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Semen Analysis – What a Clinician Should Know

1 POINT

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INTRODUCTION

Infertility is defined as failure to achieve pregnancy after one year of regular unprotected intercourse. It is a significant global problem with a prevalence of approximately 1 in 7 couples.¹ Male factor is a common diagnostic category, and as a single factor it accounts for approximately 20% of the infertile couples.^{2,3}

Semen analysis provides information on sperm production from the seminiferous tubules. The parameters determined in a semen analysis serve as surrogate markers of reproductive function of the male partner. Although the value of the conventional semen analysis in the diagnosis of male infertility has been questioned in a number of studies,⁴ semen analysis when being done correctly can provide useful information for management of infertile couples.

There are four objectives in this article. The first is to provide information that the patients should know on collection of semen. The second is to discuss the method used for the semen analysis and the reference ranges for various semen parameters. The third is to summarize the relationship between semen quality and reproductive outcome in the literature. The fourth is to share the experience of Queen Mary Hospital in Hong Kong in using sperm parameters for management of infertile couples.



Semen Analysis Provides Information on Sperm Production from the Seminiferous Tubules.

SEMEN SAMPLE COLLECTION

A proper semen analysis starts from appropriate semen collection. Clinicians should give clear instructions on the method of semen collection to their patients. These include:

1. The need of sexual abstinence from 2 to 7 days.⁵ Prolonged abstinence may increase the sperm concentration and ejaculate volume; while the sperm concentration may decline if the abstinence period is too short.⁶
2. Semen should be collected wholly in sterile and non-toxic container preferably provided and tested by the clinic

- or laboratory performing the analysis. The early portion of the ejaculate contains mainly of the prostatic secretion and is rich in sperm, while the later portions are mostly secretion from the seminal vesicle.⁷ Incomplete collection will affect the reliability of the result and should be reported to the laboratory, especially when the earlier part of the ejaculate is lost.
3. The ejaculate should be produced by masturbation preferably in a semen collection room adjacent to the laboratory. Stress affects semen parameters.⁸ For men experiencing stress in

Table 1. Checklist for Receiving Semen Sample**Checklist**

- To check the patient identity document
- To check the semen collection bottle identity label
- To check if the following information is recorded in the semen submission form:
 - Date, time and place of sample collection
 - Duration of abstinence
 - Method of sample collection
 - Complete/incomplete collection
 - Any anomalies in collection and/or transportation
 - Patient signature

Table 2. Lower Reference Limits (WHO 2010) and Reference Value (WHO 1999) for Semen Characteristics

Parameter	Lower Reference Limits (WHO 2010)	Reference Value (WHO 1999)
Semen Volume	1.5 ml	≥ 2.0 ml
Sperm Concentration	15 million sperm/ ml	≥ 20 million sperm/ ml
Progressive Motility	32%	≥ 50%*
Total Motility	40% [^]	n/a
Vitality	58% live	≥ 75% live
Morphology (Strict criteria)	4% normal form	(≥ 15% normal form) [#]

[^]Grade a + b according to the WHO 1999 manual.

^{*}Progressive motile + non-progressive motile sperm according to the WHO 2010 manual.

[#]No actual value given. Value was concluded from multi-centre studies.

the clinic, the semen can be collected at home but should be delivered to the laboratory within one hour. Patients should be told not to store the semen in the fridge to keep it 'fresh'. During transport, the sample has to be kept at body temperature by placing the sample close to the body.

4. Commercial condoms for contraception should not be used for semen collection for diagnostic or therapeutic purpose. There are specially designed condoms without lubricant and spermicide for semen collection. It should be noted that some of the condoms marketed as non-spermici-

dal are in fact toxic to sperm.⁹ Proper testing on the products should be conducted before recommending to patients.

5. For men with febrile illness, samples should be submitted at least 3 months after recovery.¹⁰

The laboratory staff should check against a checklist when receiving the semen sample (Table 1).

