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Semen Analysis – Problems & Pitfalls

Aanantha Lakshmi B[1], Pandiyan Natarajan[2]

[1]Senior Clinical Embryologist, Janani Trichy Fertility Centre, Trichy,
[2]Professor and Head of the Department (Retired), Department of Andrology & Reproductive Medicine, Chettinad Super Speciality Hospital, Chennai, India

Introduction:

Semen analysis, despite all the limitations, remains the single most important test in the evaluation of male infertility. Semen analysis, though seemingly straightforward, poses challenges regarding the reliability and accuracy of determining fertility in humans [1]. Semen primarily comprises two key components: spermatozoa and seminal fluid. Spermatozoa originate in the seminiferous tubules of the testicles, while seminal fluids are produced in the accessory sex glands and excurrent ducts. Both elements are scrutinised in semen analysis, focusing on spermatozoa count and semen volume [2]. This examination offers insights into the normal functioning of the testicular machinery for spermatozoa production and evaluation of the seminal fluid. Moreover, it aids in assessing spermatozoa quality for fertility [3] or evaluating the outcomes of procedures like vasectomy [4]. Semen analysis involves assessing various physical characteristics of semen, like colour, odour, pH, viscosity, and liquefaction, along with volume, Spermatozoa concentration, morphology, and progressive motility.[5].

In this article, we will navigate through the intricacies of WHO 2021 semen analysis guidelines, focusing on key parameters such as semen volume, concentration, motility and morphology and their interpretation. We will explore common interpretation problems and the pitfalls physicians often face, which can lead to diagnostic inaccuracies or oversight.

Semen Collection:

Masturbation: This is the most preferred method. The individual is provided with a private room and a sterile collection container. He can have his wife with him if the couple so desire. If not, He must be provided with pornographic material. Some men have difficulty in collecting semen samples at the hospital. These men can be encouraged to bring semen samples from Home, provided he is able to maintain the Warm Chain and bring the sample within 1 hour from the collection. Bathrooms are not suitable places for semen collection. He must be instructed to collect the complete semen sample and not spill any portion of the sample. If he were to spill the sample, he must be advised to report to the laboratory personnel. An incomplete sample is not suitable for analysis. No final decision should be based on such a semen sample.

Collection During Intercourse: Some men are unable to collect semen samples by masturbation but can ejaculate during intercourse. These men are encouraged to collect semen samples at ejaculation during intercourse. Specially designed condoms (without spermicidal lubricants) can be used to collect semen during intercourse. This method may be preferred if masturbation is culturally or personally unacceptable.

Electroejaculation can be used for men who cannot ejaculate normally due to spinal cord injuries or other conditions.[6]

Precollection Instructions to the Patient & Their Pitfalls:

Abstinence from ejaculation for 2-7 days before collection is the recommendation by WHO 2021.[6] This recommendation by WHO is not evidence-based.

Avoid alcohol, caffeine, and drugs that may affect semen quality.

Maintain a healthy lifestyle, including a balanced diet and regular exercise.

Collection Environment:

- Privacy and comfort are important.
- The collection container must be clean, sterile and dry.
- The sample should be kept at body temperature and delivered to the lab as soon as possible.
- Semen collected outside the laboratory environment may be subject to temperature changes or contamination, affecting the integrity of the sample.
- Improper containers or contamination during collection can lead to inaccurate results.

Handling and Transport:

- Semen should be kept at room or body temperature.
- Avoid extreme temperatures.
- Label the container with the date, time of collection, and any relevant personal information.
- Delayed transport or improper handling during transportation can compromise the sample quality.

Semen Analysis - Procedure:

Semen evaluation is divided into two parts: macroscopic and microscopic analysis (figure 1).

