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WHO 6th Edition and 5th Edition Semen Examination Guidance

Introduction, core concepts, laboratory workflow and responsible interpretation

BEGINNER | FOUNDATION EDITION

Major category	ART Standards, Laws and Ethics
Subcategory	ART Standards and Guidelines
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Educational and medical disclaimer

This learning guide is intended for education and professional development. It does not replace patient-specific medical advice, clinical examination, informed consent, institutional standard operating procedures, laboratory accreditation requirements, manufacturer instructions, national legislation or professional judgement.

Educational role The WHO manual standard laboratory procedures and reporting. It is not a treatment algorithm and does not establish an absolute border between fertile and infertile men.	**Results need context** Semen characteristics vary within the same person and can be influenced by abstinence, fever, medications, sample loss, transport and laboratory technique.
Local governance applies In India, ART clinics and banks must also comply with the Assisted Reproductive Technology (Regulation) Act, 2021, the 2022 Rules and institutional quality systems.	**No guarantee language** An isolated count, motility result or morphology percentage cannot guarantee natural conception, fertilisation, embryo development, pregnancy or live birth.

Responsible use

Laboratories should use validated methods, trained personnel, equipment maintenance, internal quality control, external quality assessment where available, traceable documentation and clear reporting. When a local SOP or legal requirement differs from a general educational summary, the applicable local requirement takes priority.

All scientific illustrations and diagrams in this guide are original educational visuals created for Inside Embryo. Numerical claims and interpretive statements are cited to the WHO manuals or peer-reviewed literature.

Contents and learning objectives

Contents	Learning objectives
1. What semen examination means 2. Why it matters in fertility care and ART 3. Essential terminology and sperm biology 4. Collection and pre-examination factors 5. Step-by-step laboratory workflow 6. Core semen parameters 7. WHO 5th edition versus WHO 6th edition 8. Responsible interpretation 9. Quality, safety and common misunderstandings 10. Case scenario and key takeaways 11. Self-assessment and glossary 12. References and further reading	• Define semen analysis and its role within male fertility assessment. • Describe correct sample collection, timing and initial laboratory handling. • Explain the principal semen parameters in beginner-friendly language. • Compare the major interpretive messages of the WHO 5th and 6th editions. • Explain why lower reference values are not strict pass/fail fertility cut-offs. • Recognise essential quality-control, biosafety and reporting principles.

Estimated reading time	Approximately 20–25 minutes.
Who this guide is for	Undergraduates, new embryology or andrology trainees, fertility-team members and allied-health learners.
Prerequisite knowledge	Basic biology only; specialist terms are explained before repeated use.

1. READ	2. REVIEW	3. CHECK
Study one section at a time and define unfamiliar terms.	Use the diagrams and tables to connect the laboratory steps.	Complete the self-assessment before reading the answer key.

1. What is semen examination and why is it important?

Semen examination, commonly called semen analysis, is the standard laboratory evaluation of an ejaculate. It describes both the seminal fluid and the spermatozoa within it. The test supports investigation of the male partner, monitoring of selected interventions and planning before some assisted reproductive technology procedures [1,2].

A semen result is a profile rather than a single score. It includes the amount of semen produced, the concentration and total number of sperm, movement, vitality and structural form. No single parameter directly measures the complete fertility potential of an individual, and results from fertile and infertile men overlap [2-4].

Why it matters in ART and fertility care

Semen analysis is often the first laboratory window into male reproductive function. The pattern may prompt repeat testing, male-factor assessment, endocrine or genetic investigation, counselling and planning of insemination, IVF or intracytoplasmic sperm injection. The female partner's assessment and the couple's duration of trying remain essential clinical context [6,7].

Ejaculate The complete semen sample released during ejaculation.	**Sperm concentration** The number of sperm in one millilitre of semen.
Total sperm number The number of sperm in the entire ejaculate.	**Motility** The pattern and proportion of sperm movement.
Vitality The proportion of live sperm.	**Morphology** The structural appearance of sperm cells.
Reference value A value derived from a reference population; it is not a guarantee or diagnosis by itself.	**SOP** Standard operating procedure used by the laboratory.

2. Basic biological background

Semen is a mixture of spermatozoa and secretions from the male reproductive tract. The testes produce sperm, the epididymides support maturation and storage, and the seminal vesicles, prostate and other accessory glands contribute most of the final fluid volume [1,2].

