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Relationships between semen quality and fertility in a population of infertile men in Erbil city

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ABSTRACT

The evaluation and the relationships between human semen quality and fertility in infertile males in Erbil city of Iraq is the aim of the present study. Semen quality and fertility were estimated by using semen analysis. The semen analysis parameters were including; the volume of the semen, and sperm (count, motility, morphology, and viability). For this purpose, one hundred fifty infertile and fifty fertile adult males participated. The study was performed from September 2021 to April 2022 in the Infertility care and In vitro fertilization center (IVF). A significant negative correlation was found between infertility% with decreased semen volume (r = - 0.58, p≤0.05), sperm concentration (r = - 0.74, p ≤0.001), total sperm count (r = - 0.68, p≤0.001), sperm morphology (r = - 0.57, p ≤0.01), sperm viability (r = - 0.80, p ≤0.001), total sperm motility (r = -0.80, p ≤0.001), and progressive motility (r = - 0.78, p≤0.001). Regarding fertility. A significant positive correlation was found between fertility% with increased semen volume (r = 0.64, p ≤0.05), sperm concentration (r = 0.76, p ≤0.001), total sperm count (r = 0.78, p ≤0.001), sperm morphology (r = 0.48, p ≤0.01), sperm viability (r = 0.70, p ≤0.001), total sperm motility (r = 0.84, p ≤0.001), and progressive motility (r = 0.75, p ≤0.001. The prevalence of hypospermia, oligozoospermia, teratozoospermia, low sperm viability, and low sperm motility kinetics (asthenozoospermia) in infertile males is significantly higher than that of fertile men.

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Introduction

Infertility is still a very common problem in the second decade of the new millennium around the world (1). Following 12 months of regular and unprotected sexual activity, infertility is defined as the failure to produce clinical pregnancy. Between 8% and 12% of reproductive-age couples worldwide are considered to be impacted. Males are alone responsible for 20–30% of infertility cases, whereas females are responsible for 50% of cases overall (2). The population of infertile patients is difficult to estimate for three reasons: differences in definitions of infertility (1, 2, or 5 years of unsuccessful attempts to conceive), significant differences in selected populations (large populations vs. epidemiological studies), and defining who the diagnosis affects (women, couples, or individuals) (3). The number of infertile couples was predicted to be 48.5 million in 2010 (approximately 24 million infertile women), a 6 million raise from 1990 (4). In 2002, 186 million married women in developed countries (excluding China) were infertile (5). This gap is due to disparities in the age of the evaluated population, as well as differences in the time and place of estimations and the use of different calculating methodologies. Mascarenhas et al. focused on women aged 20 to 44, whereas Rutstein and Johnson looked at women aged 15 to 49.

The cornerstone of determining male fertility is sperm analysis. There are various factors of semen quality according to the World Health Organization's (WHO) guidelines for semen quality (6); nevertheless, sperm concentration and motility (total and progressive) have the most important of these parameters (7). The etiology of infertility is heavily influenced by sperm parameters including sperm (concentration, morphology, viability, and motility). The fertilization process is known to be influenced by sperm factors. If these factors fall below a level that is indicative of fertility, they will be significantly impacted (8, 9). These characteristics have a significant impact on assisted reproductive treatment outcomes, including the rates of (fertilization, embryo development, embryo quality, pregnancy, and implantation) (10-12). Many causes of male infertility in many individuals, can be recognized and treated like Idiopathic situations, in which the source of aberrant sperm parameters cannot be determined. Patients with normal sperm quality may have sperm that are either unable to fertilize oocytes or have genetic defects that prevent them from doing so (13).

From 1973 to 2011, sperm numbers and total sperm count in men from North America, Europe, and Australia decreased by 50–60%, according to a comprehensive meta-analysis. These data strongly suggest a decrease in male reproductive health, which has far-reaching consequences beyond worries about reproduction. This decline necessitates urgent research into the reasons and consequences (14). The decline in male fertility, which is linked to advancing age, poor lifestyles, and environmental variables, has a significant impact on natality, and its implications for the future of the human population make it a critical

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Table 1. Pearson's correlation (r) between semen quality with fertility and infertility percent.

