ORIGINAL ARTICLE

Semen Analysis and Insight Into Male Infertility

Doi: 10.36129/jog.2021.01

Batool Mutar **Mahdi** *

Research Unit, Department of Microbiology, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq.

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 the1970s 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 .[1] 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.[2] 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.[3] Other factors are infection and oxidative stress and increase in inflammatory cytokines in seminal plasma that decrease sperm quality and damage sperm DNA.[4,5] Nutritional factor had an important role in sexual health and semen quality especially vitamin D deficiency.[6] 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. [7] 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.[8] 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 Collage 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).[9] 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 clinical trail.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.

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 Asthenospemia 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=0.024-0.048).

Table-1- Main characteristics of the study population.

Characteristics	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 (ds.) X±SD	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	0.811-0.857
Oligospermia	120	12.0	0.100-0.142
Azoospermia	045	4.5	0.032-0.058

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** **(A)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**	**8.04±1.02**	**22.81±5.8**	**38.26±9.57**	**74.13±8.64**	**25.86 ±12.4**
95% Confidence interval	**51.21667 - 51.38333**	**3.18476-3.49524**	**7.97145-8.10855**	**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±0.15**	**6.66± 0.5**	**8±1.51**	**34.1±5.72**	**28.5±11.8**	**21.5±7.5**
95% Confidence interval	**6.50519 - 7.65481**	**0.77289 -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
Asthenospemia	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 agglutination	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.[11] In addition to that, studies an 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.[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%), Asthenospemia (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 normospeermia , 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. [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 [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 [18] and geographical differences.[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. [20]

Azoospermia affects about 4.5% of the study male populationand 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% [21] while in other study was 33% . [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%. [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 Asthenospemia 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. [25] 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. [26] Other studies showed asthenospermia was in 25% ,21.42% and 18% . [13,27,28]

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. [29] 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 .[30]Other affecting factors are decrease in level of vitamin D and physical exercise.[31]

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.

1. Ethical approval : none applicable
2. No funding from any organization
3. Conflicting interests: none
4. Informed consent : none applicable

References

1. Patel AS, Leong JY, Ramasamy R. Prediction of male infertility by the World Health Organization laboratory manual for assessment of semen analysis: A systematic review. Arab journal of urology. 2017 ;16(1):96-102.
2. Morin SJ, Scott RT. Knowledge gaps in male infertility: a reproductive endocrinology and infertility perspective. Transl Androl Urol. 2018;7(Suppl 3):S283-S91.

3. Harmoosh SK, and Almadfaiee ZA. Estimation of the seminal fluid of subfertile patients of different age groups. Journal of the faculty of medicine Baghdad.2011;53:428-431.

4. Seshadri S, Bates M, Vince G, et al. The role of cytokine expression in different subgroups of subfertile men. Am J Reprod Immunol. 2009;62:275–82.

5. Lazem A, Al-Kaseer E, Al-Diwan J, Al- Hadithi T. Effect of infection on semen parameters in a sample of Iraqi infertile males. J Fac Med Bagdad. 2010;52:274-276.

6. Arab A, Hadi A, Moosavian SP, Askari G, Nasirian M. The association between serum vitamin D, fertility and semen quality: A systematic review and meta-analysis. Int J Surg. 2019 Sep 24;71:101-109.

7. Tocci A, Lucchini C. WHO reference values for human semen. Human reproduction update. 2010;16(5):559.

8. Ahadi M, Aliakbari F, Latifi S, Hosseini SJ, Gharib A, Movafagh A, Abdolalian Z et al. Evaluation of the Standardization in Semen Analysis Performance According to the WHO Protocols Among Laboratories in Tehran, Iran. Iran J Pathol. 2019 Spring;14(2):142-147.

9. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT, Vogelsong KM (May–Jun 2010). World Health Organization reference values for human semen characteristics. Human Reproduction Update. 2010;16 : 231–45.

10. Agha RA, Borrelli MR, Vella-Baldacchino M, Thavayogan R and Orgill DP for the STROCSS Group.The STROCSS Statement: Strengthening the Reporting of Cohort Studies in Surgery . International Journal of Surgery . 2017; 46:198-202.