REFERENCE RANGES

The procedure described by the World Health Organization (WHO) in the 'WHO laboratory manual for the examination and processing of human semen' is generally regarded as the gold standard for performing semen analysis. The reference ranges suggested in the previous versions of the manual were criticized on their usefulness and lack of evidence.¹¹ WHO addressed the concerns in the recent edition of the manual published in 2010.⁵ In this latest edition, the reference ranges were derived from data of over 4,000 recent fathers recruited in several cross sectional studies of semen analysis collected from fertile populations in Europe, America, Asia and Australia; these men became fathers within 12 months after stopping contraception.¹² The fifth centile of semen volume, sperm count, motility, vitality, and morphology were considered as the lower reference limits (Table 2). Nomenclature for semen variables were also included (Table 3).

Caution should be exercised when interpreting the reference range. As the reference ranges are derived from recent fathers, it is important to be aware that men with semen parameters that fall below the limits are not necessarily infertile nor have a lower probability of fathering a child. On the other hand,

men with semen parameters above the limits do not guarantee fertility because semen analysis does not directly measure sperm fertilizing potential. A significant portion of men with defective sperm-zona pellucida interaction and therefore failure of fertilization in IVF can have normal semen parameters.¹³ Moreover, these reference values are valid only when the protocols stated in the manual are followed. Due to considerable overlap of semen parameters between fertile and infertile populations,¹⁴ diagnosis of the fertility potential of men should be made by combining the results of semen analysis, medical history and fertility history.

SEMEN ANALYSIS PROCEDURES

In the laboratory, a semen analysis begins with physical examination of the sample including determination of pH, volume, viscosity and visual appearance of the sample. Semen is ejaculated as coagulum, and should liquefy within 60 minutes.⁵ The clinical significance of delayed liquefaction remains controversial. Semen volume is measured by a graduated pipette or by weight when the semen is collected in a pre-weighted specimen container, assuming the density of semen to be 1g/mL.⁵ Semen pH is measured by pH paper, and it tends to increase with time. There is no reliable reference range for semen pH, and WHO used the consensus value of 7.2 as the lower reference range.⁵ Semen viscosity is assessed by estimating the length of the thread formed when releasing a drop of semen from a pipette. Viscous seminal plasma impairs accurate measurement of sperm motility.

The main sperm parameters to be determined are motility, concentration,

Table 3. Nomenclature for Semen Variables (WHO 2010)

Aspermia	No semen
Asthenozoospermia	Percentage of progressively motile sperm < 32%
Azoospermia	No spermatozoa in the ejaculate
Normozoospermia	Normal ejaculates with parameters above the lower reference limit
Oligoasthenoteratozoospermia	Sperm concentration and percentage of progressively motile sperm and morphologically normal sperm below the lower reference limit
Oligozoospermia	Sperm concentration fewer than 15 million / ml
Teratozoospermia	Percentage of morphologically normal sperm fewer than 4%

morphology and vitality. Sperm concentration is measured by volumetric dilution and hemocytometric counting of the sperm. The new edition of the manual classifies sperm motility into 'progressively motile', 'non-progressively motile' and immotile,⁵ instead of the four categories defined in the earlier versions. Sperm with forward motion or moving in large circles are defined as having progressive motility, while those with other patterns of motion without any progression as having non-progressive motility. Computer-aided sperm analysis systems are not recommended for routine semen analysis because some of the systems cannot accurately distinguish sperm from debris in the semen and special quality control procedures including specimen preparation are required to provide high quality results.

The fifth edition of the manual recommends the use of the Tygerberg strict criteria⁵ for assessing the percentage of sperm with normal morphology. The size

and structure of the head, mid-piece, tail and cytoplasmic droplet of each sperm are assessed. Only spermatozoa without any morphological abnormalities of all components are classified as normal. Sperm vitality determination is clinically important if a large proportion of spermatozoa are immotile because immotile sperm are not necessarily dead though motile sperm must be viable. Sperm vitality is assessed by dye exclusion test; dead cells, but not viable sperm with an intact membrane, will take up the dye.

