ESHRE recommendations on Good Practice in the IVF laboratory

Authors

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Abstract

STUDY QUESTION: What is the currently good practice in the IVF laboratory, based on the best available evidence in the literature if available, and the expertise of the working group?

SUMMARY ANSWER: The updated ESHRE Recommendations on Good Practice for the IVF laboratory provides recommendations on all activities in the IVF laboratory.

WHAT IS KNOWN ALREADY? The previous version of this document was published in 2015. After more than a decade, a thorough review and update was needed.

STUDY DESIGN, SIZE, DURATION: This document was developed according to a predefined methodology for ESHRE good practice recommendations. The working group reviewed the document of 2015, and based on this assessment, each group member updated one or more sections. Recommendations are supported by data from the literature, if available, and the expertise of the working group and discussed until consensus was reached within the working group.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The working group included 10 members representing the ESHRE Special Interest Groups for Embryology, Safety and Quality, and Andrology, with different areas of expertise and representing different European countries and settings. Based on the available evidence and the expertise of the working group, recommendations were formulated.

MAIN RESULTS AND THE ROLE OF CHANCE: The ESHRE IVF labs working group updated the recommendations on the general organization of an IVF laboratory (staffing and direction, quality management, laboratory safety), and on the specific aspects of the procedures performed in IVF laboratories (Identification of patients and traceability of their reproductive cells, consumables, handling of biological material, oocyte retrieval, sperm preparation, insemination of oocytes, scoring for fertilisation, embryo culture and transfer, cryopreservation and emergency procedures). The section on embryo culture and transfer was split up, the section on sperm preparation was expanded to cover all general andrological procedures and a new section on biopsy procedure was introduced.

LIMITATIONS, REASONS FOR CAUTION: Not all recommendations are supported by evidence. Other recommendations published in legal documents and relevant and recent documents, manuals and consensus papers were taken into account when formulating the recommendations.

WIDER IMPLICATIONS OF THE FINDINGS: The guideline group is confident that this document will be helpful to directors and managers involved in the management and organisation of IVF laboratories, but also to embryologists and laboratory technicians performing daily tasks.

STUDY FUNDING/COMPETING INTEREST(S): The guideline was developed by ESHRE, who funded the guideline meetings, literature searches and dissemination of the guideline. The

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Introduction

The ESHRE Recommendations on Good Practice in IVF Laboratories serve as a comprehensive guide to all procedures performed within the IVF laboratory, aiming to promote the highest standards of safety, quality, and effectiveness in medically assisted reproduction (MAR). Originally published in 2008 and subsequently updated in 2015, these guidelines have evolved in response to ongoing scientific advancements, technological innovations, and the changing regulatory landscape.

Since their inception, the recommendations have been developed through extensive literature review and expert consensus, establishing themselves as a valuable resource for MAR laboratory professionals. The adoption of new procedures and technologies has led to significant improvements in laboratory outcomes, while the introduction of updated European legislation has necessitated further revision of IVF laboratory procedures to ensure compliance and best practice.

This latest edition brings together the expertise of the ESHRE working group, integrating the most recent scientific evidence, stakeholder feedback, and regulatory developments. The document systematically lists and describes all relevant laboratory procedures, supporting harmonisation of practices across different settings and fostering continuous improvement in patient care.

By providing clear, evidence-based recommendations, these guidelines are intended to assist laboratory directors, embryologists, technicians, and all members of the IVF team in delivering high-quality, patient-centred care. The present updated recommendations aim to promote excellence, safety, and accountability, ensuring that IVF laboratories remain at the forefront of innovation and best practice in MAR.

This Good Practice Recommendations paper was published before the 6th edition of the EDQM guidance was published. However, the working group tried to take changes in the EDQM guideline into account based on the stakeholder version of the 6th edition. The 6th Edition will

be aligned with the new regulation and that compliance with this guide will equal compliance with the regulation.

Methodology

The current document was developed according to the manual for development of ESHRE Good Practice Recommendations (Vermeulen et al., 2019).

A working group was composed of members of the ESHRE Special Interest Group (SIG) Embryology, Safety and Quality, and Andrology, ensuring representation of different laboratory expertise, and geographical balance, supported by a methodological expert (NLC). In the first meetings, the working group reviewed the topics that were covered in the 2015 version of the guideline and missing topics were identified. A literature search of PUBMED/MEDLINE and Cochrane library was performed. Papers published up to 13 January 2026 were included. All titles and abstracts were screened to identify relevant papers, for which full text papers were collected and summarized. At working group meetings, the evidence and draft recommendations were presented by the assigned working group member and discussed until consensus was reached within the group.

