruction in the seminal ducts, thus spermatogenesis is normal, while NOA is failure of sperm production or maturation by the testis, thus there is deficient spermatogenesis (NOA (+)) or absent spermatogenesis (NOA (−)) (Ginsburg and Racowsky, 2012). In early 1990s, testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) were introduced for the treatment of OA, later these techniques are applied on NOA (Gardner et al., 2011), where it was reported that sperm retrieval chance for each biopsy is 20–50% in the NOA patients (von Eckardstein et al., 1999).

Many predictive markers that could predict the presence of sperm in testicular tissue were proposed. It was reported that follicular stimulating hormone (FSH) and testicular volume constitute the best options to make such a prediction (Toulis et al., 2010), in addition to serum Inhibin B (Inh-B) level (Ballesca et al., 2000; Bohring et al., 2002; Bailly et al., 2003), seminal Inh-B level (Nagata et al., 2005), serum and seminal levels of anti-Müllerian hormone (AMH), detection of round spermatids in seminal fluid (Amer et al., 2001), as well as the testicular histopathological findings (Su et al., 1999; Abdel Raheem et al., 2013). None of the aforementioned markers has gained universal acceptance (Isikoglu et al., 2006; Mostafa et al., 2007; Duvilla et al., 2008; Mitchell et al., 2010; Toulis et al., 2010), and with regard to taking diagnostic testicular biopsies, it is an invasive procedure. Therefore the search for a better spermatogenic non-invasive marker and more accurate method of predicting TESE outcome is still in process (Vernaeve et al., 2002; Ma et al., 2010).

Leptin is an adipocytokine, synthesized as a 167-amino acid hormonal protein and it is the product of the obesity gene. Zhang et al. have reported that leptin was originally discovered in 1994 by Jeffrey M. Friedman and colleagues. They also reported that mature leptin is 146 amino acids in length, with a molecular weight of approximately 16 kDa (Zhang et al., 1994). Originally leptin was defined in association with satiety and energy balance and claimed to be an anti-obesity factor that functioned via a feedback effect from adipocytes to hypothalamus. In addition it was found to play a role in the regulation of metabolism, sexual development, angiogenesis, hematopoiesis, immunity, gastrointestinal functions, sympathetic activation, and reproduction (Ziylan et al., 2009).

Previously, some studies have linked leptin and male reproduction; puberty, spermatogenesis, functional regulation of the male gonadal axis, sperm maturation, sperm capacitation and sperm motility (El-Hefnawy et al., 2000; Kiess et al., 2000; Kratzsch et al., 2000; Glander et al., 2002; Tena-Sempere and Barreiro, 2002; Aquila et al., 2005; Cervero et al., 2006; Ishikawa et al., 2007; Zorn et al., 2007; Haron et al., 2009; Nicopoulou et al., 2009). Only a handful of studies have dealt with leptin association with azoospermia (Steinman et al., 2001; Zorn et al., 2007; Ma et al., 2010; Gao et al., 2011). Very few studies have focused on the use of leptin to increase the prediction accuracy for sperm retrieval in azoospermic men; Ma et al. (2010) studied the use of seminal leptin as a marker to differentiate between sperm negative and sperm positive NOA patients and Gao et al. (2011) studied the use of both serum and seminal leptin concentrations for the differential diagnosis of OA and NOA.

In this study we investigated the correlation between serum, seminal and testicular levels of leptin and sperm retrieval in functional azoospermic men.

Materials and method

Patients

The research included four groups of 61 men with mean age 33.557 ± 0.626 (24–48 years). Group 1 (n = 6) was NOR, group 2 (n = 15) was OA, group 3 (n = 11) was non-obstructive azoospermic men with positive TESE (NOA (+)) and group 4 (n = 29) was non-obstructive azoospermic men with negative TESE (NOA (−)). Both OA and NOR groups were used as control groups (n = 21).

Group 1 was subjected to history taking, full examination, determination of body mass index (BMI), measurements of blood sugar level, semen analysis, hormonal evaluation; including FSH, LH, Prolactin, Free and Total testosterone, and measurement of leptin level in serum and seminal plasma. The same protocol was applied to other groups in addition to scrotal duplex, karyotyping, TESE, measurement of leptin level in the testicular tissue.