There is no universally accepted method for assessment of antisperm antibodies. The clinical evidence for its use in prediction of natural conception and assisted reproduction outcome is thin, and a recent systematic review and meta-analysis showed that semen antisperm antibodies are not related to IVF and ICSI outcome.¹⁵ The National Institute for Clinical Excellence (NICE) does not recommend routine screening of antisperm antibodies.¹⁶

OTHER SPERM FUNCTION TESTS

Routine semen analysis gives no clue about the fertilizing activities of sperm. A number of sperm function tests have been developed. For instance, the hemizona binding assay is used to determine the zona pellucida binding ability of sperm.¹⁷ Sperm acrosome reaction can be evaluated by the acrosome reaction-ionophore challenge test.¹⁸ Despite the potential predictive value of the sperm function tests on IVF outcome, these tests are used for research purposes mainly due to the complexity of the tests and the need of expertise for their interpretation.

There is no well-defined semen parameter that distinguishes infertile from fertile men

Sperm DNA integrity tests refer to a panel of tests using different methodologies to measure the extent of DNA damage in sperm. There is no consensus on the reference values of results derived from the various methodologies. The association of the test results with reproductive outcome in studies is conflicting. The Special Interest Group in Andrology of the European Society of Human Reproduction and Embryology questioned the validity of some of the test protocols as they might induce DNA damage.¹⁹ The American Society for Reproductive Medicine does not recommend the tests for routine clinical use.²⁰

VARIATION OF SEMEN PARAMETERS

There is no well-defined semen parameter that distinguishes infertile from fertile men. This is partly due to the fact that the

parameters determined in a conventional semen analysis are not directly measuring the function of sperm in fertilizing an oocyte. In addition, semen parameters are known to vary widely between samples from the same man. The variations are derived from two sources. The first source is the inherent fluctuation of the semen parameters within the same men. Many conditions such as duration of abstinence, illness and activity of accessory sex glands affect the semen parameters. Therefore, semen analysis is recommended to be performed on at least two samples collected 2 to 4 weeks apart to obtain representative results.⁵ However, multiple semen analyses to

further compensate for the variation may not be cost-effective. In an assisted reproduction program, clinical evaluation does not necessarily require analysis of multiple samples. NICE recently recommended that a second semen analysis is required only if the result of the first sample is abnormal.¹⁶ Single-off semen analyses are generally predictive of fertility problem in cases of extreme severe semen abnormality or azoospermia.

The second source of variation originates from the laboratories. There are wide variations in the protocols used and the technical standard of laboratories performing semen analysis,²¹ resulting in large inter-laboratory variation of the semen analysis parameters. In a survey in United Kingdom, only 5% of the laboratories followed all the WHO guidelines.²² There are also variations among technicians within the same laboratory.

Most of the semen analysis procedures are done manually and some, such as morphology assessment, depend on subjective judgment. Training can reduce the variation among technicians.²³ Therefore, semen analysis should ideally be performed by experienced technicians in a dedicated andrology laboratory with internal and external quality control. Internal quality control and standard operation procedure are important to standardize and stabilize the manual process of semen analysis. In this regard, WHO recommends laboratories to set up their own reference ranges. Participation in external quality control will help to compare results from different laboratories.

SEMEN QUALITY AND NATURAL CONCEPTION

A number of reports studied the relationship between semen parameters and natural conception. No single semen parameter is predictive of natural conception. Bonde et al. (1998) studied the semen parameter of 430 first-pregnancy planners, and concluded that the probability of conception increased with sperm concentration up to $40 \times 10^6/\text{mL}$, above which the chance of conception increased with increase in percentage of sperm with normal morphology.²⁴ Semen volume and motility were not predictive. Guzick et al. (2001) compared the semen parameters of 765 infertile and 696 fertile couples.¹⁴ There were extensive overlaps in the semen parameters between the two populations. None of these parameters was a good discriminator, but morphology had the greatest discriminatory power. The available data suggest that the chance of pregnancy increases with increasing numbers of motile sperm with normal morphology in the ejaculate.