Abbreviations used throughout this article are listed in Supplementary Table S1.

Results

1. Staffing and direction

Personnel are one of the most important parts of an IVF laboratory. An IVF laboratory is both a diagnostic and therapeutic laboratory service unit, and an integral part of the process of assisted reproduction. Appropriate human and logistic resources should provide an adequate climate to perform all laboratory tasks in a timely and safe manner, to ensure patient safety and quality care. The number of laboratory staff should reflect the number and complexity of tasks assigned to the embryologists at the specific clinic (see more in 1.4), including duties such as administration, training, education, quality management, witnessing and communication. Leave after weekend rotas, parental leave, illness etc. also needs consideration in the number of staff. A minimum of two qualified clinical embryologists is always recommended in every laboratory, irrespective of the size and workload.

The hierarchical laboratory organisation depends on staff size. Larger facilities can delegate responsibilities to different staff levels, e.g. supervisors, clinical embryologists, laboratory technicians and administrative personnel (2015).

1.1 Laboratory director

The laboratory should be directed by a person with officially recognised qualifications and expertise in clinical embryology and biological/medical sciences. In accordance with the ESHRE certification programme, this would include a higher academic degree (MD, MSc, PhD) with a

minimum of 6 years of documented human embryology experience, and preferably attainment of the ESHRE senior clinical embryologist certification (ESHRE Working Group on Embryologist Training Analysis et al., 2023, Kovačič et al., 2020) or similar. The laboratory director should also participate in CPD (Continuing Professional Development) programs.

Laboratory directors should be able to evaluate and interpret the significance of medical and laboratory findings, and communicate them to laboratory staff members, clinical colleagues, patients and the public. They should proactively seek clinical and scientific updates, promote science and participate in clinical studies and research, where possible.

Laboratory director responsibilities include ensuring:

- Selection and implementation of the most adequate materials and procedures to reach the highest standards in clinical IVF.
- Maintenance of, and safe and appropriate laboratory facilities and equipment according to European and/or national regulations.
- Implementation of a quality management system (QMS), including trouble-shooting.
- Implementation of a laboratory risk management and prevention policy.
- Sufficient laboratory staff members with the appropriate skills.
- A comprehensive orientation and introduction programme for all new staff members.
- Management of laboratory staff training and continual scientific and biomedical education.
- Implementation and review of key performance indicators (KPIs) for all laboratory procedures for quality control and quality assurance purposes.
- Involvement in inspections from authorities and internal/external audits.
- Reporting of clinical data and adverse events (biovigilance) according to European and/or national regulations.
- Where relevant, participating in authorisation and application of substances of Human origin (SoHO) preparation processes.
- Where relevant, participating in approval of research projects by competent authorities.

1.2 Laboratory supervisors/manager

Some laboratories may require additional managerial positions, working in close collaboration with the laboratory director. These require specific qualifications, e.g. at least a BSc in biomedical sciences, 3 years of documented human embryology experience and preferably attainment of the ESHRE clinical embryologist certification or similar.

Laboratory manager/supervisor responsibilities could include ensuring:

- Efficient organisation of daily work of their areas of responsibility.
- Effective communication with laboratory staff and clinical colleagues.
- Continuous improvement work.
- Structured training of staff members and students.

1.3 Clinical embryologists

Clinical embryologists represent the first line of participation in daily clinical practice. These positions require at least a BSc in biomedical sciences. New staff should follow a structured training programme supervised by experienced clinical embryologists.

Clinical embryologists with 3 years of experience should endeavour to apply for the ESHRE clinical embryologist certification, whereas those with higher degrees and 6 years of experience should endeavour to apply for the ESHRE senior clinical embryologist certification.

Clinical embryologist responsibilities include:

- Adherence to and execution of standard operating procedures (SOPs).
- Documentation of all relevant clinical and laboratory data (database management).
- Participation in organisation of daily practice.
- Communication with both patients and other healthcare professionals.
- Contribution to laboratory clinical decisions.
- Training of staff members and students.

All procedure should be performed by experienced practitioners who are skilled in general embryology and micromanipulation, after appropriate training and following SOPs.

Detailed recommendations on embryo biopsy training are outlined in the ESHRE recommendation paper (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group et al., 2020).

1.4 Number of staff

Performance and safety of the IVF laboratory is dependent upon the number and competence of staff. Some IVF laboratories may be handling only routine *in vitro* fertilisation/intracytoplasmic sperm injection (IVF/ICSI) cycles, others all complex treatment cycles such as embryo biopsies, gamete donation or fertility preservation. It is also different between clinics and countries whether the andrology lab is included in the IVF lab and handled by the embryologists, or separate and run by other staff (technologists) (Shirasawa and Terada, 2025).