Semen quality parameters	Fertility %	Infertility %
Volume (ml)	0.64*	− 0.58*
Sperm concentration ($\times 10^6$/ml)	0.76***	− 0.74***
Total sperm count ($\times 10^6$/volume of semen)	0.78***	− 0.68***
Normal Sperm morphology %	0.48**	− 0.57**
Sperm Viability %	0.70***	− 0.80***

Where, * Correlation is significant at p≤0.05; ** Correlation is significant at p≤0.01; *** Correlation is significant at p≤0.001; − the negative correlation was found.

public health issue in this century. As a result, a planned program of educational, environmental, nutritional/physical activity, and behavioral adjustment is necessary (15).

The present study aimed to evaluate the relationships between human semen quality parameters and infertility in males in Erbil city of Iraq. This study also included the comparison of the prevalence of abnormal sperm parameters between fertile and infertile males.

Materials and Methods

Subjects

The study included infertile males, who after a year or longer of unprotected intercourse, not being able to get pregnant. and 50 fertile males who have a child. The mean age of infertile males was 38.24 ± 3.46 years, while for fertile males was 40.22 ± 5.64, with no significant differences between the two groups.

Semen collection

Semen samples were collected handly in disposable plastic containers after 3 days of abstinence. Semen samples were incubated at 37 °C for 30 minutes to liquefy (16). The following routine parameters were examined in the liquefied semen samples according to the WHO methodology (6, 17). The volume of the semen, and the sperm (morphology, viability, and motility).

Seminal fluid analysis

A graduated cylinder tube was used to measure the volume of the ejaculate. To determine sperm concentration, ten liters of each semen sample were deposited in a Makler chamber with a covered glass to determine sperm concentration and then studied at 200X magnifications (6). Sperm concentration divided by volume equals total sperm count. A drop of semen, covered with a cover glass, was examined under 400X of power of a microscope fitted with a heat plate at 37 °C to determine sperm motility. At least 200 spermatozoa should be counted in at least 5 separate fields, including both motile and immotile sperms. (17). Motility % = number of motile spermatozoa/total number of spermatozoa (motile and immotile) ×100. Counting spermatozoa with straight-line forward movement only was used to determine the percentage of motile spermatozoa with straight-line forward movement. The sperm viability was evaluated by using a hypoosmotic swelling (HOS) test and according to the procedure illustrated in (6). The HOS test was created by (18). The HOS test is a straightforward laboratory procedure for determining the functional integrity of the sperm membrane. When sperm are placed in a hypoosmotic solution, water travels through the cell membrane, causing the membrane to expand. There will be no swelling of the membrane if it has already been injured. The intact membrane is measured by the percentage of swollen spermatozoa (19). Each sample was immediately incubated at 37°C, and all measurements were taken after 30 minutes. When the sample had completely liquefied, 1 ml of warmed 150 mOsm hypo-osmotic swelling solution containing sodium citrate (25 mmol/l) and fructose was added to 0.1 ml of liquefied semen for each subject.

Sperm viability = the number of viable sperm/total number of spermatozoa × 100. Hematoxylin and eosin staining was used to determine the spermatozoal morphology (20).

The excluded criteria

The exclusion criteria included; smoking, necrospermia, azoospermia, antioxidant therapy, varicocele, diabetes, hypertension, obesity, underweight, and any disease which can interfere with the results of the semen analysis.

Statistical analysis

Statistical Package for Social Science (SPSS), version 17 was used for the analysis of the data. The standard error of the mean (SEM) is used to express all of the results. Pearson's correlation (r) was used to founding the relationships between semen quality parameters (volume of semen, sperm concentration, motility, morphology, and viability) with fertility and infertility percent. Pearson's Chi-square test was used to compare the prevalence of normal and abnormal semen quality parameters between the fertility and infertility groups. Statistical significance was defined as a P-value of less than 0.05.

Results

The correlation between semen parameters with fertility and infertility percent is presented in Table 1. A significant negative correlation was found between infertility% with semen volume (r= - 0.58, p≤0.05), sperm concentration (r= - 0.74, p≤0.001), total sperm count (r= - 0.68, p≤0.001), sperm morphology (r= - 0.57, p≤0.01) and sperm viability (r= - 0.80, p≤0.001. A significant positive correlation was found between fertility% with semen volume (r= 0.64, p≤0.05), sperm concentration (r= 0.76, p≤0.001), total sperm count (r= 0.78, p≤0.001), sperm morphology (r=

Table 2. Pearson's correlation (r) between sperm motility kinetics parameters with fertility and infertility percent.