Patients with genetic abnormality, varicocele or any other known cause for azoospermia, as well as patients with diabetes or an abnormal BMI were excluded.

Ethical approval

All patients signed a consent form before proceeding with procedures. The Research Ethical Committee (REC) at the Department of Andrology, Cairo University, Egypt has approved this research.

Serum samples preparation

Fasting blood samples were collected by venipuncture, allowed to clot, and then centrifuged at 2000*g* for 10 min at room temperature for serum separation. Sera were aliquoted and stored at −80 °C until assayed.

Semen analysis and seminal plasma preparation

According to the WHO guidelines, two or three specimens over several weeks were collected after 2–7 days of sexual abstinence and examined. Semen specimens were obtained and were allowed to liquify at temperature of 20–40 °C. Azoospermia was confirmed by examination of sperm of the centrifuged specimens. Seminal plasma was obtained by centrifugation at >3000*g* for 15 min and then aliquoted and rapidly stored at −80 °C until assayed (World Health, 2010).

Testicular sperm extraction and tissue homogenate preparation

Single or multiple testicular biopsies were taken to obtain sperm in both NOA and OA patients. Single site biopsy was performed first. If positive, the procedure is terminated. If negative, microsurgical TESE is performed. Testicular tissues were taken in a Petri dish containing 1 ml HEPES-buffered Ham's F10. Under sterile conditions, testicular biopsies were minced and shredded then examined immediately under an inverted microscope for the presence of testicular spermatozoa in the entire Petri dish. Tissue (0.1 g) was homogenized in 1 ml HEPES-buffered Ham's F10. The resulting suspension was subjected to two freeze–thaw cycles to further break the cell membranes. After that, the homogenates were centrifuged for 15 min at 3000*g*, and supernatants were aliquoted and stored at −80 °C until assaying leptin levels in them.

Hormonal assays

Serum luteinizing hormone (LH), follicular stimulating hormone (FSH), prolactin (PRL) and total testosterone (TT) levels were determined by the chemiluminescence method using an automated immunoassay analyzer (Immulite analyzer) (Siemens Healthcare Diagnostics, Inc., USA).

Free testosterone was measured by enzyme-linked immunoassay (ELISA) kit (CAN-fTE-260, Diagnostics Biochem Canada Inc., 1020 Hargrieve Road, London, Ontario, Canada, N6E 1P5).

Leptin concentrations in serum, seminal plasma and testicular tissue homogenate were measured using a solid-phase sandwich ELISA (EAI-2395, DRG Instruments GmbH, Germany).

Statistical analysis

By using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA), all mean values among groups were compared by Duncan's test for pairwise comparison. Pearson's correlation coefficient (r) with P-value was used to determine significant differences between all hormones studied. Values were given as mean ± S.E. and $P < 0.05$ was considered statistically significant.

Plotting receiver operating characteristic (ROC) curves were performed for serum, seminal and testicular leptin levels to evaluate the ability of serum, seminal or testicular leptin discriminate between positive and negative TESE of NOA men. Binary logistic regression analysis was performed to select the best combination of these variables in addition to FSH for predicting testicular sperm retrieval, using the MedCalc software version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Results are summarized in Tables 1–4. Values were presented as mean ± standard error (S.E.) and $P < 0.05$ was considered statistically significant.

Duncan's multiple range test

From Table 1, Duncan's test shows that leptin concentrations in sera of all studied groups were comparable.

Duncan's test revealed that there was a statistically significant difference in the seminal leptin concentrations of the two control groups (NOR and OA), where the OA group was 29.0% higher than the NOR group. NOA (+) and NOA (−) groups differed significantly, where the NOA (−) group was 15.8% higher than the NOA (+) group. There was a statistically significant difference between the NOA (−) group and the two control groups (NOR and OA), where the NOA (−) group was 53.0% higher than the NOR group and was 18.6% higher than the OA group. There was a statistically significant difference between NOA (+) and NOR groups, where the NOA (+) group was 32.1% higher than the NOR group. But there was no statistically significant difference between NOA (+) and OA groups.