SEMEN QUALITY AND INTRAUTERINE INSEMINATION (IUI)

Available reports suggest that male factors affect IUI outcome, though well conducted randomized controlled trials is lacking limiting the quality of the evidence. Wainer *et al.* (2004) retrospectively analyzed 2,564 IUI cycles, and showed improved pregnancy rates when the numbers of motile sperm inseminated were above 5×10^6 and the percentage of sperm with normal morphology exceeded 30%.²⁵ Ombelet *et al.* (1997) examined 792 IUI cycles and concluded that a combination of an inseminated motile sperm concentration below 1×10^6 and a normal morphology less than 4% predicted failure.²⁶ There is no consensus on a threshold of semen parameters above which IUI may be offered. Many authors agreed that a minimum total motile sperm count of 1×10^6 sperm after sperm preparation are required to obtain satisfactory outcome.^{27, 28}

SEMEN QUALITY AND IN VITRO FERTILIZATION (IVF) / INTRACYTOPLASMIC SPERM INJECTION (ICSI)

Sperm quality is only one of the many confounding variables affecting the outcomes of IVF/ICSI. This together with variation in IVF/ICSI practice among assisted reproduction centres makes it difficult to compare studies and to isolate the influence of sperm quality on the treatment outcomes. Despite lack of evidence-based consensus on the relationship between sperm parameters and outcomes, some generalization on using semen quality as guidance for treatment path of the subfertile couples is agreed upon.

1. ICSI is required for extremely poor semen quality.

a. Azoospermia or severe oligozoospermia.

Azoospermia is defined as no sperm seen under low power microscopic examination of the sediment after centrifugation of the semen sample.⁵ The prevalence is about 1% in the male population, and ranges between 10-15% of infertile men.²⁹ Azoospermic men with low seminal pH (<7.0) and volume (<1.0 mL) may suggest congenital bilateral absence of the vas deferens (CBAVD) or ejaculatory duct obstruction.^{30, 31} There is no consensus cutoff value for severe oligozoospermia. When azoospermic or severe oligozoospermic results were encountered, additional tests should be performed to diagnose the cause of the conditions. These tests include physical examination of the male reproductive tract, determination of serum testosterone and follicle stimulating hormone (to distinguish between hypogonadotropic hypogonadism, testicular failure and obstructive causes), and genetic tests such as karyotype and Y-chromosome microdeletion.³² In Hong Kong, the prevalence of chromosomal abnormalities and Y-chromosome micro-deletion is 10.5% and 8.3%, respectively for men with sperm count $< 2 \times 10^6$ /mL and azoospermia.³³ Mutation of cystic fibrosis transmembrane conductance regulator gene should be checked for in Caucasian men with CBAVD. Microsurgical retrieval of sperm with ICSI and use of donor sperm are the treatment options.

b. Globozoospermia

This refers to presence of only sperm with round head in morphological assessment. The prevalence of men with total or partial globozoospermia is less than 0.1% in infertile males.³⁴ Round headed sperm usually have no or a small acrosome,³⁵ an organelle required for

fertilization. Therefore, globozoospermic men have fertilization problem. Fertilization can be achieved using ICSI, though with lower fertilization rate.³⁴

c. Immotile sperm

Kartagener's syndrome is a rare genetic disorder with an overall incidence of about 1/40,000.³⁶ The disease affects the ciliary structure of the sperm tail, thus producing viable but immotile sperm. ICSI is currently the only treatment option of infertility for these men.

2. Couples who fail in IUI and those who fail to meet the sperm quality requirements for IUI should be offered IVF. The British Fertility Society recommend that IVF should be used when less than 5×10^6 motile sperm are recovered in a 'diagnostic sperm preparation' procedure.³⁷

3. Couples requiring surgical sperm retrieval from epididymis or testes, having severe asthenozoospermia (no consensus threshold) or severe teratozoospermia (no consensus threshold) or a combination of severe oligo-astheno-teratozoospermia and those having failed fertilization or low fertilization rate (no consensus threshold) in previous IVF treatment should be offered ICSI.¹⁶

There are controversies on the use of sperm morphology as predictor of assisted reproduction outcome. While a positive correlation of fertilization rate and pregnancy rate with percentage of normal morphology using the strict criteria was found in some studies,^{38, 39} no correlation was found in other studies. A recent meta-analysis⁴⁰ showed that isolated teratozoospermia was not associated with reduced IVF/ICSI outcome. The discrepancies in the results are probably due to subjectivity of assessing sperm morphology.