11. Ajeel NA and Abdul-Kader RA. Female Infertility: A Study of Risk Factors . Journal of the Faculty of Medicine.Baghdad.2014;56:200-204.

12. Brugh VM, Lipshultz LI . Male factor infertility. Medical Clinics of North America.2004; 88 : 367–85.

13. Butt F and Akram N. Semen analysis parameters: Experiences and insight into male infertility at a tertiary care hospital in Punjab. J Pak Med Assoc.2013; 63: 558-562.

14. Mortimer D, Templeton AA, Lenton EA, Coleman RA. Semen Analysis Parameters and Their Interrelationships in Suspected Infertile Men. Arch Androl 1982; 8: 165-71.23

15. Shoaib KM, Irshad A, Musa KB, Faheem T. Evaluation of the gonadotropic ratios among men with varying sperm quality. Pak J Med Res 2005; 44: 19-22.

16. Yu S, Rubin M, Geevarughese S, Pino JS, Rodriguez HF, Asghar W. Emerging technologies for home-based semen analysis. Andrology. 2018;6:10-19.

17. AL-Marayaty SS, Saeed GT, AL-Ahmed HI. Effect of Swim Up Techniques on Sperm Motility and DNA Integrity Versus Unprepared Semen. 2017; 59:151-155.

18. Virtanen HE, Jørgensen N, Toppari J. Semen quality in the 21st century. Nat Rev Urol. 2017 ;14:120-130.

19. Elbardisi H, Majzoub A, Al Said S, Al Rumaihi K, El Ansari W, Alattar A, Arafa M. Geographical differences in semen characteristics of 13 892 infertile men .Arab Journal of Urology.2018; 16: 3–9.

20. Fisch Harry. Declining Worldwide Sperm Counts: Disproving a Myth. Urol Clin N Am 2008; 35: 137-46.

21. Khan DA, Khan FA, Sattar A, Naveed AK, Malik IA. Azoospermia in clinical practice at Rawalpindi. Pak Armed Forces Med J 1992; 42:93-5.

22. Subhan F, Tahir F, Ahmed R, Khan ZU .Oligospermia and its relation with hormonal profile. J Pak Med Assoc 1995; 45: 246-7.

23. Imam MEI, Siuf A, Mansour MM, Khalid KE, Yosif N, Elhasan EM,Miskeen E. Semen Analysis of Infertile Sudanese Males in Gezira State. Central Sudan. SJPH 2009; 4: 340-4.

24. Nwafia WC, Igweh JC, Udebuani IN.. Semen analysis of infertile igbo males in enugu, eastern Nigeria. Niger J Physiol Sci 2006; 21:67-70.

25. Akhtar M S, Akhtar F K. Causes of male infertility. Pak J Med Res .1991; 30: 159-62.

26. Sharma R, , Agarwal A, Esteves SC. Cigarette Smoking and Semen Quality: A New Meta-analysis Examining the Effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. Eur Urol. 2016;70:635-645.

27. Subhan F, Tahir, F, Alam W, Sultan S, Shahab M. Seminal and hormonal profiles of fertile and subfertile Pakistani men -a study of infertility cases. Pak J Med Res 2000; 39: 42-5.

28. Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardisegovia LM, et al. Asthenozoospermia: analysis of a large population. Arch Androl 2003; 49: 343-9.

29. Dua AA, Vaidya SR. Sperm motility and morphology as changing parameters linked to sperm count variations. J Postgrad Med.1996; 42: 93-6.

30. Ahadi M, Aliakbari F, Latifi S, Hosseini SJ, Gharib A, Movafagh A, Abdolalian Z, Dehghan A, Moradi A, Kazeminejad B, Rakhshan A, Jamali E, Allameh F, Moradi A. Evaluation of the Standardization in Semen Analysis Performance According to the WHO Protocols Among Laboratories in Tehran, Iran. Iran J Pathol. 2019;14:142-147.

31. Yan X, Dong L, Liu Y, Yang F, Tan K, Li J, Chang D, Yu X, Effects of physical exercises on semen quality and reproductive outcomes in male infertility: A protocol for systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2019 ;98:e17494.