The time needed for embryologists to perform standard tasks was calculated in a study from Spain by Veiga *et al.* (Veiga et al., 2022), with Spanish working conditions as the example and based on the calculation that a full-time person works 214 days per year. In the study, a full-time clinical embryologist (8 hours/day) was recommended for every 119 IVF/ICSI cycles with time-lapse (TL) culture per year, although always with a minimum of two persons for back-up reasons. For the andrology laboratory a full-time person was needed for every 549 annual semen samples (diagnostic or therapeutic). On top of this comes the laboratory director (+possibly a coordinator/manager). (For a complete overview of the numbers, please see (Veiga, et al., 2022)). In the review by Shirasawa and Terada 2025, it was found that the most common standards for minimum staffing for embryologists was 2-3 for up to 150 cycles, 3-4 for up to 300 cycles, 4-5 for up to 600 cycles, and then one additional embryologist for each 150-200 additional cycle (Shirasawa and Terada, 2025).

However, it is important to take into account the fact that all laboratories have different set-ups of staff, and different logistics and workflow. It is therefore recommended that laboratories perform their own calculations of the number of staff needed to run a safe, efficient and high performing laboratory, based on their actual organisation and day-to-day processes. These calculations should be documented for quality and inspection purposes. There are several published tools to aid in these calculations (for example (Alikani et al., 2014) and https://asebir.com/cassandra-calculadora-de-rrhh/?idioma_cassandra=en).

1.5 Continuing education

Over four decades after the first IVF cycles, the procedures and timelines involved in completing a cycle have become dramatically more complex. Techniques such as ICSI, extended embryo culture, TL imaging, vitrification, testicular and embryo biopsies, as well as comprehensive quality management and witnessing systems, have all been incorporated into routine practice. At the same time, regulatory requirements, technological and informatics competencies, and the volume of necessary documentation and reporting have increased substantially.

It is vital that an embryologist learn not only the practical “how’s” but also the theoretical “why’s” of the processes being performed. Some countries have formalized structured training including theoretical parts, while many others rely on in-house training (Nijs et al., 2025). Additional and continuous training is of great importance, such as workshops.

One of the drawbacks of clinical embryology is the lack of regulation concerning the criteria required to be a specialist in the field in many countries. In fact, clinical embryology is practiced by a wide variety of professionals who have trained in very different disciplines, including biology, medicine, pharmacy, veterinary science, etc. (Kovačič et al., 2015). Trainees should begin with a solid academic background in biological or biomedical sciences, followed by a formal, written training plan that includes observation, supervised performance, and progressive responsibility. Training activities should be logged in detailed records (logbooks) documenting the number and type of procedures performed and reviewed by a senior embryologist or laboratory director. Competence validation is mandatory before independent

work—this involves parallel assessments, direct observation, and demonstration of consistent technical proficiency across a minimum number of procedures (typically 30–60 supervised ART cycles, depending on the skill). Mentorship and structured feedback throughout this process, ensuring that each step from observation to autonomy is evidence-based and recorded is considered good practice. Periodic evaluations are required to confirm readiness for independent practice, and laboratories should maintain defined experience thresholds for different professional levels (trainee, junior, senior, supervisor). ESHRE provides a CPD program via campus courses and an e-learning platform (https://www.eshre.eu/Education).

2. Quality management

According to the SoHO regulation (European Parliament, 2024, European Committee on Organ Transplantation), establishing, maintaining and updating a quality management system (QMS) is mandatory. IVF laboratories should designate one or more persons to design, implement and revise a QMS, so called quality managers.

The requirements of the QMS cover personnel and organisation, premises, equipment and materials, documentation, change control, complaints, recall, quality review, and so on. The QMS should be documented and designed to assure quality safety and effectiveness of our processes. It should incorporate risk management principles and written procedures of all critical processes which need to be reviewed systematically in light of new knowledge and significant changes to processes should be identified and verified or validated. Good documentation is an essential part of the QMS and hence a document control system should be established to ensure that documentation is complete, current, accurate and easily available to all staff.

The QMS should include at least the following:	
2.1	The provision for unique identification of patients and their reproductive cells and tissues. Traceability records, whether maintained electronically or in handwritten form, should be clearly readable, permanent, securely stored, and readily accessible. Data should be electronically captured and validated; manual transcription should be double-checked. In absence of regulation stating otherwise, these records should be maintained securely for 30 years; critical materials and equipment data should be retained for at least 10 years.
2.2	A documentation system for dealing with non-conformities, emergencies, incidents, adverse events or reactions and complaints. Non-compliances should be analysed, and appropriate corrective and/or preventive actions (CAPA) should be identified. Non-conformities should be discussed with the teams regularly to highlight learning points and reviewed at least annually with the laboratory management.