Sperm motility kinetics	Fertility %	Infertility %
Total sperm motility %	0.84***	−0.82***
Progressive motility %	0.75***	−0.78***
Non-progressive motility %	0.84***	−0.76***

Where, *** Correlation is significant at p≤0.001; − the negative correlation was found

0.48, p≤0.01), and sperm viability (r= 0.70, p≤0.001). The relations between sperm motility kinetics parameters (total motility%, progressive motility%, and non-progressive motility%) are observed in Table 2. A significant negative correlation was found between total sperm motility (r= -0.80, p≤0.001), progressive motility (r= - 0.78, p≤0.001), and non-progressive motility (r= - 0.76, p≤0.001) with infertility%. A significant positive correlation was found between fertility% with total sperm motility (r= 0.84, p≤0.001), progressive motility (r= 0.75, p≤0.001), and non-progressive motility (r= 0.84, p≤0.001).

The results are represented in Tables 3 to 8, which demonstrate Pearson's Chi-square test to compare the prevalence of normal and abnormal semen quality parameters between the fertility and infertility groups. The prevalence of low sperm volume (hypospermia), low sperm count (oligozoospermia), low normal sperm morphology (teratozoospermia), low sperm viability, and low sperm motility kinetics (asthenozoospermia) in infertile males (semen volume <1.5ml, 16.7%, p-value = 0.05, Table 3), (sperm concentration <15 $\times 10^6$/ml, 25.3%, p-value = 0.01, Table 4), (sperm morphology < 36.0% normal, p-value = 0.01, Table 5), (sperm viability < 50% normal, 43.3%, p-value = 0.001, Table 6), (total sperm motility < 50% motile, 35.7%, p-value = 0.001, Table 7), and (sperm progressive motility < 35.5%, p-value = 0.001, Table 8) is significantly higher than that of fertile men (semen volume <1.5ml, 4.0%, p-value = 0.05, Table 3), (sperm concentration <15 $\times 10^6$/ml, 6.0%, p-value = 0.01, Table 4), (sperm morphology < 14.0% normal, p-value = 0.01, Table 5), (sperm viability < 50% normal, 8.0%, p-value = 0.001, Table 6), (total sperm motility < 50% motile, 4.0%, p-value = 0.001, Table 7), (sperm progressive motility < , 4.0%, p-value = 0.001, Table 8). Figure (1) showed different abnormal sperm morphology (teratozoospermia) including sperm with two tails, a small head, rounded head, sperm without a head, and amorphous head with a bent tail.

Table 3. The prevalence of semen volume in fertile and infertile men.

Semen volume	Fertile	Infertile
≥ 1.5ml	96.0% (48)	83.3% (125)
< 1.5ml (hypospermia)	4.0% (2)	16.7% (25)

Pearson's chi-square = 5.15 p- value = 0.05

Table 4. The prevalence of sperm concentration in Fertile and infertile men.

Sperm concentration	Fertile	Infertile
≥ 15 ×106/ml	94.0% (47)	74.7% (112)
< 15 ×106/ml (Oligozoospemia)	6.0% (3)	25.3% (38)

Pearson's chi-square = 8.60 p- value = 0.01

Table 5. The prevalence of sperm morphology in fertile and infertile men.

Sperm morphology	Fertile	Infertile
≥ 4% normal	86.0% (43)	64.0% (96)
< 4% normal (Teratozoospermia)	4.0% (7)	36.0% (54)

Pearson's chi-square = 8.56 p- value = 0.01

Table 6. The prevalence of sperm viability in fertile and infertile men.

Sperm viability	Fertile	Infertile
≥ 58% normal	92.0% (46)	56.7% (85)
< 58% normal	8.0% (4)	43.3% (65)

Pearson's chi-square = 20.71 p- value = 0.001

Table 7. The prevalence of sperm motility in fertile and infertile men.

