Effect of stress on semen quality in a population of infertile human

Dr. Edress M. Ameen
Dept of Biology, College of Science,
University of Salahaddin- Erbil

Abstract
The aim of the present study was to assess the relationships between the stress which results from the work and human semen quality. The work stress which was studied includes two types; quality stress (easy and moderate to hard performing work/day) and quantity stress (number of hours performing work/day). Results are elaborated by using independent t-test statistical method. From fifty two men which has an easy and moderate work and forty eight men with daily hard job, we determined significant negative (p<0.05) influence of stress on sperm concentration, sperm grade activity, percentage of sperm motility, and percentage of sperm viability. Quantity stress showed that a men with 7-10 hrs performing job/day has a significant (p<0.05) lower; sperm concentration, sperm grade activity, percentage of sperm motility, and percentage of sperm viability compare with 4-6 hrs performing job/daily. No significant differences were recorded between quality and quantity stress in which means that both types of stress has the same effect on semen quality.

Keywords: semen quality, stress, sperm motility, sperm concentration, sperm viability.

Introduction
Stress is one of the most important health and social problems. It is responsible for various diseases such as atherosclerosis and gastrointestinal pathology (1, 2). Although stress has been implicated as a cofactor in the severity and progression of a number of diseases, the potential role of stress in semen quality has received very little scientific attention (3). There is a long history of the interest in the effects of psychological stress on the seminiferous epithelium, male hormone axis, and semen quality (4). Psychological stress can depress testosterone (5, 6, 7) and, thereby, interfere with spermatogenesis (8). Clinical observations and experimental studies have shown that stress suppresses the sexual/reproductive function (9). Previous studies have indicated that stress has a negative impact on various parameters associated with semen quality, including sperm concentration, motility and morphology (10, 11). Several studies suggest that stress associated with workplace, family, and other factors has adverse effects on semen quality (12, 13, 14, 15). Decreased semen quality has long been associated with decreased fertility (16, 17). Some evidence suggested a decreasing of normal sperm...

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parameters trend of male fertility in the last 50 years such as decreased mean sperm count \(^{(18)}\).

High proportions of motile sperm have been associated with fertilization \(^{(19)}\). Important information about sperm function is also contained in the morphology of spermatozoa \(^{(20)}\).

Sperm morphology gives information for the function of the reproductive tract and is a predictor of men fertility potential \(^{(21)}\). In addition to their clinical application, sperm motility and morphology have been shown to be sensitive indicators of germ-cell toxicity due to environmental, occupational, and therapeutic agents \(^{(22)}\). Reduced percentage of motility with a high percentage of viable sperm may reflect structural or metabolic abnormalities of spermatozoa that derived from abnormalities in testicular function or antimotility factors in the seminal plasma \(^{(23)}\).

Different types of stress were found and may be affects on semen quality such as psychological stress, sedentary work position, work place position, severity of working and others. Globally most studies are confined to psychological stress and little studies were performed on working stress. In Kurdistan region of Iraq, there is no studies on all types of stress and their effects on semen quality. So that this work was done and considered as a first trial to objectives the influence of two kinds of working stress on human semen quality.

Improvement of our understood about the effect of stress on semen quality is very important because any negative changes in standardized semen measurements may be lead to infertility, and according to this it is necessary to improve our knowledge about how to change lifestyle and working toward the best.

**Materials and Methods**

**Semen samples collection**

The study included one hundred and ninety selected infertile men who attended to Infertility care and in vitro fertilization center in Erbil city. This work was carried out between Junes to November 2007. The men were divided into four groups. Group 1 include 52 men which has easy and moderate work/daily with mean age \((32.456 \pm 0.892)\) years. Group2 include 48 men which has hard work/daily with mean age \((33.785 \pm 0.653)\) years. Group3 include 45 men which working about 4-6hrs/daily with mean age \((34.327 \pm 1.211)\) years. Group4 include 45 men which working about 7-10 hrs/daily with mean age \((34.674 \pm 0.935)\) years. Group 3 and group 4 has an easy to moderate work/ day. All other factors which affects semen quality was excluded from this study until they don’t interferes with the results such as varicocele, inguinal hernia, pubertal mumps, smoking, alcohol drinking and others.

Semen samples were collected by masturbation after 3 days of abstinence, in wide mouth disposable plastic container. The semen samples were incubated at \(37^\circ C\) for 30 minutes to liquefy \(^{(24)}\).