2.3	Procedures to address the withdrawal of release or (temporary) restriction of reproductive material in case of a recall or serious adverse events/reactions. Monitoring of performance indicators (PIs) which are objective and relevant, regularly checked and discussed, and communicated to all staff. PIs can be based on a reference patient group with good prognosis, as well as on the whole patient population. Appropriate statistics can be used to account for patient variation, and the number of previous treatment cycles patients may have already undertaken. Critical performance levels should be defined for each PI with reference to national data and European registry data collected by the European IVF-monitoring programme for ESHRE. If necessary, appropriate action should be taken. In addition to laboratory and clinical performance, operator performance should be checked regularly to ensure competence, compliance and consistency, via direct observation of procedural skills (DOPS) and/or individual PIs. Retraining should be implemented after a longer period of absence (e.g. a longer parental or sick leave).	(ESHRE Clinic PI Working Group et al., 2021, ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine, 2017)
2.4	Participation to Internal Quality Control (IQC) and External Quality Assurance (EQA) programmes, either commercial or in collaboration with other laboratories, at least for diagnostic semen laboratories. These records should be maintained and reviewed, including documentation of results and any corrective action.	
2.5	A risk-based change-control system to describe steps to be taken to plan, record, analyse and implement changes in the IVF-laboratory.	
2.6	An audit system, both internal and external, to verify compliance of all procedures with SOPs and requirements. And any findings, corrective actions and their effectiveness should be documented.	
2.7	A documented validation of all critical processes and the qualification of all critical equipment used in these processes.	
2.8	A documented risk assessment strategy to identify, evaluate and mitigate risks to ensure the protection of donor, recipients and children born from MAR. Several risk management tools are available such as hazard analysis and critical control points analysis (HACCP), Root cause analysis (RCA), Failure mode and (critical) effects analysis (FME(c)A), EuroGTPII tool, and the EDQM microbiological risk of contamination assessment tool (MiRCA). Since there is no obligation to use a specific tool and since each tool has its specificities and possibilities to assess risks, it is recommended to document which tools are used in which scenarios in your IVF-laboratory. QMS reviews should be performed periodically with the aim of having an overall review of the whole system ideally every three years to verify the	

	consistency and appropriateness of the QMS. This so-called quality management system review will consider the review of all critical QMS items, define trends and identify risks for the QMS.

3. Laboratory safety

3.1 Laboratory design

Laboratory design should ensure optimal workflow over minimal distances while handling reproductive cells during all treatment phases.

The IVF laboratory should have adequate functionalities to minimise any damaging effects upon reproductive cells and tissues. At any time, laboratory design should ensure aseptic and optimal handling of reproductive cells and tissues (Council of Europe, 2022).

Ideally, the laboratory should be adjacent to the operating room where clinical procedures are performed in order to ensure optimal workflow over short distances. Technical facilities as well as staff and storage rooms should be physically separated from the laboratory reserved for the procurement, processing and release of human cells and tissues. A separate laboratory with a safety fume hood should be provided for analyses using fixatives and other toxic reagents. Cryopreservation storage facilities need special precautionary measures and should be safely located outside but close to the laboratory.

In a scenario of newly constructed or renovated laboratories sufficient time should be scheduled for off-gassing of construction materials (Cairo Consensus Group, 2020). In any case, critical equipment should be qualified, appropriate in number and fit for its purpose (European Commission, 2022).
Special attention should be given to workplace ergonomics and operator comfort to provide an effective and safe working environment that minimises the risk of distraction, fatigue and thereby making a mistake due to human error (Rienzi et al., 2015). Taking into account local, national and European occupational health and safety requirements, such considerations should include adequate work space per person, bench height, adjustable chairs, microscope eye height, efficient use of space and surfaces. Sufficient environmental lighting and air-conditioning with controlled humidity and temperature are advised.

More specifically:

3.1.1	Materials used in laboratory construction, painting, flooring and furniture should be appropriate for clean room standards, thus, minimising Volatile Organic Compounds (VOC) release and embryo toxicity.
3.1.2	Laboratory access should be restricted to authorised personnel. Entry of non-specialist staff should be documented.
3.1.3	A double door system for clean access of personnel and materials to the laboratory is recommended.