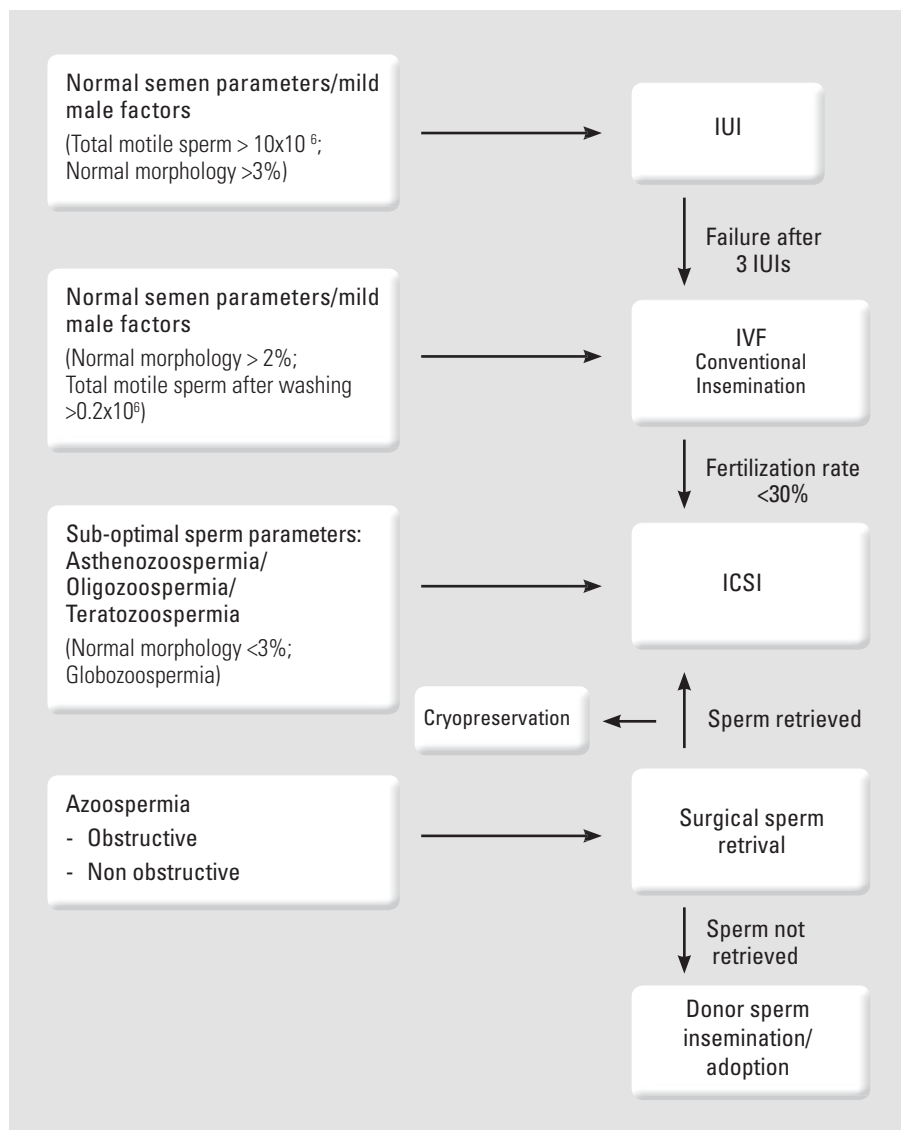


Chart 1. Patient Management for Male Subfertility (Subfertility Clinic, QMH, HKSAR) IUI Should be Offered to Couples with Patent Tubes and Normal Ovulation

of infertility, except in extreme conditions such as azoospermia, globozoospermia and totally immotile sperm.

