INTRODUCTION

Leptin, an adipocyte derived protein hormone consists of 167 amino acids, enables the body to maintain homeostasis. It has emerged as a metabolic link between nutrition and fertility by its involvement in regulation of reproductive function in both experimental animals and humans. Deficiency was a causative agent of infertility in ob mice. Serum leptin level strongly relates to fat mass and subcutaneous fat. It is expressed mainly on spermatocytes in the seminiferous tubules and its expression increases with spermatogenic dysfunction. Leptin receptors are present in testicular tissue. The mechanism of leptin action seems to be the inhibition of testosterone secretion as demonstrated in vitro studies in the adult rat testis. Apart from the well-defined role in food uptake, leptin has a less clear regulatory function on the male reproductive axis. In particular, the association between leptin and testicular function remains to be elucidated and the question is still unclear whether leptin acts through hypothalamic or by directly targeting the effector organs. The present study was designed to investigate a possible relationship or association between leptin and male fertility dysfunction.

METHODOLOGY

This prospective study was conducted from April to December 2009. There were 154 male subjects including 24 (15.58%) fertile, 19 (12.34%) polyzoospermic (PZs), 26 (16.88%) teratozoospermic (TZs), 27 (17.53%) astheno-teratozoospermic (ATZs), 18 (11.69%) oligozoospermic (OZs), 18 (11.69%) oligo-astheno-teratozoospermic (OATZs), 11 (7.14%) obstructive azoospermic (OBST-AZOOs) and 11 (7.14%) non-obstructive azoospermic (NON-OBST-AZOOs). BMI was also determined, divided into groups of greater than 24. Hormonal concentrations were compared by ANOVA and correlation was performed by using Graph pad prism version 5.

RESULTS: Significantly high levels of leptin concentrations were found in fertile (p < 0.001) as well as TZs, PZs, OZs, ATZs, (p < 0.001), OATZs (p < 0.01) and NON-OBST-AZOOs (p < 0.05) male subjects with BMI > 24 compared to fertile and infertile male patients with BMI < 24. Serum testosterone concentrations were significantly lower in PZs, OATZs, OZs, NON-OBST-AZOOs male patients with BMI < 24 compared to PZs (p < 0.05), OATZs, OZs (p < 0.01), OBST-AZOOs (p < 0.001) male patients with BMI > 24. Leptin showed a significant positive correlation with LH (p < 0.01) and FSH (p < 0.002) and a significant negative correlation with testosterone (p < 0.001).

CONCLUSION: Abnormal leptin level was significantly associated with fertility problems in males. Providing a link between leptin and reproduction factors contributing in control of testosterone and gonadotropins secretion in many aspects depending on fertility status in male subjects. BMI appears to have significant association with serum leptin levels.

Key words: Leptin, Testosterone, FSH, LH, Male infertility.
Patients provided semen sample by masturbation which were analyzed and categorized according to the World Health Organization’s guidelines. Blood samples were drawn by venous puncture with sterile syringes. Serum was separated from centrifuged blood and stored at 2-8°C until hormonal analysis.

LH (leutinizing hormone), FSH (follicle stimulating hormone) and testosterone were quantitatively determined by EIA kits (Amgenix International.Inc, USA), while human leptin was determined by EIA kit (AssayMax, Germany). All the kits were used according to the manufacturer's instruction.

Data were expressed as mean ± S.E.M. To compare fertile and all infertile categories of male subjects, one way ANOVA followed by Tukey's test was used. The correlation between leptin and other hormones was performed using statistical analysis package, Graph pad prism version 5. Statistical significance was set at \( p \leq 0.05 \).

**RESULTS**

Mean age, weight, height and body mass index of fertile and infertile polyzoospermics (PZs), teratozoospermics (TZs), astheno-teratozoospermics (ATZs), oligozoospermics (OZs), oligo-astheno-teratozoospermics (OATZs), obstructive azoospermics (OBST-AZOOs) and non-obstructive azoospermics (NON-OBST-AZOOs) of male subjects are given in Table I.