3.1.4	Staff should be provided with a dedicated space for changing into laboratory clothing and washing hands before entering the laboratory.	
3.1.5	The area for cleaning and sterilisation of materials should be separate from the laboratory.	
3.1.6	If possible, systems connected to main water and drainage should be outside of the laboratory due to potential infection risks associated with standing water.	
3.1.7	Separate office space for administrative work should be available outside the laboratory.	(Barrese et al., 2014)
3.1.8	Potential distractors, such as private cell phones should be removed from the laboratory.	(de los Santos and Ruiz, 2013)

3.2 Laboratory air quality

3.2.1	To optimise environmental conditions, laboratory air should be subjected to high-efficiency particulate air (HEPA) and VOC control.	
3.2.2	Positive pressure is recommended to minimise air contamination. Air conditioning should ensure a sufficient number of fresh air changes.	(Cairo Consensus Group, 2020)
3.2.3	Procedures involving gamete or embryo manipulation should be performed in a controlled environment. Background and processing air quality should comply with national and European guidelines and should be regularly monitored.	
3.2.4	The level of air cleanliness in the IVF laboratory should be risk-based and regularly assessed in “at rest” and “in operation” states.	
3.2.5	The microbial contamination level of the laboratory should be regularly determined. Frequency of controls and number of sampling locations should be based on a documented risk assessment.	

3.3 Laboratory equipment

3.3.1	The laboratory should contain all essential items required for IVF and ICSI, in a number appropriate to the workload. Critical items of equipment should be in duplicate.	
3.3.2	The incubator number is critical and should be based on the number of cycles and embryo culture duration. Gametes and embryos should be conveniently distributed across incubators to minimise door openings.	
3.3.3	Equipment should be adequate for optimal laboratory work, easy to disinfect and kept clean to avoid contamination.	
3.3.4	Critical equipment should be validated to meet quality requirements and to guarantee quality assurance. This process usually is composed of design,	

	installation, operational and performance qualification. Measured parameters should be verified by calibrated probes and instruments.
3.3.5	Equipment should preferably be CE-marked for the intended use.
3.3.6	Gas cylinders should be located outside the laboratory. There should be an automatic change-over system and sufficient cylinders stocked for immediate replacement. High-purity/medical grade gas and inline HEPA and VOC filters are highly recommended.
3.3.7	Regular maintenance should be scheduled and documented for all critical equipment, at least according to suppliers' recommendations.
3.3.8	Heating devices, such as tube warmers or heated stages, should be in place to maintain the optimal temperature of media as well as reproductive cells and tissues during handling.
3.3.9	Accepted ranges of use for all measured parameters should be determined and monitored. If measurements are out of range, corrections should be made, documented and their effectiveness verified.
3.3.10	For every item of equipment, the instruction manual, and simplified instructions where needed, should be easily available.
3.3.11	Malfunctioning equipment should be labelled as "out-of-use" to avoid its use by mistake.
3.3.12	Critical items of equipment, including incubators and cryostorage tanks, should be continuously monitored and equipped with external alarm systems.
3.3.13	A backup power system should be in place for critical equipment.

3.4 Cryopreservation facilities and material

3.4.1	For safety reasons cryopreservation facilities should allow visible access to the interior (e.g. via a window, camera).
3.4.2	Adequate ventilation and low oxygen alarms should be installed. Personal low oxygen alarms can be used, as an additional security measure.
3.4.3	Cryostorage tanks should be continuously monitored and equipped with alarm systems, detecting and logging any out of range temperature and/or levels of liquid nitrogen (LN2).
3.4.4	Protection devices (e.g. glasses, face shield, cryogloves, apron, footwear) should be used during LN2 handling.
3.4.5	All staff dealing with LN2 should be trained in safety aspects of its use.
3.4.6	All liquid nitrogen would preferably be clinical grade.

3.5 Infectious agents

All MAR technologies involve handling of biological material, and pose a potential hazard of transmitting diseases to personnel and to other patients' biological material (cross-contamination).

3.5.1	Procedures to ensure personnel safety and prevent cross-contamination should be established, taking national and European safety regulations into consideration. Therefore:	
	• Vaccination of all personnel against hepatitis B or other viral diseases, for which a vaccine is available, is strongly recommended.	(The ESHRE guideline group on viral infection/disease et al., 2021)
	• Patients should be screened for infectious diseases according to national and European regulations.	(The ESHRE guideline group on viral infection/disease, et al., 2021)
	• Staff should be informed when a viral-positive patient is to be treated and be aware of the risks of handling infected biological material, as well as the correct procedures of disposal. However, crude samples (such as follicular fluid and semen) and patients without serology at the time of donation (oncological patients who come for sperm freezing e.g.) should always be treated as potentially infectious.	
	• SOPs should be in place to manage eventualities where infection might take place, e.g. needlestick injuries.	
3.5.2	To ensure adequate safety measures, the treatment of viral-positive patients should be only performed in IVF laboratories with dedicated areas and equipment. Alternatively, such patient treatments could be allocated to specific time slots provided processing of their biological materials is followed by a thorough disinfection of the allocated areas and equipment. Procedures to handle gametes from viral-positive patients are covered in the ESHRE Viral infection/disease guideline.	(The ESHRE guideline group on viral infection/disease, et al., 2021)
3.5.3	Whenever biological material is imported into the IVF laboratory from another clinic, full screening results should be obtained in advance. If any transported material is viral-positive, a dedicated dry shipper may be needed, depending on European and national regulations.	