Table 1 also shows concentrations of leptin in the homogenate of testicular tissue for three groups only; OA, NOA (+) and NOA (−), with no data for testicular leptin in NOR men, because ethically it is forbidden to take a testicular biopsy from normal men. Duncan's test suggested that NOA (−) group was significantly different from OA and NOA (+) groups, where the NOA (−) group was 25.1% higher than the OA group and also was 14.9% higher than the NOA (+) group. No statistically significant difference between OA and NOA (+) groups was found.

Correlations analysis

In Table 2, correlations between the three leptin levels; serum, seminal and testicular leptin levels, with each other, with BMI and with concentrations of LH, FSH, PRL, total testosterone, and free testosterone in all investigated patients are summarized.

In all samples ($n = 61$), there was a highly significant positive correlation between concentration of serum leptin and BMI value, $P < 0.01$ and there was a statistically significant negative correlation between concentration of serum leptin and concentration of serum total testosterone, $P < 0.05$. No statistically significant correlations were found between concentration of serum leptin and LH, FSH, PRL, free testosterone, seminal leptin and testicular leptin concentrations.

Table 1 Statistical comparisons between groups by Duncan's range test.

	NOR (n = 6)	OA (n = 15)	NOA (+) (n = 11)	NOA (−) (n = 29)
Age (years)	32.000 ± 1.2383 (29–37)[a]	33.867 ± 1.8149 (24–48)[a]	33.364 ± 0.7419 (30–36)[a]	33.793 ± 0.8715 (25–45)[a]
Glucose (mg/dl)	95.667 ± 3.3233 (86–107)[a]	99.333 ± 2.8329 (73–110)[a]	92.818 ± 4.3271 (60–110)[a]	93.069 ± 2.0985 (70–110)[a]
BMI (kg/m^2)	22.917 ± 1.0173 (18.7–25.6)[a]	24.847 ± 0.7485 (20.2–29.4)[ab]	24.518 ± 0.6169 (20.2–27.5)[ab]	25.759 ± 0.4789 (18.6–29)[b]
LH (mIU/ml)	6.083 ± 1.3398 (1.6–11.6)[a]	5.059 ± 0.8726 (0.19–12.2)[a]	10.918 ± 1.4401 (5.5–20.2)[b]	7.123 ± 1.0208 (0.37–23.4)[ab]
FSH (mIU/ml)	4.85 ± 1.7068 (1.7–13.2)[ab]	3.848 ± 0.6685 (0.18–10.9)[a]	31.127 ± 5.9426 (14.3–80)[c]	13.657 ± 1.7598 (0.75–47.7)[b]
PRL (ng/ml)	6.117 ± 0.8228 (3.7–8.9)[a]	16.793 ± 3.3755 (4.7–51)[ab]	25.941 ± 10.9942 (4.5–130)[b]	17.252 ± 2.0372 (5.7–50)[ab]
Total testosterone (nmol/l)	13.217 ± 2.5268 (5.7–21.2)[a]	15.640 ± 1.7531 (6.3–26.2)[a]	13.918 ± 1.9804 (5.5–24.2)[a]	11.203 ± 1.3677 (1.1–35.1)[a]
Free testosterone (pmol/l)	29.683 ± 1.8895 (24–36)[a]	29.680 ± 3.5173 (13–53.1)[a]	28.936 ± 1.7858 (17.5–38)[a]	35.324 ± 3.9465 (6–103.1)[a]
Serum leptin (ng/ml)	2.829 ± 0.6691 (0.965–5.506)[a]	3.861 ± 0.6547 (1.398–8.958)[a]	3.819 ± 0.7441 (1.143–8.221)[a]	4.605 ± 0.4261 (0.784–9.058)[a]
Seminal leptin (ng/ml)	0.813 ± 0.0497 (0.659–0.947)[a]	1.049 ± 0.0294 (0.714–1.183)[b]	1.074 ± 0.0430 (0.842–1.268)[b]	1.244 ± 0.0434 (0.473–1.716)[c]
Testicular leptin (ng/ml)	No samples	1.353 ± 0.0378 (0.921–1.564)[a]	1.473 ± 0.0529 (1.217–1.81)[a]	1.692 ± 0.0392 (1.253–2.115)[b]

In the row, mean values marked with the same superscript letters are similar (insignificant, $P > 0.05$) whereas different superscripts denote significance ($P < 0.05$).

