Semen Analysis and Insight Into Male Infertility

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Abstract

Objectives: Semen analysis is the cornerstone for the valuation of the male partner in the infertile couples. This test has been standardized throughout the world through the World Health Organization (WHO) since the 1970s by producing, editing, updating, and disseminating a semen analysis manual and guidelines. A retrospective study to give an insight about male infertility.

Methods: This retrospective study assessed the semen findings of 1000 men evaluated at the Department of Urology, Al-Kindy Teaching Hospital in Baghdad-Iraq between January 2016 and May 2019. Semen analysis were done for them.

Results: According to WHO standard for semen normality, 1000 samples that were analyzed, normospermia was shown in 835 (83.5%) males (95% CI=0.811-0.857) and 12% had oligospermia and the rest 4.5% was azospermia. The normospermic samples had significantly higher levels regarding the following parameters: count per ml (51.30±1.24) (P= 0.001), volume(3.34±2.31)(P=0.0001), pus cell (8.04±1.02)(P=0.0001), motility (22.81±5.8)(P=0.0001), abnormal motility (22.81±5.8)(P=0.0001) and normal (V)(P=0.0001) or abnormal morphology (25.86 ±12.4)(P=0.0002) when compared with oligospermia.

Conclusions: Semen analysis is the keystone of infertile couple. Semen parameters like sperm concentration, motility and morphology, are indicators for male reproductive function. Sperm concentration is declining and there is a significant association between sperm concentration and sperm parameters.

Keywords: Infertility; male; semen.

Introduction

Infertility is a global health problem in the community with physical, psychological and social influences. Infertility can be defined as failure in achieving a successful pregnancy of a couple after twelve months or one year of regular sexual intercourse without using a protection or contraceptive methods. It represents about 10-15% of couples that seen in clinical daily practice and constitutes about 40-50% of the 70 million cases worldwide and caused by male factors and from each infertile six
couples, one of them either husband or wife experiences a primary or secondary infertility. According to the records from WHO, about 40% of infertility cases are due to male factors which is due to aging processes that leads to decrease sperm motility, sedentary work and lack of exercise. Other factors are infection and oxidative stress and increase in inflammatory cytokines in seminal plasma that decrease sperm quality and damage sperm DNA. Nutritional factor has an important role in sexual health and semen quality especially vitamin D deficiency. Semen or sperm analysis after three days of abstinence is usually the first laboratory test that done and one of the most important test for fertility tracking and follow-up. Meanwhile this test has to be conducted in the laboratory, many men patients are unwilling to be tested for this simple test as a result of social stigma and embarrassment in certain regions of the world. The characteristics of male infertility are abnormality in sperm motility, PH, color, morphology, velocity, semen volume, sperm concentration, and sperm count that done using visual examination, microscope and counting chambers. This method is complex, labor intensive, subjective and liable to human error so other method was used which is computer assisted semen analysis (CASA) which is effective in tracking sperm and many laboratories do not follow the instructions and guidelines of WHO in doing semen analysis and do not follow the recommended methods in the test. So this study tries to shed a light on frequency of male factor infertility in the last ten years.

Patients and methods

This retrospective study assessed the semen findings of 1000 men evaluated at the Department of Urology, Al-Kindy Teaching Hospital in Baghdad-Iraq between January 2016 and May 2019 and were referred for semen analysis to the laboratory as part of male infertility investigation and venereal infection. History was taken from them regarding age, duration of marriage, first or second marriage, occupation, type of infertility whether primary or secondary drug intake, symptoms of any venereal infection, surgical and medical history. Males excluded from study were those who received treatment like antioxidants therapy, surgical treatment like varicocelectomy and seminal tract reconstruction, patients were unable to pass specimen by masturbation.

The study protocol was reviewed by the Scientific and Ethical Committee of Al-Kindy Medical College without funding.

Patients were instructed to give a sample after abstinence from coitus for three to four days and collected aseptically by masturbation into sterile wide-mouthed container within hospital. Semen analysis was performed according to the methods and standards outlined by the World Health Organization (WHO). The parameters included the following: appearance (grey to opalescent); Volume (2.0ml or more); PH (7.2-7.8); Sperm concentration (>15x10^6 spermatozoa/ml); Total sperm count (39x10^6 or more / ejaculate); Motility (50% or more with forward progression); Morphology (4% or more with normal form); White cell count or pus cell (<1x10^6/ml).