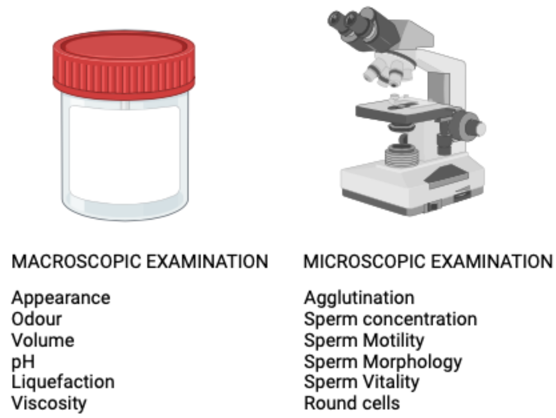

Figure 1: Diagrammatic representation of components of semen analysis.

Every laboratory should participate in External Quality Control (EQC) programs when interpreting semen analysis reports.

Macroscopic Examination:

Macroscopic evaluation examines the chemical and physical parameters of the ejaculate, including liquefaction, viscosity, appearance, odor, volume, and pH. The primary macroscopic characteristics are summarised in Table 1.

Table 1: Primary macroscopic findings in a semen analysis [7]

Macroscopic Examination	
APPERANCE	Grey Opalescent
VISCOSITY	Forms small discrete drops (thread < 2cm long)
VOLUME	1.4 mL – 2.3 mL
pH	7.2

Liquefaction

Liquefaction of semen takes 15–30 minutes, a process regulated by prostatic secretions rich in citric acid, which act in synergy with proteolytic enzymes such as lysozyme, α-amylase, β-glucuronidase, and prostate-specific antigen (PSA). PSA, a trypsin-like protease, cleaves semenogelin proteins. PSA levels can be altered due to congenital or acquired factors, such as prostatitis.[7]

Viscosity:

Viscosity refers to the homogeneity and stickiness of an ejaculate specimen, which can vary under different conditions. A normally liquefied sample has an opalescent appearance with a cream/grey colour. This parameter is influenced by sperm concentration: a transparent ejaculate may indicate an extreme reduction in sperm count, while a highly concentrated specimen will be opaque. High viscosity may suggest prostatitis. [7]

Colour:

A red-brown appearance of the ejaculate should alert the clinician to the presence of blood. The presence of erythrocytes in the semen, known as hematospermia, can have different etiologies depending on the patient's age. In younger men (under 40), it is often due to inflammation or urogenital infections, whereas in older men (over 40), it may indicate more serious conditions, such as prostate cancer.[7]

Volume and pH:

Seminal plasma primarily consists of secretions from the accessory glands (about 90%), with minimal contributions from the epididymis and bulbourethral glands. Semen volume reflects the secretory activity of these glands and their responses to autonomic nerve stimulation from sexual arousal, which causes smooth muscle contractions to empty each gland. The prostate and seminal vesicles are target organs for androgens; thus, severe androgen deficiency is associated with lower semen volume. If the seminal fluid volume is markedly reduced, the clinician should confirm with the patient whether the collection was complete. Semen volume will be markedly reduced in conditions like Congenital Bilateral Absence of Vas Deferens (CBAVD) also known as Vasal Aplasia and in Ejaculatory Duct Obstruction.

Both the prostate and seminal vesicles contribute to the semen pH through their secretions: the prostate produces an acidic fluid, while the seminal vesicles produce an alkaline fluid, resulting in a typical neutral pH of around 7.2–7.4. Though this parameter is also susceptible to analytical biases. The pH generally increases over time after ejaculation, so if a highly alkaline specimen is observed, poor sample handling and delays in analysis should be ruled out before interpretation.[7]

Microscopic Analysis:

The World Health Organization (WHO) 2021 has established reference values for semen parameters based on a comprehensive study involving 3,589 fertile men whose Time to Pregnancy (TTP) was one year or less. (Table 2)

Table 2: Reference range in Who 2021 (6th Edition) [6]

Microscopic Examination		
	Lower reference	Upper reference
Sperm Concentration	16 million /mL	66 million/mL
Total Sperm Count	39 million	210 million
Total Motility	42%	64%
Progressive motility	30%	55%
Normal Morphology	4%	14%
Peroxidase positive leucocytes	<1 million/mL	

Problems & Pitfalls:

Each part of the analysis considers different parameters, and depending on any alterations, various clinical conditions may be suspected. If normozoospermia is observed, a second analysis is not required. However, if one or more semen parameters are abnormal, repetition of the analysis is necessary to eliminate potential pre-analytical and analytical factors, as well as biological variability.