A normal sperm cell has a head, neck, midpiece and tail. The head contains the nucleus and the acrosome. The midpiece contains mitochondria that support energy production, while the tail produces movement. These structures help learners understand why motility and morphology are assessed separately [1,2].

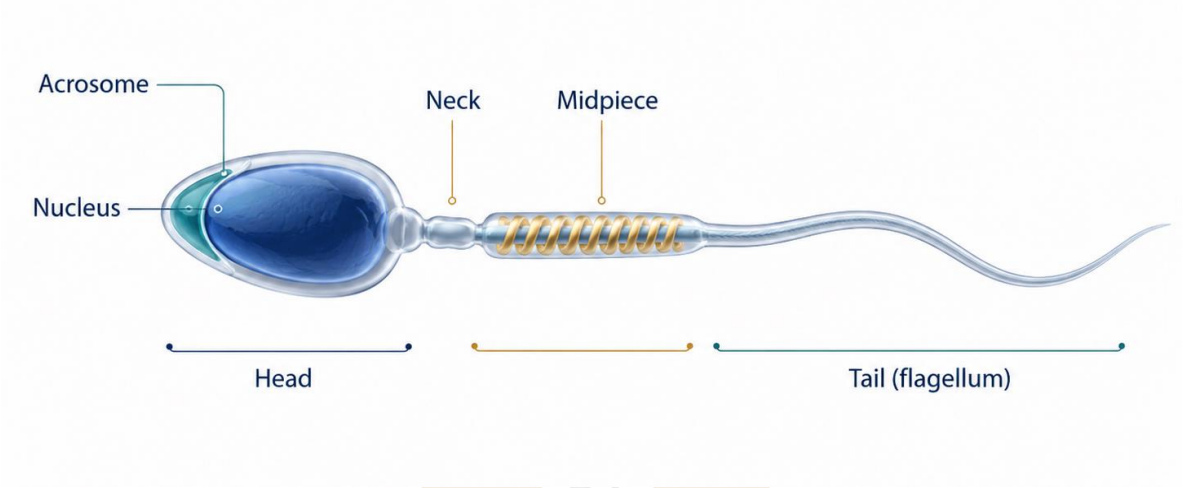

Figure 1. Basic sperm structure. The principal structural regions of a spermatozoon and their functional relevance.

Practical importance: the laboratory observes measurable features of the sample at the time of testing. It does not directly observe future pregnancy potential. Structural or movement findings therefore need careful interpretation rather than absolute conclusions.

3. Collection and pre-examination factors

Many apparent abnormalities begin before the microscope is used. WHO manuals describe a documented abstinence interval, correct patient identification, collection of the complete ejaculate into an appropriate container and timely delivery to the laboratory [1,2].

The first portion of the ejaculate can contain a high concentration of sperm. If it is lost, measured concentration and total sperm number may be reduced. Delayed transport, unsuitable lubricants, fever and incomplete documentation can also alter interpretation.

Pre-examination factor	Why it matters
Abstinence interval	A very short or long interval can change volume, concentration and motility.
Incomplete collection	Loss of the first fraction may cause a misleadingly low sperm number.
Transport delay	Movement and liquefaction may change with time and temperature.
Recent fever or illness	Spermatogenesis and motility may be temporarily affected.
Lubricant or contamination	Some substances harm sperm or interfere with the test.
Missing clinical details	The report becomes harder to interpret safely.

Practical laboratory check: before interpreting an unexpected result, confirm the collection details, sample completeness, timing and any recent illness.

IDENTITY AND LABEL Patient identifiers match the request and sample container.	**TIME AND ABSTINENCE** Collection time and abstinence interval are documented.
COMPLETE EJACULATE Any lost fraction is recorded before interpretation.	**TRANSPORT CONDITION** Delay, temperature and off-site collection are documented.

4. Step-by-step laboratory workflow

A reliable examination follows a defined sequence. The workflow below separates patient preparation, specimen traceability, macroscopic observations, microscopic measurements and final quality review. Straight arrows show the order without crossing or reversing direction.

Figure 2. Semen examination workflow. A clear, single-direction process from patient preparation to quality review and reporting.