The semen analysis was done within 60 minutes after collection then after liquefaction, the semen specimen was thoroughly mixed with the help of a pipette for the following parameters: volume was measured with a graduated disposable pipette; appearance, PH was estimated with pH paper, liquefaction, concentration, motility, morphology, viability and the presence of pus cells was assessed by microscope.

Semen samples were divided on the basis of sperm count per milliliter of semen in accordance with WHO: normospermia, oligospermia, and azoospermia. The samples grouped were compared for ejaculated volume, pus cells, motility and morphology. The following definitions were used according to WHO definitions: Normospermia: Sperm count 15 million/ml to 120 million/ml, Oligospermia: Sperm count below 15 million/ml, Azoospermia: Absence of spermatozoa in the ejaculation,
Astheno-spermia: Reduced sperm motility, Terato-zoospermia: Abnormal sperm morphology, Oligo-astheno-terato-spermia: All sperm variables abnormal, Hypospermia: Volume <2ml. Hyperspermia: Volume >5ml.

The study was registered in clinicaltrial.gov with NCT04178954 and link was [https://register.clinicaltrials.gov/prs/app/template/Home.vm?uid=U0004R9N&ts=45&cx=fvia6f, https://register.clinicaltrials.gov/prs/app/action/ReleaseProtocol?uid=U0004R9N&ts=37&sid=S0009ERV&cx=cfbgkt, https://register.clinicaltrials.gov/prs/app/action/ViewOrUnrelease?uid=U0004R9N&ts=43&sid=S0009ERV&cx=gjr3ax.](https://register.clinicaltrials.gov/prs/app/template/Home.vm?uid=U0004R9N&ts=45&cx=fvia6f)

The work has been reported in line with the STROCSS criteria (10).

Statistical analysis: The data was analysed using MiniTab version 3.0 software. Frequencies were determined by direct counting. Mean ± Standard deviation (SD) were estimated for sperm count, volume, pus cells, motility and morphology; 95% Confidence interval was calculated for proportions and for means. Mean values were compared for statistical significance using student t-test. The value with level of significance was (P-value) <0.05.

**Results**

The study include 1000 male patients, their age ranged from 15 to 60 years with mean age were (32±1.43). The highest age frequency was between 31 to 40 years (39.5%) with 95%CI was 0.365-0.426. Mean ejaculation abstinence time was 3±0.26 as shown in table-1-

According to WHO standard for semen normality, 1000 samples that were analyzed, normospermia was shown in 835 (83.5%) males (95% CI=0.811-0.857) and 12% had oligospermia and the rest 4.5% was azospermia as demonstrated in table - 2-. Table-3- revealed the distribution of semen volume, 74% of total sample study had normospermia (2-5 ml) and 24.5% had hypospermia (<2ml) and the rest (1.5%) was hyperspermia (>5ml) . Other semen parameters were compared in oligospermic and normospermic samples for count per ml, volume, pus cell, motility and normal or abnormal morphology. The normospermic samples had significantly higher levels regarding the following parameters(Table-4-): count per ml (51.30±1.24) (P= 0.001), volume(3.34±2.31)(P=0.0001), pus cell (8.04±1.02)(P=0.0001), motility (22.81±5.8)(P=0.0001) , abnormal motility (22.81±5.8)(P=0.0001) and normal (V)(P=0.0001) or abnormal morphology (25.86 ±12.4)(P=0.0002) when compared with oligospermia. Other semen abnormalities was shown in table-5- like Asthenospermia that present in 13% of the total samples with 95% CI= 0.109-0.151, Terato-spermia (11.1%) 95% CI=0.092-0.13) , Oligo-astheno-terato-spermia (4.5%) (95% CI=0.032-0.058 and agglutination present in 3.6% of the patients (95% CI=0.024-0.049).