3.6 Protective measures

All body fluids (blood, follicular fluid, semen, etc.) should be treated as potentially contaminated.

Protective measures for laboratory staff to ensure aseptic conditions for gametes, embryos and tissue include:

- Strict adherence to staff hygiene regulations and aseptic techniques.
- Use of protective laboratory clothing, preferably with low particle-shedding.
- Use of hairnets and non-toxic, non-powdered gloves and masks where appropriate.
- Use of appropriate vertical laminar flow benches for handling biological material.
- Disposal of single-use consumables immediately into proper waste containers. Potentially infectious materials should be disposed of in a manner that protects staff from exposure.
- Needles, glassware and other sharps should be handled with extreme caution and discarded into sharps containers.
- Non-alcoholic disinfectants with proven compatibility and efficacy for an IVF laboratory should be used.
- Food, gum, drinks and tobacco are strictly forbidden.
- Use of cosmetics should be minimised and perfumes should be avoided.

4. Identification for patients and traceability of their reproductive cells

The new EU Regulation on standards of quality and safety for substances of Human origin (SoHO), published on 17 July 2024, reinforced measures to enhance traceability throughout the lifecycle of SoHO, from donation to clinical application. This means that MAR centres should fulfil, among others, with one of the key traceability provisions that consists in having systems to enable the tracking of SoHO from the donor to the recipient and vice versa, ensuring full traceability across the supply chain.

Traceability is defined as the ability to trace every tissue or cell from donor to recipient or offspring, and to identify all associated entities and processes—procurement, testing, processing, storage, distribution, and application.

A proper identification and traceability system should therefore ensure that the main characteristics of patients (or donors) and their tissues and cells, together with relevant data regarding products and materials coming into contact with them, are available at all times. With traceability, risks related to mix-ups or donor-transmitted genetic conditions can be mitigated. Moreover, reporting exercise and communication of severe adverse reaction and events between SoHo entities and/or regulatory bodies are facilitated.

Regular traceability audits ensure compliance and facilitate biovigilance and quality improvement. Traceability enables long-term biovigilance, including recipient follow-up and health monitoring of children born through ART treatments. All stakeholders - tissue establishments, ORHAs, MAR centers, and families - should collaborate to preserve the

traceability chain. The core components of an effective traceability system are the following ones:

4.1. Unique Identification code

Before commencing any procedure, each patient, sample, and product should be uniquely labelled throughout the process to prevent duplication across organizations. The laboratory should be provided with each patient's unique identification code which has to clearly and easily refer to the patient's documentation. Each treatment cycle should be assigned a unique code. When samples are to be distributed beyond the clinic, SoHO entities should implement a coding system that uniquely identifies each donation within the EU. Importantly this code should be machine-readable and should not reveal the identity of the donor. This is also relevant in the context of cross-border exchanges, where the complexity of maintaining traceability increases, highlighting the need for standardised global identification. Despite the existence of some gaps and challenges of using the Single European Code (SEC) (Alteri et al., 2020) its use can be very helpful in the context of surveillance and safety purposes across EU member states. Gametes and embryos from within-relationship use as well as reproductive cells and tissues of third party donation can be excluded from the SEC as long as they remain in the centre of origin.

Rules concerning the correct identification and processing of reproductive cells should be established in the laboratory by a system of codes and checks including:

- Direct verification of patient identity and correspondence with their assigned unique identification code is required at every critical step. Patients should be directly asked to give their own identifying information (at least full name and date of birth) before procurement or artificial insemination/embryo transfer.
- All devices containing biological material should be clearly and permanently labelled with the unique patient identification code.
- Biological material from different patients should not be processed in the same working area at the same time.
- Incubators and cryostorage systems should be organised to ensure easy access and identification of the biological materials therein.
- During critical steps (such as first identification of cells and tissues, each time biological material is moved from one container to another and at final destination e.g. embryo transfer, cryocontainer), double-checks by a second person (witness) and/or an electronic identification system is strongly advised.
- Products and materials used with biological materials should be traceable.
- The date and time of each manipulation and identity of all operators and witnesses should be documented throughout the treatment.