Table 2 Correlations between serum, seminal and testicular leptin with each other and with LH, FSH, PRL, total testosterone and free testosterone concentrations in all patients investigated (n = 61).

		BMI	LH	FSH	PRL	Total testo.	Free testo.	Serum leptin	Seminal leptin	Testicular Leptin
Serum leptin	r	+0.709**	−0.045	+0.014	−0.105	−0.305*	−0.036	+1	+0.150	+0.200
	P-value	0.000	0.729	0.913	0.420	0.017	0.784		0.249	0.143
Seminal leptin	r	0.128	−0.005	+0.112	+0.168	−0.114	+0.322*	+0.150	+1	+0.486**
	P-value	0.327	0.968	0.389	0.196	0.382	0.011	0.249		0.000
Testicular leptin	r	−0.009	+0.186	+0.094	−0.105	−0.242	+0.154	+0.200	+0.486**	+1
	P-value	0.946	0.175	0.497	0.444	0.075	0.262	0.143	0.000	

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

With respect to leptin of the seminal plasma, Table 2 shows statistically significant positive correlations between concentration of seminal leptin and both concentrations of serum free testosterone and testicular leptin, $P < 0.05$ and $P < 0.0001$ respectively. No statistically significant correlations were found between the concentration of the seminal leptin and BMI and concentrations of LH, FSH, PRL, total testosterone and serum leptin.

Finally, concerning leptin of the testicular tissue, there was a highly significant positive correlation between concentration of testicular leptin and concentration of seminal leptin, $P < 0.0001$. No statistically significant correlations were found between the concentration of the testicular leptin and BMI and also concentrations of LH, FSH, PRL, total testosterone, free testosterone and serum leptin.

Receiver operating characteristic (ROC) and binary logistic regression analyses

Receiver operating characteristic (ROC) curves were used to analyze the diagnostic accuracy of serum, seminal and testicular leptin to discriminate between successful and failed TESE in NOA patients and also to determine their best threshold concentrations for discrimination (Table 3 and Fig. 1). Binary logistic regression analysis was performed to select the best combination of these variables in addition to FSH for predicting testicular sperm retrieval (Table 4).

Table 3 Analysis of receiver operating characteristic curves for serum leptin, seminal leptin, and testicular leptin levels for non-obstructive azoospermia.

	AUC	S.E.	95% CI	Sensitivity	Specificity	Criterion	*P*-value
FSH	0.828	0.0685	0.675–0.928	100	58.6	>13.3	<0.0001
Testicular leptin	0.809	0.0799	0.654–0.916	81.8	79.3	⩽1.567	0.0001
Seminal leptin	0.773	0.0752	0.613–0.890	90.0	65.5	⩽1.194	0.0003
Serum leptin	0.605	0.110	0.438–0.756	72.73	62.07	⩽3.674	0.3397

AUC: Area under curve.
CI: Confidence interval.
S.E.: Standard error of mean.

Data from Table 3 show that the value of area under the curve (AUC) was the highest for FSH followed by that of testicular leptin, that of seminal leptin and finally that of serum leptin; 0.828, 0.809, 0.773 and 0.605 respectively. This indicates that FSH is the best in discrimination between positive and negative TESE followed by testicular leptin, seminal leptin then finally serum leptin. The best FSH and testicular, seminal and serum leptin discriminative concentrations were >13.3 mIU/ml (sensitivity 100%, specificity 58.6%), ⩽1.567 ng/ml (sensitivity 81.8%, specificity 79.3%), ⩽1.194 ng/ml (sensitivity 90.0%, specificity 65.5%) and ⩽3.674 ng/ml (sensitivity 72.73%, specificity 62.07%) respectively.