5. Core parameters examined in semen analysis

Routine examination combines macroscopic observations with microscopic measurements. The values provide complementary information: volume describes the ejaculate, concentration and total sperm number describe quantity, motility describes movement, vitality identifies live sperm and morphology describes structural form [1,2].

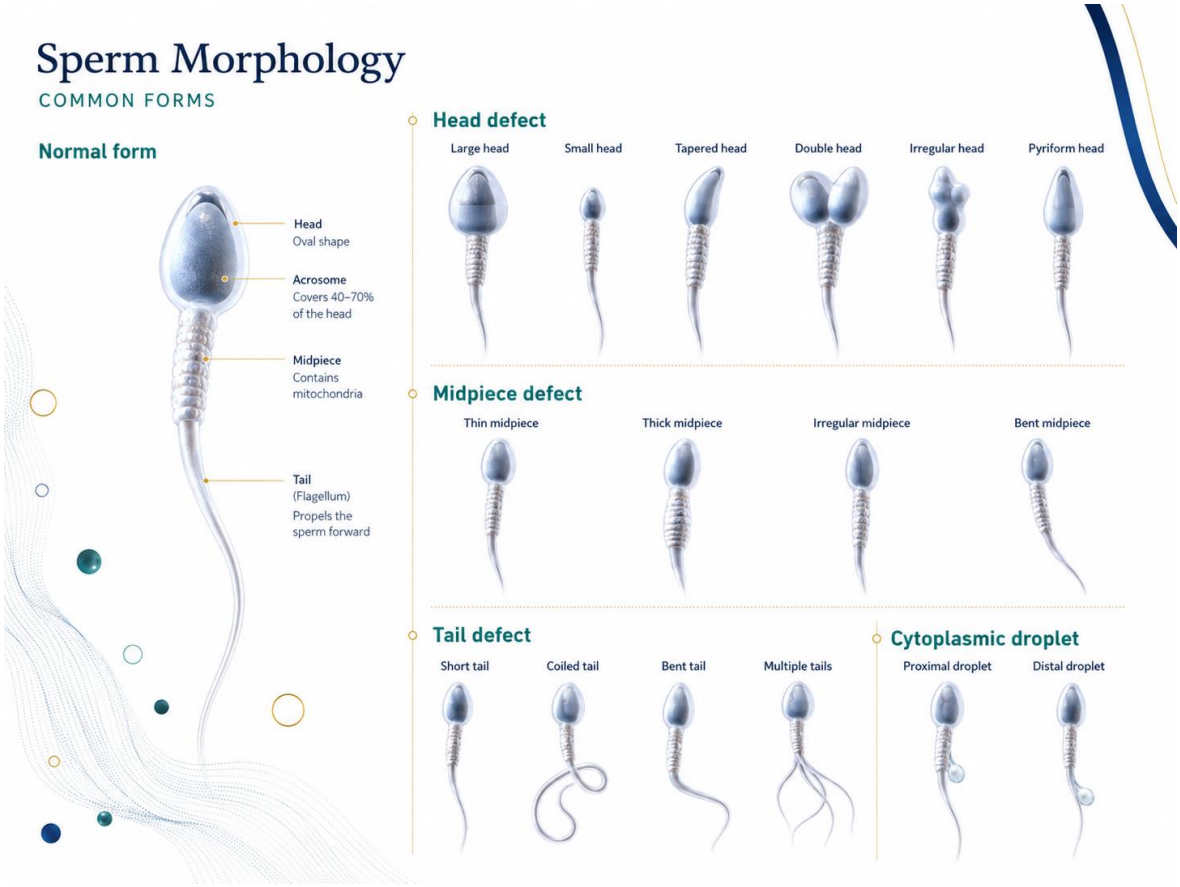

Figure 3. What semen examination evaluates. Overview of the five principal beginner-level parameters commonly discussed in a semen report.

Parameter	What it describes	Beginner interpretation point
Volume	Amount of ejaculate produced	Low volume may reflect collection loss, ejaculatory dysfunction or accessory-gland factors; interpret in context.
Concentration	Number of sperm in one millilitre	Concentration is not the same as the total number in the whole ejaculate.
Total sperm number	Number of sperm in the complete ejaculate	Calculated from concentration and volume; both inputs must be reliable.
Motility	How many sperm move and how they move	Delay, temperature and illness can affect the observed movement.
Vitality	Percentage of live sperm	Especially useful when motility is very low, because immotile sperm may still be alive.
Morphology	Structural appearance of sperm	Should not be interpreted alone or used as a guarantee of fertilisation or pregnancy.