Significantly higher leptin concentrations were found in fertile (\( p < 0.001 \)) males as well as different categories of infertile, TZs, PZs, OZs, ATZs, (\( p < 0.01 \)), OATZs (\( p < 0.01 \)) and NON-OBST-AZOOs (\( p < 0.05 \)) male subjects with BMI > 24 compared to fertile and infertile male patients with BMI < 24. However, no significant difference was found in serum leptin concentrations with increase in BMI of OBST-AZOOs patients.

Serum leptin concentrations in fertile males were significantly lower than in NON-OBST-AZOOs (\( p < 0.001 \)), OZs (O < 0.05) and OATZs (\( p < 0.01 \)) OZs (p < 0.05) and OATZs (p < 0.01) patients with BMI < 24. All other infertile categories showed no significant difference as compared to the fertile male subjects.

In the NON-OBST-AZOOs, OATZs and OZs male patients, serum leptin concentrations were found significantly high (\( p < 0.01 \)) as compared to the fertile subject with BMI > 24. However, no significant difference was noticed in fertile and all other infertile male patients with BMI > 24 (Table II).

Serum testosterone concentrations in patients with BMI < 24 were significantly lower in PZs (\( p < 0.05 \)), OZs (\( p < 0.01 \)), OBST-AZOOs (\( p < 0.001 \)) as compared to PZs, OATZs, OZs, NON-OBST-AZOOs male patients with BMI > 24 respectively.

In the patients having BMI < 24, serum testosterone concentrations (ng/ml) were significantly high in fertile subjects as compared to NON-OBST-AZOOs (\( p < 0.01 \)) and OATZs (p < 0.05) male patients. NON-OBST-AZOOs, OATZs and OZs patients having BMI > 24 showed significantly high (\( p < 0.05 \)) testosterone concentrations as compared to fertile subjects (Table II).

LH and FSH concentrations (mIU/ml) were not significantly different in fertile and all categories of infertile patients with BMI < 24 as compared to the patients with BMI > 24.

Significantly high LH concentrations (\( p < 0.05 \)) and FSH concentrations (\( p < 0.001 \)) (mIU/ml) were found in NON-OBST-AZOOs male patients with BMI < 24 compared to fertile male subjects with BMI < 24.

Significantly high LH concentrations were found in NON-OBST-AZOOs patients with BMI > 24 as compared to the fertile (\( p < 0.05 \)), PZs, TZs and ATZs (\( p < 0.01 \)) male subjects with BMI > 24, while FSH concentrations were significantly (\( p < 0.001 \)) high in NON-OBST-AZOOs with BMI > 24 as compared to fertile male subjects with BMI < 24 (Table II).

The subjects with BMI < 24 and BMI > 24 showed a positive correlation between LH (\( r = 0.45 \), \( p = 0.003 \)) and (\( r = 0.198 \), \( p = 0.23 \)), FSH (\( r = 0.52 \), \( p = 0.001 \)) and (\( r = 0.29 \), \( p = 0.05 \)) respectively, while a significant negative correlation between leptin and testosterone concentrations (\( r = -0.397 \), \( p = 0.01 \)) and (\( r = -0.313 \), \( p = 0.04 \)) (Figure 1).

In Table I: Mean age, weight, height and body mass index of fertile and infertile polyzoospermics (PZs), teratozoospermics (TZs), astheno-teratozoospermics (ATZs), oligozoospermics (OZs), oligo-astheno-teratozoospermics (OATZs), obstructive azoospermics (OBST-AZOOs) and non-obstructive azoospermics (NON-OBST-AZOOs) of male subjects.