Data from Table 4 summarizes values of AUC for combined variables which were the highest for combination of the four predicting variables with 0.909 AUC value, then combination of FSH, testicular leptin and seminal leptin with 0.897 AUC value, then combination of FSH and testicular leptin and combination of FSH, serum leptin and testicular leptin with the same AUC value; 0.893, then combination of FSH and seminal leptin with 0.887 AUC value, then combination of FSH, serum leptin and seminal leptin with 0.881 AUC value, then FSH with 0.828 AUC value, then combination of seminal leptin and testicular leptin and combination of serum leptin, seminal leptin and testicular leptin with the same AUC value; 0.824, then combination of FSH and serum leptin with 0.821 AUC value, then testicular leptin with 0.809 AUC value, then combination of serum leptin and testicular leptin with 0.781 AUC value, then testicular leptin with 0.773 AUC value, then combination of serum leptin and seminal leptin with 0.759 AUC value and then finally serum leptin with 0.605 AUC value.

Discussion

In this study we evaluated the leptin levels in serum, seminal plasma and testicular tissue homogenate and their relationships to spermatogenesis, and evaluated the predictive value of leptin hormone concentrations in serum and seminal plasma as markers for the existence of sperm within the testicular tissue in NOA cases compared to control.

To the author's knowledge this study was the first to determine the level of leptin quantitatively in the testicular tissue. Moreover, for the first time the best threshold concentration for testicular leptin for discrimination between positive and negative TESE in NOA men was determined.

Sperm retrieval became the solution for paternity in NOA men. A previous study has reported that sperm retrieval chance for each biopsy is 20–50% in these patients (von Eckardstein et al., 1999). In our study, the chance of sperm retrieval for NOA men was 27.5% (11 out of 40 patients).

Table 4 Analysis of binary regression for FSH, serum leptin, seminal leptin, and testicular leptin levels for non-obstructive azoospermia.

	AUC	S.E.	95% CI
FSH + serum leptin + seminal leptin + testicular leptin	0.909	0.0456	0.775–0.977
FSH + seminal leptin + testicular leptin	0.897	0.0500	0.759–0.970
FSH + testicular leptin	0.893	0.0583	0.755–0.969
FSH + serum leptin + testicular leptin	0.893	0.0531	0.755–0.969
FSH + seminal leptin	0.887	0.0511	0.747–0.965
FSH + serum leptin + seminal leptin	0.881	0.0540	0.739–0.962
FSH	0.828	0.0685	0.675–0.928
Seminal leptin + testicular leptin	0.824	0.0665	0.672–0.926
Serum leptin + seminal leptin + testicular leptin	0.824	0.0674	0.672–0.926
FSH + serum leptin	0.821	0.0674	0.668–0.924
Testicular leptin	0.809	0.0799	0.654–0.916
Serum leptin + testicular leptin	0.781	0.0797	0.622–0.896
Seminal leptin	0.773	0.0752	0.613–0.890
Serum leptin + seminal leptin	0.759	0.0793	0.597–0.880
Serum leptin	0.605	0.110	0.438–0.756

AUC: area under curve.
CI: confidence interval.
S.E.: standard error of mean.

Many recent studies have focused on the use of non-invasive markers; FSH, Inh-B and AMH concentrations, to predict the presence of the spermatozoa in the testicular tissues (Isikoglu et al., 2006; Tunc et al., 2006; Nistal et al., 2007; Goulis et al., 2009), while few studies have pointed the use of leptin for this purpose (Ma et al., 2010; Gao et al., 2011).

The results of the present study have shown that serum leptin concentrations were comparable among all studied groups; NOR, OA, NOA (+) and NOA (−) groups. This finding is compatible with Zorn et al. who found no significant difference in serum leptin concentrations of non-obstructive azoospermic, obstructive azoospermic, oligoasthenoteratozoospermic and normozoospermic men (Zorn et al., 2007), and with Chen et al. who reported no significant difference in serum leptin concentrations between varicocele-related (low spermatogenic) patients and the control group (Chen et al., 2009). On the other hand, Steinman and his collogues have reported higher serum leptin concentration in azoospermic men than in normozoospermic men (Steinman et al., 2001), stating that serum leptin has a direct effect on testis function, specifically on spermatogenesis. However, they have shown no significant correlation between serum leptin and testosterone concentrations. They also have shown no correlation between serum leptin concentration and LH, FSH and prolactin concentrations which is in agreement with the present results.