These records should be kept for a specified period of time according to European and/or national legislation.
• Steps considered to be critical should be identified and documented.

4.2. Witnessing Protocols

Incidents may occur for a variety of reasons (de los Santos and Ruiz, 2013). Mix-ups such as incorrect embryo, sperm, or egg transfers are extremely rare but serious when they occur and translates to approximately 0.001% to 0.002% of cases (Sterckx et al., 2023).

To control for human errors, manual or electronic witnessing protocols are considered a best practice in assisted reproductive technology (ART) because it introduces a layer of verification that helps prevent critical errors involving patient samples management. Each laboratory should identify critical steps to be witnessed and document them. Examples can be found in (Cimadomo et al., 2016, de los Santos and Ruiz, 2013, Rienzi et al., 2017a, Rienzi, et al., 2015).

Each ART laboratory should:	
• Encourage use of **standardized witnessing protocols** across all procedures and personnel to minimize variability.	
• Encourage a **blame-free culture** for reporting witnessing mismatches or electronic witnessing systems (EWS) anomalies.	
• Use reported cases and near misses as **training material** for continuous improvement.	
• **When using EWS** take into consideration:	
◦ The importance of staff training to maintain **critical thinking and manual verification** skills, even with EWS in place.	(Alteri et al., 2025, Gupta et al., 2020, Intra et al., 2016)
◦ The creation of indicators to minimize mismatch tolerance and clear user alerts to prevent overlooked errors.	(Holmes et al., 2021, Intra, et al., 2016)
◦ The reiteration among team members about the **complementary role of EWS** as it does not replace human vigilance.	
◦ To emphasize the importance of **proper data entry and accurate sample labelling.**	
◦ The implementation EWS audits for **design flaws**, **unclear alerts**, or **normalization of deviation** (e.g., ignored warnings).	(Rienzi, et al., 2017a, Rienzi, et al., 2015, Vujisic et al., 2024)

5. Consumables

5.1. Critical reagents and materials

Specifications of critical reagents and materials should be in compliance with European and/or national regulations and intended for IVF use. SoHO entities are mandated to mitigate risks associated with consumables that may be transferred to recipients and potentially harm their health.

• All medical devices used in the collection, processing, storage, and application of SoHO materials should comply with the stringent safety and quality standards established by the CE/Medical Device Regulation (MDR) ((EU) 2017/745).	
• In-house manufactured medical devices may be used by IVF laboratories; however, under the MDR ((EU) 2017/745), health institutions may only manufacture and apply such devices internally if a comprehensive set of regulatory conditions is met.	
• It is strongly recommended that consumables lacking CE/MDR are tested with an appropriate MEA/HSSA bioassay before using the materials in the clinical setting.	(Chronopoulou and Harper, 2015, Delaroche et al., 2024, Delaroche et al., 2020, Nijs et al., 2009, Togola et al., 2021)
• It is advisable to confirm that the pH of new batches of all culture media fall within the laboratory's established acceptable range before use.	

IVF culture media, cryopreservation media, polyvinylpyrrolidone (PVP), sperm separation media are generally classified as a medical device Class IIa (low to medium risk), whereas mineral oil and other consumables used in IVF procedures, such as culture dishes, denudation capillaries, etc, are typically classified as Class I (low risk) medical devices as they do not include any active therapeutic or diagnostic elements. However, the specific classification can vary depending on the intended purpose and risk profile of the consumable.

5.2. Consumable delivery entry control

Laboratories should define criteria to accept delivered consumables. Upon receipt, the integrity of product packaging should be thoroughly inspected to ensure no compromise during shipping. Proper delivery conditions, including temperature check upon arrival are needed to assure that the cold chain has not been broken. Documentation demonstrating quality control testing should accompany all commercially supplied media and should specifically correspond to the delivered batch.

5.3. Use of Sterile, Single-Use Disposables

To reduce the risk of contamination and ensure consistent performance, only sterile, single-use disposable consumables should be used in ART procedures. These disposables should meet

regulatory sterility standards and be handled in a controlled environment. This practice minimizes the potential for introducing reprotoxic substances that could adversely affect gametes and embryos. In-house-made or sterilised devices for handling human gametes and embryos should be avoided. Tips or capillaries for pipetting devices (for handling or denudation pipettes) should be used for one procedure only.