The following routine parameters were assessed in the liquefied semen samples according to the methods described in the laboratory WHO manual \(^{(25)}\). These parameters includes; semen volume, sperm conc., total sperm count, sperm grade activity, sperm motility percent, sperm vitality percent, and sperm morphology percent. The semen was centrifuged at 3000 (rpm) for 10 minutes to obtain the plasma. The plasma was stored in \(-80^\circ C\) (Sanyo ultra Low MDF-142 Japan) for further examinations, such as osmolality, and malondialdehyde determination.

**Semen analysis**
The volume of the ejaculate was measured with a graduated cylinder. The sperm concentration was estimated by multiplying the mean of sperm number in ten fields with \(10^6\). Total sperm count = Sperm conc. \times volume\(^2\). For assessed of sperm motility a minimum of around 200 sperm should be counted, both motile and immotile sperms are counted in at least 5 separate microscopic fields\(^3\).

The motility of spermatozoa in each sample is graded 0, 1, 2, 3, or 4 according to whether it shows: 0 = no movement, 1 = movement but not forward progression, 2 = movement with slow forward progression, 3 = movement in an almost straight line with good speed, 4 = movement in a straight line with high speed\(^2\). Sperm viability was determined by using vital stains eosin 1% and Nigrosin 10% and sperm morphology was measured by using haematoxylin and Eosin stains according to the methods described by\(^2\). Myeloperoxidase cytochemical test (Endtz test) was used for seminal plasma leucocytes determination with using working benzidine solution\(^2\).

Semenal plasma osmolality was measured by the freezing point depression method and using an osmometer type (Knauer, D- 14163, Berlin, Germany). This method requires samples of semen to be centrifuged free of particulate matter\(^2\). The assessment of lipid peroxidation process was achieved via determination the end product, malondialdehyde MDA\(^2\). The level of seminal plasma MDA was determined by a modified procedure described by\(^3\) with using of trichloroacetic acid.

**Data analysis**

Data were reported as means ± standard errors of means (SEM). Comparisons of the data between the stress and non-stress groups were made by the independent t-test. Analysis of the data was performed by using SPSS (Version 10) in the home computer. A P-value of less than 0.05 was considered to be statistically significant.

**Results**

**Effect of hard work on semen quality**

The effect of hard work on semen quality was observed in table 1. Men with hard work has significantly (p<0.05) lower; sperm concentration \((53.123 \times 10^6/ ml)\), sperm grade activity (1.987), sperm motility (32.472 %), and sperm viability (48.870 %) compare with men which has an easy and moderate work/ daily. The value of their semen parameters are higher and are as follows; sperm concentration \((62.222 \times 10^6/ ml)\), sperm grade activity (2.670), sperm motility (38.207 %), and sperm viability (54.756 %).

There are no significant differences between the two groups in other semen parameters such as; semen volume, total sperm count, normal sperm morphology, seminal leucocytes, seminal plasma osmolality, and seminal plasma malondialdehyde.

**Effect of long period work on semen quality**

The long period work has the same effect on semen quality as hard work do. Results which are recorded in table 2 showed that men with 7-10 hrs working daily has significantly (p<0.05) lower; sperm concentration \((56.213 \times 10^6/ ml)\), sperm grade activity (2.120), sperm motility (34.282 %), and sperm viability (48.201 %) versus sperm concentration \((65.123 \times 10^6/ ml)\), sperm grade activity (2.832), sperm motility (40.561 %), and sperm viability (58.321 %) of men which working 4-6 hrs daily.

There are no significant differences between the two groups in other semen parameters such as; semen volume, total sperm count, normal sperm morphology, seminal leucocytes, seminal plasma osmolality, and seminal plasma malondialdehyde.

**Comparison between hard work and long period work**
Table 3 demonstrates the comparison between the 2 types of stress on their effects on semen quality. No significant differences appears between the hard work and long period stress on their effects on all seminal parameters, that is means that both stress has the similar effects on semen quality.

Table (1): Effect of hard work (quality stress) on semen quality (Means ± S.E).