Zorn et al. have reported that there is a link between serum leptin and testicular function independent of FSH and LH, and they attributed serum leptin direct action on testis to testosterone and sex hormone binding globulin (SHBG) through a regulation of Leydig cell function, since they have shown a significant inversely proportional relationship between serum levels of leptin and total testosterone and SHBG (Zorn et al., 2007).

Although the present study and that of Zorn et al. (2007) have reached the same conclusion as Steinman et al. (2001) who reported that leptin affects testes function and that there is no correlation between serum leptin and LH and FSH, the present study and that of Zorn et al. have reported that serum leptin affects steroidogenesis through a regulation of Leydig cell function while Steinman et al. reported that serum leptin affects spermatogenesis.

In the present study, a negative correlation between serum leptin and total testosterone concentrations was found, this is in agreement with many authors (Luukkaa et al., 1998; Tena-Sempere et al., 1999; Hanafy et al., 2007; Zorn et al., 2007; Jahan et al., 2011). It is also in agreement with others who reported that serum leptin affects the testes directly through steroidogenesis (Tena-Sempere et al., 2001; Tena-Sempere and Barreiro, 2002) and with the review which has reported that leptin negatively regulates steroidogenesis through direct actions on Leydig cells (Phillips and Tanphaichitr, 2010).

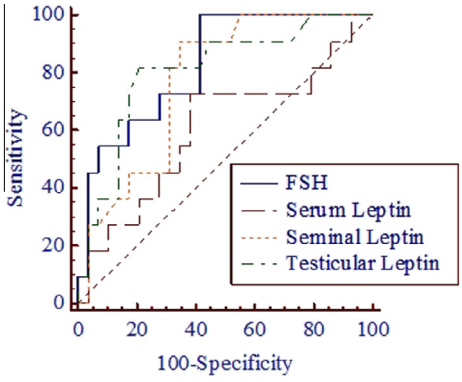

Fig. 1 Receiver operating curves for FSH, serum leptin, seminal leptin and testicular leptin concentrations for discriminating successful and failed testicular sperm extraction in non-obstructive azoospermic patients.

This negative correlation between serum leptin and total testosterone may explain the decreased serum testosterone level observed in obese males in some studies (Zumoff et al., 1990; Vettor et al., 1997; Isidori et al., 1999; Aggerholm et al., 2008). Some studies have shown a negative correlation between BMI and serum testosterone concentration (Fejes et al., 2006; Aggerholm et al., 2008), which was not the case with the present study, possibly due to the exclusion of the obese men.

From the results of the present study no correlation was observed between neither serum and seminal leptin nor serum and testicular leptin, which might be due to the testis–blood-barrier that prevents many large molecules from passing from the interstitial tissue and the basal compartment of the tubule to the adluminal compartment of the tubule and the lumen. Contrarily, some studies have shown a positive correlation between serum leptin and seminal leptin concentrations (Banks et al., 1999; von Sobbe et al., 2003; Ando and Aquila, 2005; Ishikawa et al., 2007) and some of them suggested that leptin is not actively transported but rather leaks through the testis–blood-barrier (Banks et al., 1999; von Sobbe et al., 2003).

Previously, it has been shown that leptin exists in the seminal plasma (Camina et al., 2002; Glander et al., 2002; Jope et al., 2003) and in the seminiferous tubules (Glander et al., 2002). Also a soluble leptin receptor in the seminal plasma and a leptin receptor isoform in the ejaculated spermatozoa have been detected which confirms a role for leptin action in the male genital tract (Jope et al., 2003). Aquila et al. (2005) have reported that the origin of seminal plasma leptin is not exactly defined but concluded that seminal leptin is secreted by the ejaculated spermatozoa itself, whereas other studies have attributed the presence of leptin in the seminal plasma to the leakage of leptin through the testis–blood-barrier (Banks et al., 1999; von Sobbe et al., 2003). The present study found detectable levels for leptin in the seminal plasma of azoospermic and normozoospermic men which affirm that leptin of seminal plasma may be secreted by the whole genital tract and/or by the testicular tissue itself.