Table-1- Main characteristics of the study population.

| Characteristic                        | Frequency No.=1000 | Percentage % | 95% CI          |
|--------------------------------------|--------------------|--------------|-----------------|
| Mean age (ys) X±SD                   | 32±1.43            | ---          | 31.911-32.088   |
| Age 15-20 Ys.                        | 46                 | 4.6          | 0.034-0.061     |
| Age 21-30 Ys.                        | 287                | 28.7         | 0.259-0.316     |
| Age 31-40 Ys.                        | 395                | 39.5         | 0.365-0.426     |
| Age 41-50 Ys.                        | 194                | 19.4         | 0.170-0.220     |
| Age 51-60 Ys.                        | 78                 | 7.8          | 0.062-0.096     |
| Mean ejaculation abstinence time     | 3±0.26             | ----         | 2.983-3.016     |
Table 2: Frequency of sperm concentration/ ml.

| Group         | Frequency No=1000 | Percentage (%) | 95% Confidence interval |
|---------------|-------------------|----------------|-------------------------|
| Normospermia  | 835               | 83.5           | 0.811-0.854             |
| Oligospermia  | 120               | 12.0           | 0.100-0.142             |
| Azoospermia   | 045               | 4.5            | 0.032-0.068             |

Table 3: Distribution of seminal volume.

| Volume                  | Frequency No=1000 | Percentage (%) | 95% Confidence interval |
|-------------------------|-------------------|----------------|-------------------------|
| Normospermia (2-5ml)    | 740               | 74.0           | 0.712-0.767             |
| Hypospermia (<2ml)      | 245               | 24.5           | 0.219-0.273             |
| Hyperspermia (>5ml)     | 15                | 1.5            | 0.008-0.025             |

Table 4: Comparisons of semen parameters between Normospermia and Oligospermia.

| Group         | Count /ml X±SD      | Volume X±SD | Pus cell X±SD | Motile sperms (% rapid progressive) X±SD | Non motile sperms(D) X±SD | Normal sperms X±SD | Abnormal sperms X±SD |
|---------------|---------------------|-------------|--------------|------------------------------------------|--------------------------|-------------------|----------------------|
| Normospermia  | No.=853             | 51.30±1.24  | 3.34±2.31    | 8.74±1.02                               | 22.81±5.8                | 38.26±9.57       | 74.13±8.64          |
| 95% Confidence interval | 51.21667 - 51.38333 | 3.18476 - 3.49524 | 8.97145 - 8.10855 | 22.42022 - 23.19978 | 37.61686 - 38.90314 | 73.54936 - 74.71064 | 25.02668 - 26.69332 |
| Oligospermia  | No.=120             | 7.08±3.18   | 0.8±0.40     | 6.66±0.5                                | 8±1.5                    | 34.1±5.72        | 28.5±11.8           |
| 95% Confidence interval | 6.50519 - 7.65461 | 0.7289 - 0.82711 | 6.56962 - 6.75038 | 7.72706 - 8.27294 | 33.06607 - 35.13393 | 26.36706 - 30.63294 | 20.14432 - 22.85568 |
| *P-value      | 0.0001              | 0.0001       | 0.0001       | 0.0001                                  | 0.0001                   | 0.0001           | 0.0002              |

*Student t-test

Table 5: Proportions of other semen abnormalities.

| Abnormal parameters | Frequency | Percentages (%) | 95% Confidence interval |
|---------------------|-----------|-----------------|-------------------------|
| Asthenospermia       | 130       | 13              | 0.109-0.151             |
| Terato-spermia       | 111       | 11.1            | 0.092-0.13              |
| Oligo-astheno-terato-spermia | 45 | 4.5            | 0.032-0.058             |
| Presence of pus cell | 168      | 16.8            | 0.145-0.191             |
| Presence of          | 36        | 3.6             | 0.024-0.048             |
Discussion