<table>
<thead>
<tr>
<th>Seminal parameters</th>
<th>Easy and moderate work/daily</th>
<th>Hard work/daily</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Volume (ml)</td>
<td>2.880 ± 0.136</td>
<td>2.998 ± 0.321</td>
<td>0.404</td>
</tr>
<tr>
<td>2- Sperm conc. (× 10^9 / ml)</td>
<td>62.222 ± 3.479</td>
<td>53.123 ± 3.423</td>
<td>0.045*</td>
</tr>
<tr>
<td>3- Total sperm count (× 10^6 / Ejaculate)</td>
<td>179.199 ± 13.422</td>
<td>159.263 ± 11.676</td>
<td>0.152</td>
</tr>
<tr>
<td>4- Grade activity</td>
<td>2.670 ± 0.358</td>
<td>1.987 ± 0.124</td>
<td>0.040*</td>
</tr>
<tr>
<td>5- Sperm motility (%)</td>
<td>38.207 ± 1.925</td>
<td>32.472 ± 1.993</td>
<td>0.043*</td>
</tr>
<tr>
<td>6- Sperm viability (%)</td>
<td>54.756 ± 0.698</td>
<td>48.780 ± 2.165</td>
<td>0.050*</td>
</tr>
<tr>
<td>7- Normal sperm morphology (%)</td>
<td>62.439 ± 1.581</td>
<td>58.022 ± 1.934</td>
<td>0.083</td>
</tr>
<tr>
<td>8- Seminal Leucocytes (× 10^6 / ml)</td>
<td>1.678 ± 0.310</td>
<td>2.011 ± 0.334</td>
<td>0.470</td>
</tr>
<tr>
<td>9- Osmolality (m OSm/ kg)</td>
<td>340.219 ± 5.735</td>
<td>337.824 ± 6.616</td>
<td>0.785</td>
</tr>
<tr>
<td>10- Malondialdehyde (µ mol / L)</td>
<td>3.340 ± 0.256</td>
<td>2.885 ± 0.245</td>
<td>0.202</td>
</tr>
</tbody>
</table>

* Significant at p<0.0

Table (2): Effect of long period work (quantity stress) on semen quality (Means ± S.E).

<table>
<thead>
<tr>
<th>Seminal parameters</th>
<th>4-6 hrs work/daily</th>
<th>7-10 hrs work/daily</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Volume (ml)</td>
<td>3.215 ± 0.224</td>
<td>3.076 ± 0.342</td>
<td>0.820</td>
</tr>
<tr>
<td>2- Sperm conc. (× 10^9 / ml)</td>
<td>65.123 ± 4.256</td>
<td>56.213 ± 3.876</td>
<td>0.038*</td>
</tr>
<tr>
<td>3- Total sperm count (× 10^6 / Ejaculate)</td>
<td>209.370 ± 12.875</td>
<td>172.911 ± 10.131</td>
<td>0.241</td>
</tr>
<tr>
<td>4- Grade activity</td>
<td>2.832 ± 0.256</td>
<td>2.120 ± 0.289</td>
<td>0.050*</td>
</tr>
<tr>
<td>5- Sperm motility (%)</td>
<td>40.561 ± 2.342</td>
<td>34.282 ± 1.876</td>
<td>0.045*</td>
</tr>
<tr>
<td>6- Sperm viability (%)</td>
<td>58.321 ± 3.875</td>
<td>48.201 ± 1.987</td>
<td>0.048*</td>
</tr>
<tr>
<td>7- Normal sperm morphology (%)</td>
<td>65.328 ± 2.321</td>
<td>62.321 ± 4.321</td>
<td>0.987</td>
</tr>
<tr>
<td>8- Seminal Leucocytes (× 10^6 / ml)</td>
<td>1.562 ± 0.342</td>
<td>1.943 ± 0.421</td>
<td>0.662</td>
</tr>
<tr>
<td>9- Osmolality (m OSm/ kg)</td>
<td>330.821 ± 8.872</td>
<td>335.231 ± 10.216</td>
<td>0.827</td>
</tr>
<tr>
<td>10- Malondialdehyde (µ mol / L)</td>
<td>2.567 ± 0.201</td>
<td>2.432 ± 0.275</td>
<td>0.562</td>
</tr>
</tbody>
</table>
Table (3): Comparison between the effect of hard work and long period work on semen quality (Means ± S.E).