<table>
<thead>
<tr>
<th>General parameters</th>
<th>Fertile (n=24)</th>
<th>PZs (n=19)</th>
<th>TZs (n=26)</th>
<th>ATZs (n=27)</th>
<th>OZs (n=18)</th>
<th>OATZs (n=18)</th>
<th>OBST-AZOOs (n=11)</th>
<th>NON-OBST-AZOOs (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (year)</strong></td>
<td>31.63±1.37</td>
<td>31.95±1.71</td>
<td>30.30±1.14</td>
<td>32.33±1.09</td>
<td>33.71±1.5</td>
<td>32.30±1.55</td>
<td>32.45±1.95</td>
<td>33.45±2.18</td>
</tr>
<tr>
<td><strong>BMI (kg/m2)</strong></td>
<td>22.50±0.58</td>
<td>23.44±0.76</td>
<td>25.41±1.29</td>
<td>24.83±0.71</td>
<td>23.54±0.86</td>
<td>22.84±0.73</td>
<td>23.78±1.43</td>
<td>24.09±0.95</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>67.30±1.70</td>
<td>68.55±2.28</td>
<td>73.44±3.73</td>
<td>71.76±2.05</td>
<td>68.86±2.33</td>
<td>66.80±2.08</td>
<td>70.35±4.95</td>
<td>70.45±3.51</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.73±1.15</td>
<td>1.71±1.37</td>
<td>1.70±1.06</td>
<td>1.70±0.80</td>
<td>1.71±1.24</td>
<td>1.71±1.44</td>
<td>1.72±0.07</td>
<td>1.71±0.06</td>
</tr>
</tbody>
</table>

Values= Mean ± S.E.M.
DISCUSSION

Human fertility is linked to sperm quality and hormonal regulation of spermatogenesis. Standard semen parameters analysis has remarkable ability to predict male fertility potential and has prognostic ability to evaluate infertile male patient for assisted reproductive procedure.11 Fertile male subjects in this investigation had normal semen parameters as previously reported.12 The infertile male subjects were categorized into subgroups on the basis of semen parameters. 13 In this study, astheno-teratozoospermics (ATZs) 17.53% and teratozoospermics (TZs) 16.88% had the highest prevalence among the infertile males. In another Pakistani population, the high incidence of teratozoospermics (39.36%) and astheno-teratozoospermics (17.64%) males were reported.14 However, a low percentage (8%) of PZs was observed from the population in UK.15 In the present study, percentage of obstructive azoospermics was 7.14% and non-obstructive azoospermics was 7.14%. These findings were also in conformity with the population from Greece and UK.15,16

Previously, many studies were carried out to determine the role of leptin in regulation of reproductive axis.17,18 However, no data regarding the relationship of Leptin concentrations with male infertility has been reported. In current study, serum leptin levels were measured in normal (BMI < 24) and overweight (BMI > 24) male subjects and in males with different categories of infertile patients. Significant elevated leptin concentrations were observed in infertile male patients with disease affecting the testis parenchyma of the seminiferous tubules (NON-OBST-AZOOS, OZs and

### Table II: Comparison of serum concentration of leptin and reproductive hormone in fertile and infertile polyzoospermics (PZs), teratozoospermics (TZs), astheno-teratozoospermics (ATZs), oligozoospermics (OZs), oligo-astheno-teratozoospermics (OATZs), obstructive azoospermics (OBST-AZOOS), non-obstructive azoospermics (NON-OBST-AZOOS) male subjects (ANOVA followed by Tukey’s test).