Semen quality is an important factor in determining infertility and females remain a target of society for this dilemma and there are many risk factors for female infertility like previous CS, menstrual cycle disturbance, regular daily caffeine intake and obesity.\(^\text{11}\) In addition to that, studies and researches with time proved that males have equal contribution to this trouble. Male infertility is inability to cause pregnancy in a fertile female and constitutes about 40–50% of infertility.\(^\text{12}\) Male infertility is either pre-testicular, testicular and post-testicular. Semen quality is a surrogate measure of male productiveness and defining thresholds for normal ranges is so difficult and sperm count is declining in the world. Thus screening of males by simple semen analysis test gives an idea about the pathological infertility problems. This study showed the frequency of normospermia (83.5%), oligospermia (12%) and azoospermia (4.5%) in male infertile subjects, and the distribution of other abnormal semen parameters were Hypospermia (<2ml)(24.5%), Hyperspermia (>5ml) (1.5%), Asthenospermia (13%), Terato-spermia(11.1%). There were a significant difference (P=0.0001) between normospermic count per ml (51.30±1.24), volume(3.34±2.31)(P=0.0001), pus cell (8.04±1.02)(P=0.0001), motility (22.81±5.8)(P=0.0001), abnormal motility (22.81±5.8)(P=0.0001) and normal (V)(P=0.0001) or abnormal morphology (25.86 ±12.4)(P=0.0002) when compared with oligospermia. This indicates that there was an association between sperm count and abnormalities in other parameters. Other study done by Butt F and Akram N. 2013 showed that mean sperm count was 135.41±70.6 in normospermia , another study in UK showed mean sperm count was 84.3±78.3.7 while other research demonstrated that sperm count was 86.8±7.5 million/ml .\(^\text{13,14,15}\) These differences with our study may be due to sample size, method use in semen study like home based semen analysis and swim up technique for sperm preparation that is increased motility and decreased DNA damage\(^\text{16,17}\), time of the study because sperm count and quality is declining in 21st century because of some associations with chemical exposures leading to endocrine disruption \(^\text{18}\) and geographical differences.\(^\text{19}\) This study was in accordance with meta-analysis study that showed sperm density has decreased all over the world around 50% over the last 60 years leading to more attraction and controversy.\(^\text{20}\)

Azoospermia affects about 4.5% of the study male population and may be due to sperm production or transport while oligospermia about 12%. Other study showed that the prevalence of azoospermia was 14.28% and oligospermia was 21.43%\(^\text{21}\) while in other study was 33% .\(^\text{22}\) Thus there were a controversy between the results which may be due to sample size.

Regarding the ejaculated volume, about 24.5% showed hypospermia while other studies showed hypospermia was 10.3%, 9%.\(^\text{23,24}\) This may be due to associated abnormalities in accessory sex glands fluid synthesis like seminal vesical, defect in the transport like physical obstruction in the genital tract, retrograde ejaculation or duration of abstinence.

According to sperm motility in this study was 22.81±5.8 in normospermia and Asthenospermia was 13% which is important in sperm travel a long very long distance.
to reach oocyte. Good motility occurs from sperm maturation in their way through the epididymis which is under the effect of epididymal proteins. So motility is an indicator of posttesticular epididymal function. Cigarette smoking had an association with decreased sperm count, motility and semen quality which is more marked in moderate and heavy smokers because toxins from tobacco can affect sperm development and function. Other studies showed asthenospermia was in 25%, 21.42% and 18%. Morphology of the sperm is other important parameter like two heads or two tails and other abnormal shapes which is the function of testes and epididymis. In this study mean normal morphology in normospermia samples was 74.13±8.64 while in oligospermic samples were 28.5±11.8 (P=0.0001). This was in opposing with other study that showed abnormal morphology was 53% and abnormal motility in 60% oligospermic males. This because of sperm motility and morphology are changing parameters and their levels depend on the sperm count in an individual. In addition to that some laboratories do not follow the orders of the WHO in performing semen analysis, and most of them do not do the instruction and methods in doing the test. Other affecting factors are decrease in level of vitamin D and physical exercise. Infection of the male genital tract, presence of pus cells and agglutination of the sperms are an important morbidity factors. It may affect seminal quality through a direct action on spermatozoa or their environment.

Conclusions:
Semen analysis is the keystone of infertile couple. Semen parameters like sperm concentration, motility and morphology, are indicators for male reproductive function. Sperm concentration in our country is declining as in other parts of world and there is a significant association between sperm concentration and sperm parameters.

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3. Conflicting interests: none
4. Informed consent : none applicable

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