6. WHO 5th edition versus WHO 6th edition

The fifth edition was published in 2010 and the sixth edition in 2021. The sixth edition retains the core objective of reproducible laboratory examination but updates technical procedures, reference information and the emphasis placed on responsible interpretation [1,2,4,5].

Figure 4. WHO 5th and 6th edition comparison. A structured comparison of the central educational messages rather than a dense numerical table.

7. Interpreting results responsibly

Interpretation means reading the whole pattern rather than focusing on one isolated value. A reduced concentration may reflect a biological problem, but it can also be influenced by incomplete collection. Reduced motility may be affected by delay or temperature. Morphology can add information but does not independently predict an individual outcome [2,4,6,7].

Variation is expected within the same individual. Repeat testing may be appropriate when a result is borderline, inconsistent with the clinical history or obtained during illness. Reports should document relevant limitations and avoid absolute claims.

Figure 5. Factors influencing the result. A two-column factor map designed to support a systematic review of unexpected findings.

8. Quality, safety and common misunderstandings

Quality begins with correct patient identification, sample traceability and documented collection details. It continues through validated methods, trained personnel, maintained equipment, timing control, internal quality control and external quality assessment where available [1,2,5,8].

Semen is a biological specimen and must be handled under the laboratory's biosafety procedures. Reports should be clear, readable and specific about limitations such as incomplete collection, delay or technical difficulty.