In general, IUI is advised as a first line treatment for young subfertile couples (female age <35) with normal or mild male factor, bilateral patent tubes and normal ovulation. For couples who fail IUI or with morphologically normal sperm > 2% and more than 0.2×10^6 motile sperm recovered in a 'diagnostic sperm preparation' procedure will be advised for IVF with conventional insemination.

Our unpublished experience showed that the percent of morphologically normal sperm below 5% according to WHO 1999 criteria⁴² were associated with decreased fertilization rate and pregnancy rate, and that ICSI could help to improve the outcome. Recent study also demonstrated that sperm morphology by strict criteria positively correlated with the hemizona binding assay, zona-induced acrosome reaction and negatively with sperm chromatin packaging assay.⁴³ Our current practice is to perform ICSI for men with sperm morphology of $\leq 2\%$ by strict criteria, which is roughly equivalent to 5% by WHO 1999 criteria according to our unpublished internal correlation study.

INTERPRETATION AND PATIENT MANAGEMENT IN QUEEN MARY HOSPITAL, HONG KONG (CHART 1)

In Queen Mary Hospital, Hong Kong, semen analysis is performed by three experienced technical staff. Compliance to standard operation procedures and internal quality control program ensures semen analyses are performed according to the WHO 2010 protocols with minimal variability in time and among staff.

The andrology laboratory also joins the proficiency testing program of the College of American Pathologists⁴¹ to confirm that the laboratory performance is in agreement with other laboratories in the program.

As interpretation of semen parameters is laboratory specific, we always ask our patients to perform semen analysis in our own laboratory. Action is not taken simply based on mild subnormal semen parameters alone in men without history

CONCLUSION

Despite the limitation of conventional semen analysis, routine semen analysis is still the cornerstone in the diagnosis of male infertility. The analysis is cheap to perform and does not require complicated instruments and procedures, though experienced and skillful staffs are absolutely necessary for obtaining consistent and informative results. The new WHO manual provides valuable guidelines for the test. Andrology laboratories should

implement quality control and quality assurance programs to produce reliable results.

Conventional semen analysis does not directly determine the fertilization capacity of the sperm. Indeed, many infertile male have normal semen parameters. On the other hand, subnor-

mal parameters may not necessarily represent infertility. Interpretation of the semen analysis results should be made together with medical history, physical examination and reproductive history.

Semen analysis can provide a definitive indication of infertility only in cases of absolute sperm dysfunction, such

as globozoospermia and azoospermia. Due to the inherent variation of semen samples, semen parameters do fluctuate, making consensus cut-off of the parameters difficult. Nevertheless, when properly performed, low percentage of normal sperm morphology is predictive of fertilization problem.

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CME ARTICLE 1 POINT

Semen Analysis – What a Clinician Should Know

Answer True or False to the questions below.

	True	False
1. Approximately one out of five subfertile couples are affected by pure male factor infertility.	<input type="radio"/>	<input type="radio"/>
2. Patient should refrain from any sexual activities for as long as possible before submitting a semen sample for analysis.	<input type="radio"/>	<input type="radio"/>
3. Patients are not allowed to collect the semen at home.	<input type="radio"/>	<input type="radio"/>
4. The WHO lower reference limit is derived from data collected from both the fertile and subfertile populations.	<input type="radio"/>	<input type="radio"/>
5. A man with semen parameters that fall below the WHO lower reference limit can still father a child.	<input type="radio"/>	<input type="radio"/>
6. A routine semen analysis can predict the fertilization potential of sperm.	<input type="radio"/>	<input type="radio"/>
7. The latest edition of WHO semen analysis manual classifies sperm mortality into four categories.	<input type="radio"/>	<input type="radio"/>
8. Kartagener's syndrome is characterized by round-headed sperm without an acrosome.	<input type="radio"/>	<input type="radio"/>
9. Semen samples from the same patient may have very different semen parameters	<input type="radio"/>	<input type="radio"/>
10. Sperm DNA integrity test is not recommended.	<input type="radio"/>	<input type="radio"/>

Name in BLOCK CAPITALS: _____

Signature: _____

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Answers

1.T 2.T 3.T 4.F 5.F 6.T 7.T 8.T 9.F 10.T