Analytical factors, inappropriate abstinence time, incomplete collection of the ejaculate, and inadequate transport of the semen sample necessitate a short-term repetition of the analysis. Analytical variables can stem from random and systematic errors during the procedures. Endogenous factors, such as high fever or medications, may affect spermatogenesis throughout its entire duration (approximately 72 days); hence, repetition should occur after the completion of the entire spermatogenic cycle.

- *Abstinence:* The study by Dupesh, Pandiyan et al with 2037 semen sample analysis suggests that the recommended period of ejaculatory abstinence may be quite flexible, and the WHO's strict 2- to 7-day abstinence guideline could be relaxed. In both normozoospermic and oligozoospermic men, semen parameters associated with less than 24 hours of abstinence were found to be comparable to those with longer abstinence periods. These results support the elimination of conservative recommendations regarding abstinence and indicate that patients may be asked to provide a semen sample on the day of their infertility evaluation, regardless of their abstinence duration.[8] Although, seminal volume may decrease with more frequent ejaculation, total sperm count, motility, morphology and vitality are typically not significantly impacted.[9]
- *Reference Ranges:* Interpretation of results must consider the reference ranges specific to the laboratory and population demographics. WHO manual established reference value based on participants from 13 countries across six continents: Asia, America, Europe, Africa, and Oceania. It is important to note that reference ranges and 5th centiles alone are insufficient to diagnose infertility and may not be applicable worldwide. There is a significant geographic and ethnic variability that these values do not always account for, potentially leading to inappropriate interpretation of results.
- *Clinical Correlation:* Semen analysis results should be interpreted in conjunction with the patient's medical history and other diagnostic tests for a holistic evaluation of fertility potential as there can be temporary suppression of spermatogenesis under the influence of drugs and diseases.
- *Other difficulty: In* this 6th Edition, adjustments have been made to the evaluation of basic semen parameters. Notably, the assessment of semen odor has been added, with the manual indicating that "urine or putrefactive odours may be of clinical interest." However, it is important to recognize that evaluating semen odor is subjective, making standardization of this parameter highly challenging. Moreover, the inclusion of this parameter contradicts recommendations aimed at ensuring the safety of laboratory personnel.
- *Intra- and Inter-laboratory Variability:* Different laboratories may produce varying results due to differences in equipment, techniques, and personnel expertise. Even within the same

laboratory, results can vary depending on the day and the technician performing the test. This is especially so regarding assessment of spermatozoa morphology.

- *Biological Variability:* The semen sample from the same individual itself can vary significantly from one ejaculation to another. Factors such as abstinence period, stress, illness, and lifestyle can affect semen quality.
- *Lack of Universal Standards:* Although the WHO provides guidelines, not all laboratories adhere to these standards strictly. This leads to inconsistencies in sample collection, processing, and analysis.
- *Technical Challenges:* Accurate counting of spermatozoa concentration and assessment of motility require skill and experience. Low sperm concentrations can be particularly challenging to assess accurately, leading to high error rates in samples with few spermatozoa. Determining sperm concentration per millilitre of semen can be achieved through various methods such as counting sperm in a hemacytometer chamber or glass slide. However, the choice of equipment can lead to either underestimation or overestimation of spermatozoa numbers. It is crucial to calibrate these devices accurately to ensure precise measurement of spermatozoa concentration.[10]
- *Sperm motility:* When reporting sperm motility, it is important to differentiate between total motility (which includes both progressive and non-progressive motility) and progressive motility. WHO 2021 classified the motility into fast progressive, slow progressive and non – progressive. Studies indicate that the percentage of progressively motile sperm is more closely associated with pregnancy rates. Therefore, distinguishing between progressive and non-progressive motility may be simpler instead of three categories.[11]
- *Sperm Morphology:* The assessment of sperm morphology is highly subjective. The classification of sperm as normal or abnormal can vary significantly between observers, and even slight differences in technique can alter the results.
- *Misleading Norms:* Terms like "normozoospermia" or "teratozoospermia" are often used based on semen parameters alone. However, these terms can be misleading without considering the overall semen picture and the clinical context, leading to either over-treatment or under-treatment.