<table>
<thead>
<tr>
<th></th>
<th>Leptin (ng/ml)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 24</td>
<td>&gt; 24</td>
<td>&lt; 24</td>
<td>&gt; 24</td>
</tr>
<tr>
<td>Fertile</td>
<td>5.16±1.23</td>
<td>14.03±2.10***</td>
<td>7.26±0.97</td>
<td>6.21±1.77</td>
</tr>
<tr>
<td>PZs</td>
<td>2.57±0.87***</td>
<td>14.74±1.49***</td>
<td>5.95±2.03</td>
<td>4.52±0.45**</td>
</tr>
<tr>
<td>TZs</td>
<td>5.22±1.18**</td>
<td>13.26±1.08***</td>
<td>4.66±0.96**</td>
<td>4.50±0.54**</td>
</tr>
<tr>
<td>ATZs</td>
<td>3.97±0.79***</td>
<td>14.21±1.32**</td>
<td>4.74±0.91**</td>
<td>4.70±0.80**</td>
</tr>
<tr>
<td>OZs</td>
<td>9.62±2.21**</td>
<td>19.81±1.2***</td>
<td>8.02±2.20</td>
<td>8.32±1.47</td>
</tr>
<tr>
<td>OATZs</td>
<td>11.80±1.73**</td>
<td>22.41±1.5***</td>
<td>8.38±1.90</td>
<td>10.06±2.39</td>
</tr>
<tr>
<td>OBST-AZOOS</td>
<td>6.77±3.26</td>
<td>12.81±2.9***</td>
<td>5.33±1.29**</td>
<td>6.28±1.90</td>
</tr>
<tr>
<td>NON-OBST-AZOOS</td>
<td>19.25±3.14***</td>
<td>26.1±3.3**</td>
<td>11.47±1.78</td>
<td>13.49±1.8*</td>
</tr>
</tbody>
</table>

Values= Mean ± S.E.M.

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Figure 1: A significant negative correlation between serum leptin concentrations (ng/ml) and testosterone (ng/ml) concentrations (a and b), and positive correlation between leptin concentrations and LH (mIU/ml) concentrations (c and d), leptin concentrations and FSH (mIU/ml) concentrations (e and f), in infertile male subjects with BMI < 24 and > 24 (Graph pad prism version 5).
OATZs). Similarly, high concentrations of leptin were found in overweight (BMI > 24) fertile and infertile subjects (BMI > 24) than normal weight (BMI < 24). These findings can be supported by the previous findings, which it was reported that leptin has a strong correlation with obesity. It was also reported that serum leptin concentration correlates significantly with body mass index and percentage body fat in men.

Low testosterone concentrations were found in overweight infertile male patients (TZs, OATZs, OZs and OBST-AZOOS), indicating that leptin excess in these overweight patients have caused reduction in androgens concentrations and deviation from normal ranges. Inverse correlation can be explained by the paracrine inhibitory effect of leptin on testosterone production by Leydig cells, also testosterone increased lipolysis when bind with androgen binding receptor on adipocytes.

Significantly higher serum concentrations of both FSH and LH were found in NON-OBST-AZOOS and slightly higher values in OZs and OATZs, evaluation of LH and FSH reflects testicular dysfunction and results in alteration of normal feed back relation between the testis and hypothalamus. Male infertility is associated to elevated concentrations of gonadotropins which are significantly divergent from normal ranges. Non-significant increase in LH concentrations in OZs and OATZs patients as compared to fertile was in agreement with a previous report. Elevated serum FSH concentration in NON-OBST-AZOOS, OATZs and OZs patients was well established. In the present study NON-OBST-AZOOS patients had higher gonadotropins (LH and FSH) concentrations and decreased in testosterone concentrations. Normal serum concentrations of FSH, LH and testosterone in OBST-AZOOS patients reflect obstruction etiology, maturation arrest i.e. spermatogenesis not occurring beyond certain stage despite presence of germ cells. Inappropriately high serum FSH is a predictor of non-obstructive azoospermia (NOA). The current study showed increased concentrations of LH, FSH and testosterone in OATZs and OZs male patients. These results are in accordance with the work of Behr Schoor et al. and Hermanns et al., who found significantly high concentrations of LH, FSH and testosterone in OATZs and OZs males. A significant correlation was found between leptin and LH ($p < 0.01$) and FSH ($p < 0.01$) concentration in infertile males, insufficient data was found regarding such correlation of gonadotropins with leptin concentration in infertile males in literature, but the present data suggest that leptin concentrations play a significant role in gonadotropin secretion.

**CONCLUSION**

Leptin may be a contributory factor to the control of testosterone, FSH and LH concentrations. This study provides a link between leptin and male reproduction axis depending on fertility status of subjects. However, BMI has a role in determining leptin levels.

**REFERENCES**