The present study results showed a detectable leptin level in the testicular homogenate of azoospermic men and also showed a significant direct proportional relationship between testicular and seminal leptin but not between testicular and serum leptin, which may indicate that leptin in seminal plasma is synthesized and secreted by the testicular tissue itself. Detection of leptin in testicular tissue as found in our study is in contradiction to the previous opinion of Camina et al. who have stated that testicular tissues are not the source of seminal leptin but the most likely sources of seminal leptin are either seminal vesicles or prostate tissue (Camina et al., 2002).

Our results have shown that the azoospermic groups; OA, NOA (+) and NOA (−) groups, have higher leptin levels in the seminal plasma compared with that of the NOR group, which is in agreement with results of Gao et al. (2011) who

have shown a significant increase in the seminal leptin levels of OA and NOA groups.

In the study of Gao et al. (2011), the authors have reported an increase in the seminal leptin concentration of NOA men compared with that of OA men (Gao et al., 2011). Thus they investigated the role of serum and seminal leptin as a marker for obstruction, but did not address the usage of leptin to predict the existence of sperm in the testicular tissues of NOA men. This is what is being addressed by this research.

The present study showed that seminal leptin concentrations of OA and NOA (+) men were comparable while NOA (−) patients showed higher seminal leptin concentrations than those of OA and NOA (+), which indicate that seminal leptin can be used as a marker for spermatogenesis, but not as a marker for obstruction.

The current study is in agreement with Ma et al. who reported that FSH is a good discriminative marker between NOA (−) and NOA (+) men and also in agreement with them in their conclusion that the combined use of seminal leptin and FSH can significantly improve the prediction accuracy. This is confirmed by the ROC curve of the present study, where it was shown that the area under the curve (AUC) of seminal leptin for discrimination between NOA (−) and NOA (+) men was 0.773 with *P*-value (0.0003). And irrespective of its lower AUC value than that of FSH (AUC: 0.773 versus 0.828), the logistic regression confirms that the combined use of both gave AUC value higher than that of FSH alone; 0.887 versus 0.828, which could significantly improve the prediction accuracy for sperm recovery.

The results of the present study also showed that the testicular leptin concentrations in OA and NOA (+) men were comparable and both being lower than NOA (−) men, which may indicate that there is a link between testicular leptin and spermatogenesis. This is in agreement with Ishikawa and his co-workers who have worked on OA, Sertoli cell-only syndrome and oligozoospermic patients with varicocele, in comparison with five fertile men, and have observed overexpression of leptin and leptin receptors in hypospermatogenetic testes (Ishikawa et al., 2007). This indication served to favor the spermatogenic deficiency as the source and cause of elevated seminal leptin rather than pathology of the accessory sex glands; seminal vesicles and prostate.

In the current study, although testicular leptin especially in combination with FSH had a high predictive value, it is not clinically applicable considering its invasive nature for an investigation. Therefore seminal leptin with or without FSH is the proposed preoperative marker for spermatogenesis.

Prospective researches are needed to answer the question of whether seminal leptin can be used as a predictive marker for sperm retrieval in obese cases or not.

In conclusion it can be proposed that the increase in testicular leptin concentration negatively affects spermatogenesis, but more future researches should be done to reach the mechanism of this action. Also, determining the concentration of leptin in the seminal plasma of NOA patients can be used as a good assistant marker to predict the success or failure of the sperm retrieval in NOA men especially in combination with FSH.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution

M.M. Ellithy performed experiments, acquired data, and wrote the manuscript. O.K. Shaeer conceived the project, designed experiments and supervised the project. K.M. Gaafar supervised the project and revised the manuscript.

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