<table>
<thead>
<tr>
<th>Seminal parameters</th>
<th>Hard work/daily</th>
<th>7-10 hrs work/daily</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Volume (ml)</td>
<td>2.998 ± 0.321</td>
<td>3.076 ± 0.342</td>
<td>0.654</td>
</tr>
<tr>
<td>2-Sperm conc. (× 10⁹ / ml)</td>
<td>53.123 ± 3.423</td>
<td>56.213 ± 3.876</td>
<td>0.548</td>
</tr>
<tr>
<td>3- Total sperm count (× 10⁶ / Ejaculate)</td>
<td>159.263 ± 11.676</td>
<td>172.911 ± 10.131</td>
<td>0.326</td>
</tr>
<tr>
<td>4- Grade activity</td>
<td>1.987 ± 0.124</td>
<td>2.120 ± 0.289</td>
<td>0.786</td>
</tr>
<tr>
<td>5- Sperm motility (%)</td>
<td>32.472 ± 1.993</td>
<td>34.282 ± 1.876</td>
<td>0.926</td>
</tr>
<tr>
<td>6- Sperm viability (%)</td>
<td>48.780 ± 2.165</td>
<td>48.201 ± 1.987</td>
<td>1.245</td>
</tr>
<tr>
<td>7- Normal sperm morphology (%)</td>
<td>58.022 ± 1.934</td>
<td>62.321 ± 4.321</td>
<td>0.988</td>
</tr>
<tr>
<td>8- Seminal Leucocytes (× 10⁶ / ml)</td>
<td>2.011 ± 0.334</td>
<td>1.943 ± 0.421</td>
<td>0.754</td>
</tr>
<tr>
<td>9-Osmolality (m OSm/ kg)</td>
<td>337.824 ± 6.616</td>
<td>335.231 ± 10.216</td>
<td>0.967</td>
</tr>
<tr>
<td>10- Malondialdehyde (µ mol / L)</td>
<td>2.885 ± 0.245</td>
<td>2.432 ± 0.275</td>
<td>0.622</td>
</tr>
</tbody>
</table>

Discussion

One of the quantitative parameters of male fertility is sperm analysis. By measuring quality of sperm one can classify the degree of the male fertility. Therefore it is one of the methods used by investigators to estimate the effect of stress upon fertility. However, results of semen analysis can only seldom predict the fertility of the couple. Indeed, one can define male infertility with certain limits in semen analysis results, and those men having results below such limits are defined as “infertile” or “sub-fertile” (32).

Our study found that the hard working and long-period working stress has negative influence and diminished the value of sperm conc., sperm grade activity, percentage of sperm motility and sperm viability. The connection between stress and sperm parameters emerges from several studies (11, 14, 33, 34, 35, 36). Previous studies were observed different results about the effect of stress on semen quality. In one study (37), 29 subjects were exposed to stress and found that psychological stress were significantly (p<0.001) lowered of the sperm concentration and rapid progressive motility of spermatozoa, and the percentage of sperm with abnormal morphology tend to increase with stress, but this changes was not significant. These results were agreement with our results. The study of (38), observed that the stress at work and total number of life events were not related to differences in semen quality, but a reduction in straight-line velocity (p<0.002) and percent of progressively motile sperm (p<0.02) were recorded. These results are in contrast with our results that the sperm concentration...
not influenced by stress, but are in agreement with our results that sperm motility was negatively affected with stress.

Self reported stress was negatively correlated with semen measures of volume and percent normal forms (14). (12) found a decrease in semen volume, count and motility in 12 of 28 infertility patients who had all experienced psychological stress. The semen specimen of 125 men reported being exposed to work-related stress showed defects in sperm morphology and vitality compared with non-stressed men (15). Those authors suggested that the deterioration of sperm variable was associated with a rise in the prolactin levels also detected in the stressed men.

The alleged mechanisms through which stress could possibly induce male sub- or infertility are not completely clear. Basic physiology and stress tell us about negative effects of physical and psychological stress on the pituitary-gonadal axis. This could mean that long standing stress might have a negative impact on sperm production and male sex hormone in testis and in circulation. Additionally, ejaculation is under control of the autonomic nervous system, including recruitment of sperm from the epididymis as well as secretion from the accessory male sex glands. Stress interaction with the autonomic nerve functions may therefore interfere with both sperm numbers and semen volume (and thereby also sperm concentration) and probably with sperm motility (recruitment and effects of secretions from the accessory sex glands) (33, 34, 39, 40, 41).

Other mechanism by which stress influence on semen quality may be due to increases in scrotal temperature, several studies have shown that men with predominantly sedentary occupations (42), or who spend considerable time driving a vehicle (43, 44), have higher average scrotal temperatures and consequently lower average sperm production.

The major components of the ejaculate volume are made up of secretions from accessory sex glands (45). The volume of semen and seminal plasma osmolality did not affected by both types of stress in the present study and it is mean that the stress has been not effect on accessory sex glands. Formation of MDA can be assayed by the thiobarbituric acid (TBA) reaction which is a simple and useful diagnostic tool for the measurement of lipid peroxidation (LPO) for in vitro and in vivo systems (46), as shown in this study, no significant differences appears in MDA value between the hard work and long period work, this is an indicator that stress not influenced the lipid peroxidation.

References