Total sperm motility	Fertile	Infertile
≥ 40% motile	96.0% (48)	64.3% (90)
< 40% motile (Asthenozoospemia)	4.0% (2)	35.7% (50)

Pearson's chi-square = 18.64 p- value = 0.001

Discussion

The first step in determining male factor infertility is to examine the semen. Standardized sperm analysis methods are now available, allowing for accurate sperm quality assessment and comparison across laboratories. For standard sperm and sperm parameters, population-based reference ranges are available (21). Male factor infertility is defined as sperm parameters that fall below the WHO normal range (22). Low sperm concentration (oligospermia), poor

Table 8. The prevalence of progressive motility in fertile and infertile men.

Progressive motility	Fertile	Infertile
≥ 32% with forwarding progression	96.0% (48)	64.7% (97)
< 32% with forwarding progression (Asthenozoospemia)	4.0% (2)	35.3% (53)

Pearson's chi-square = 55.21 p- value = 0.001

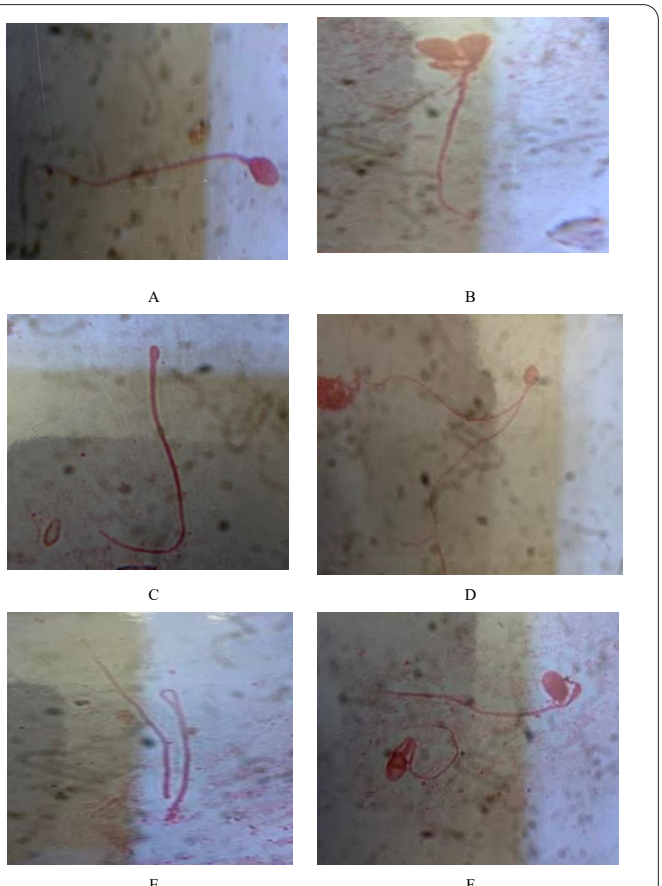

Figure 1. A: normal sperm morphology. B-F teratozoospermia. B: sperm with two tails. C: Small head. D: Rounded head with two tails. E: Sperm without a head. F: Amorphous head with a bent tail.

sperm motility (asthenospermia), and aberrant sperm morphology (teratozoospermia) are the most serious of these problems. Semen volume and other seminal markers of epididymal, prostatic, and seminal vesicle activity are less well-related to infertility (23). In the present study, a significant negative correlation was found between infertility% with semen volume. Incomplete collection, severe androgen shortage, ejaculatory duct obstruction, or bilateral vas deference should all be considered when the volume of seminal fluid is considerably reduced (21).

There is a link between aberrant sperm parameters and sperm count in up to 90% of male infertility cases (24). Our results are in agreement with the findings of (25) who observed that the mean seminal parameter values (sperm concentration, total motility, rapid linear progressive motility, normal sperm morphology, and sperm viability) for infertile Indian men were considerably lower than for fertile men. But it is in contrast with their results about semen volume, which record the higher semen volume in infertile men compared with fertile.