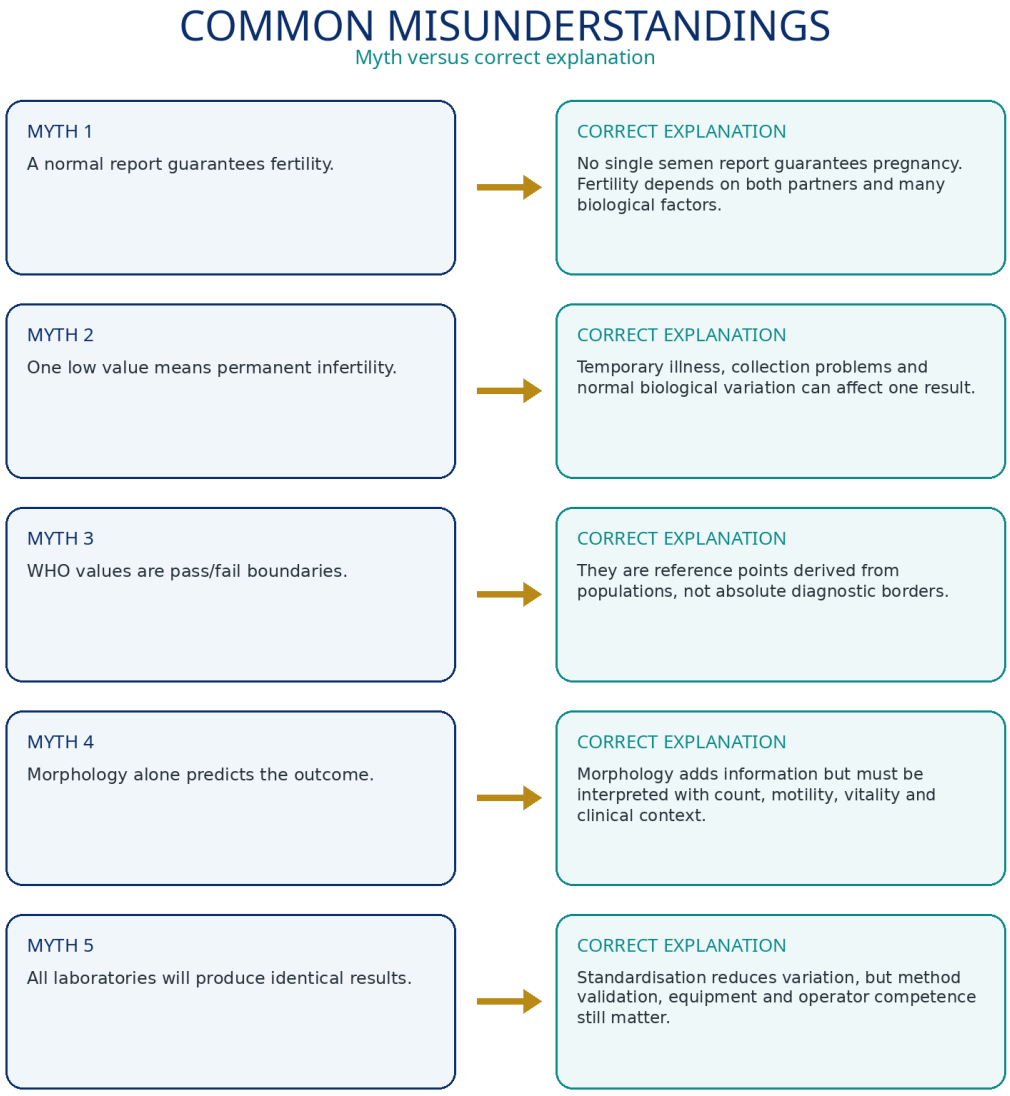

Figure 6. Common misconceptions. A myth-versus-correct-explanation revision graphic for new learners.

9. Simple case scenario and key takeaways

Case scenario: a couple presents after one year of infertility. The male partner provides a semen sample after one day of abstinence and reports that part of the ejaculate was lost. The result shows low volume and reduced total sperm number. Before concluding that severe male-factor infertility is present, the collection limitation should be documented and repeat testing under suitable conditions should be considered.

What this teaches: safe interpretation identifies the limitation, explains which values may be affected and avoids giving a definitive diagnosis from a specimen collected under imperfect conditions.

Figure 7. Key takeaways. Six concise principles that summarise the chapter.

10. Self-assessment

1. Define sperm concentration. _____ _____	**6. What does vitality measure?** _____ _____
2. How does total sperm number differ from concentration? _____ _____	**7. How does motility differ from morphology?** _____ _____
3. Name three pre-examination factors that can affect a result. _____ _____	**8. Why are WHO lower reference values not strict pass/fail cut-offs?** _____ _____
4. Why is loss of the first ejaculate fraction important? _____ _____	**9. Why may repeat testing be useful?** _____ _____
5. List two macroscopic and two microscopic parameters. _____ _____	**10. State two quality principles that improve reliability.** _____ _____

Confidence check

☐ **I can explain why collection quality changes the result.**	☐ **I can distinguish concentration from total sperm number.**
☐ **I can explain why WHO values are not pass/fail boundaries.**	☐ **I can state the main quality and reporting principles.**

11. Answer key and glossary

1. Concentration is the number of sperm in one millilitre.

2. Total sperm number refers to the entire ejaculate and combines volume with concentration.

3. Examples include abstinence, incomplete collection, delay, temperature, illness and unsuitable lubricants.

4. The first fraction can be sperm-rich, so its loss may lower measured concentration and total sperm number.

5. Macroscopic: volume, pH, appearance, liquefaction or viscosity. Microscopic: concentration, motility, vitality, morphology or round cells.

6. Vitality measures the proportion of live sperm.

7. Motility describes movement; morphology describes structural appearance.

8. Reference values are population-derived comparison points rather than absolute diagnostic borders.

9. Repeat testing helps address biological variation and temporary or collection-related influences.

10. Validated methods, training, equipment maintenance, traceability and quality control improve reliability.

Glossary

Abstinence interval Time between the previous ejaculation and sample collection.	**Acrosome** Cap-like region of the sperm head.
Ejaculate The complete semen sample released during ejaculation.	**Liquefaction** The process by which semen becomes less coagulated after collection.
Macroscopic examination Assessment of features visible without high-power microscopy.	**Motility** Assessment of sperm movement.
Progressive motility Active forward movement.	**Reference value** A population-derived comparison point.
Round cells Non-sperm cells that may include immature germ cells or leucocytes.	**Vitality** Proportion of live sperm.

12. Further reading and references

For formal laboratory implementation, consult the full WHO manual, current institutional SOPs, applicable accreditation requirements and national legislation. This guide is a teaching summary and does not reproduce the complete technical protocols.

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About Inside Embryo

Inside Embryo by Aurion is an educational platform focused on Assisted Reproduction, Fertility Care, Embryology and IVF laboratory science. It explains complex ART concepts through structured teaching resources, original visuals and practical laboratory context. Website: insideembryo.com.

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