Conclusion:

Semen analysis, while remaining as a fundamental tool in assessing male fertility, is fraught with various challenges and potential pitfalls. As medical professionals, we continually strive to enhance our diagnostic accuracy and treatment efficacy. Against this backdrop, the release of WHO 2021 guidelines for semen analysis marks a pivotal update, one that demands our thorough understanding and correct application. From the variability in collection methods and abstinence periods to the subjective nature of certain parameters like semen odor, standardizing semen analysis procedures remains complex. Despite these issues, advancements in technology and ongoing research are gradually improving the accuracy and reliability of semen analysis. To maximize the diagnostic value, it is essential to adhere to best practices, ensure proper training for laboratory personnel, and remain aware of the inherent limitations of current methodologies. Continued efforts in refining these techniques will enhance our understanding of male fertility and improve outcomes in reproductive medicine.

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References:

1. de Kretser DM, Baker HWG. Infertility in men: Recent advances and continuing controversies. J Clin Endocrinol Metab [Internet]. 1999;84(10):3443–50. Available from: http://dx.doi.org/10.1210/jcem.84.10.6101
2. 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 J Urol [Internet]. 2018;16(1):96–102. Available from: http://dx.doi.org/10.1016/j.aju.2017.10.005
3. Aitken RJ, Best FSM, Richardson DW, Djahanbakhch O, Templeton A, Lees MM. An analysis of semen quality and sperm function in cases of oligozoospermia. Fertil Steril [Internet]. 1982;38(6):705–11. Available from: http://dx.doi.org/10.1016/s0015-0282(16)46698-7
4. Lemack GE, Goldstein M. Presence of sperm in the pre-vasectomy reversal semen analysis: Incidence and implications. J Urol [Internet]. 1996;155(1):167–9. Available from: http://dx.doi.org/10.1016/s0022-5347(01)66584-4
5. Menkveld R. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. Asian J Androl [Internet]. 2010;12(1):47–58. Available from: http://dx.doi.org/10.1038/aja.2009.14
6. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. Geneva, Switzerland: WHO Press.
7. Practical clinical Andrology Carlo Bettocchi • Gian Maria Busetto Giuseppe Carrieri • Luigi Cormio.
8. Dupesh S, Pandiyan N, Pandiyan R, Kartheeswaran J, Prakash B. Ejaculatory abstinence in semen analysis: does it make any sense? Ther Adv Reprod Health [Internet]. 2020; 14:263349412090688. Available from: http://dx.doi.org/10.1177/2633494120906882
9. Mayorga-Torres BJM, Camargo M, Agarwal A, du Plessis SS, Cadavid ÁP, Cardona Maya WD. Influence of ejaculation frequency on seminal parameters. Reprod Biol Endocrinol [Internet]. 2015;13(1). Available from: http://dx.doi.org/10.1186/s12958-015-0045-9
10. Mahmoud AMA, Depoorter B, Piens N, Comhaire FH. The performance of 10 different methods for the estimation of sperm concentration. Fertil Steril [Internet]. 1997;68(2):340–5. Available from: http://dx.doi.org/10.1016/s0015-0282(97)81526-9
11. Simon L, Lewis SEM. Sperm DNA damage or progressive motility: which one is the better predictor of fertilization*in vitro*? Syst Biol Reprod Med [Internet]. 2011;57(3):133–8. Available from: http://dx.doi.org/10.3109/19396368.2011.553984