The quality of sperm is crucial for maintaining a healthy population growth rate and fertility rate (26). The etiology of infertility is heavily influenced by sperm parameters. The fertilization process is known to be influenced by sperm parameters. If these factors fall below a level that is indicative of fertility, they will be significantly impacted (8, 9). Sperm concentration, motility, and morphology have the greatest impact on infertility of all the semen parameters. Assisted reproductive methods such as intrauterine insemination (IUI), in-vitro fertilization (IVF), or intracytoplasmic sperm injection (ICSI) are used if these are found to be below the predetermined value (11, 12). The volume of seminal fluid, sperm concentration, and total sperm count have all declined over the last 20 years. In the latest decade (2003–2012), the proportion of hypospermic, azoospermic, and oligozoospermic men grew by 24.6%, 109.5%, and 9.5%, respectively, compared to the first decade (1993–2002) (26).

The recorded results in this study found that the prevalence of oligozoospermia in infertile males is higher than that of fertile males. It is unknown what the lowest limits of sperm concentration and total sperm per ejaculate are that indicate male infertility. The WHO reference value for sperm concentration is 15×10^6/ml, while the total sperm number is 39×10^6 per ejaculate. This is based on data collected from 1859 fertile men who had a pregnancy time of less than a year (27). The optimal time of abstinence to identify high or low sperm production may be between 42 and 54 hours, according to Cryobank data on 18 to 20 consecutive semen samples from 48 semen donors (28). The author also suggested that assessing the total quantity of spermatozoa per ejaculate is indicative of sperm production if the abstinence interval is appropriate and that the pace of daily sperm production may better reflect changing spermatogenesis (29). The study of (1) found a correlation between sperm quality and male reproductive hormones. Gonadotropins negatively correlated with sperm count and sperm motility while testosterone showed a positive correlation.

A spermatozoon is a motile cell that has the unique capacity to pass through the female reproductive system and fertilize an oocyte. Spermatozoa should have increasing motility to reach and pierce the oocyte. As a result, motility is a crucial factor in both natural and aided conception. Increased risk of infertility is linked to a global trend of decreasing the amount and motility of healthy spermatozoa in the ejaculate (30). Asthenozoospermic men had ejaculates with less than 40% total motile spermatozoa or 32% progressively motile spermatozoa. Asthenozoospermia is thought to be one of the most common causes of male infertility (31). The recording data in this study found that the prevalence of low sperm motility kinetics (asthenozoospermia) in infertile is significantly higher than that of fertile men, and these results are in the line with the findings of (32), who observed that the proven fertile group had considerably higher motility and progressive motility compared to the infertile male group. Also (33) observed that subfertile groups have less than 13.5×10^6 sperm concentrations, less than 32% of sperm motility, and less than 9% normal morphologic. While the fertile men have a sperm concentration of more than 48.0×10^6 per milliliter, more than 63% motility, and more than 12% normal sperm morphology. The pregnancy outcome and fertility are strongly related to motility (34). Sperm motility of 50% or higher has been linked to a better chance of getting pregnant, whereas motility of less than 50% has been linked to a poorer chance of getting pregnant (35). When the overall number of motile sperm was reduced, pregnancy rates were dramatically reduced when sperm concentration and % motility were combined (36).

Immotile sperm are either dead or alive. When using sperm for IVF, this differentiation is important. In the present study, the percentage of viable spermatozoa in fertile males is significantly higher than that of infertile ones. These results are in agreement with the finding of (37-40). Because dead cells cannot fertilize an egg, determining sperm viability is crucial if a significant percentage of spermatozoa in a semen sample is non-motile (21).

In conclusion, Infertility percent is negatively correlated with decreased semen volume, sperm concentration, total sperm count, sperm morphology, sperm viability, total sperm motility, and progressive motility. The infertile men showed a higher prevalence of hypospermia, oligozoospermia, teratozoospermia, low sperm viability, and low sperm motility kinetics (asthenozoospermia).

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Conflict interest
The authors declare no conflict of interest.

Authors' Contribution
Edrees Mohammad Ameen: had a principal role in study design and statistical analysis. All authors had an equal role in the study and manuscript writing.

Ethics approval and consent to participate
The samples of the study were taken following the Helsinki Declaration of 1975, as revised in 2000 and approved by the Human Ethical Committee of Salahaddin University, College of Science, Biology department, and numbered 4S/364.

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