5.4. Expiry Dates and Shelf Life Management

• All reagents, culture media, and consumables should be used within the manufacturer's stated expiry date to guarantee their efficacy and safety.	
• Using products beyond their validated shelf life may compromise the viability of gametes and embryos.	
• Regular inventory checks and a robust stock management system can aid in ensuring that expired products are promptly removed from use.	(2022, Malhotra et al., 2021, Vujisic, et al., 2024)

5.5. Appropriate Packaging Size

• Using appropriately sized containers reduces the risk of contamination and degradation by limiting exposure to air and temperature fluctuations between first and last use. This practice helps maintain the integrity of the products throughout their usage period.	(Chronopoulou and Harper, 2015, Delaroche, et al., 2020, Vujisic, et al., 2024)
• Consumables and media should be supplied in packaging that minimizes the need for repeated openings.	

5.6. Storage conditions

• Temperature of storage rooms should comply with the manufacturer's specifications of the products.	
• Refrigeration units that meet the specified temperature requirements should be available and monitored for the storage of media and reagents.	
• Repeated exposure to temperature fluctuations during handling and storage should be avoided to preserve product integrity, as suboptimal storage conditions can lead to the degradation of media components, potentially impacting embryo development.	(Cairo Consensus Group, 2020, Vujisic, et al., 2024)

5.7. Stock Management System

Effective stock management contributes to the overall quality control within the laboratory as it prevents use of expired, compromised, or inappropriate materials, help to link each product batch to specific procedures, patients, or dates, support audits and compliance, optimize inventory turnover and avoid overstocking or expiration, minimizing waste an also ensure availability of critical items without delays (2022, Vujisic, et al., 2024).

A robust stock management system should be in place to track all media, oil, and consumables and is essential for ensuring the traceability, safety, efficiency, and regulatory compliance of all consumables, media, and reagents used in the handling of gametes and embryos. Laboratories should:

• Record key data such as the batch number (lot number), date of entry into inventory, and expiration date to ensure traceability and prevent the use of expired materials.	
• Implement standardized labelling and identification protocols to further enhance the safety and efficiency of laboratory operations.	(Vujisic, et al., 2024)
• Conduct regular risk assessments to ensure that all consumables and media are clearly labelled and easily identifiable. This minimizes the possibility of misuse and supports traceability and accountability in laboratory procedures.	

6. Handling of biological material

6.1	Handling of biological material should be easy, simple and effective and should preferably be performed in laminar flow hoods equipped with heating stages and pre-warmed heating blocks, using aseptic techniques at all times.	
6.2	Measures should be taken to ensure that oocytes and embryos are always maintained at the appropriate temperature, pH and osmolality during culture and handling. Exposure to light, toxic substances or harmful radiation should be minimised.	
6.3	Buffered media (HEPES, MOPS or similar) should be kept in atmospheric air, whereas bicarbonate-buffered media should be kept in 5-7% CO_2.	
6.4	Traceability should be confirmed at all times (see chapter 4).	
6.5	MOPS/HEPES are preferably not used for oocyte or embryo culture. Where observations will take longer than two minutes, MOPS or HEPES (or similarly buffered solution) should be used (e.g. denudation or ICSI). Appropriately buffered medium (bicarbonate, HEPES or MOPS) should be selected based on the expected time taken for manipulation or culture as well as gas availability.	(Mendola et al., 2024, Morgia et al., 2006)

6.6	Efforts should be made to reduce mechanical and shear stress on oocytes and embryos as far as possible through use of appropriately sized pipettes and minimising handling.	
6.7	Oil overlay should be used for dishes whenever deemed appropriate ensuring appropriate volume and viscosity to reduce fluctuations in pH, temperature and osmolality.	
6.8	Warming of pipettes and catheters for gamete or embryo handling is ineffective as the temperature loss is rapid when these consumables are removed from the source of heat. Warming of these consumables could increase the risk of release of VOCs.	
6.9	Reduce blue light exposure as much as possible.	(Cairo Consensus Group, 2020)
6.10	Pipetting should be undertaken using standard hand pipettes. Mouth pipetting is not recommended.	

7. General andrology procedures

Before starting a treatment cycle, diagnostic semen analysis should be performed according to the protocols described in the World Health Organization (WHO) laboratory manual for the examination and processing of human semen, 6th ed. (World Health Organization, 2021). The WHO Guideline for the prevention, diagnosis and treatment of infertility suggests that a single semen analysis result is sufficient, unless one or more parameters lie outside the WHO reference ranges, in which case a second should be performed after a minimum of 11 weeks (Mburu et al., 2025). In practice, sperm count and motility are the parameters vulnerable for febrile illness. Therefore, a repeat semen analysis is only necessary when sperm count or motility parameters are out of WHO reference ranges in case of febrile illness.

In addition, where assisted conception is planned, a test sperm preparation may also be advisable in order to confirm the most adequate insemination technique (IVF/ICSI), or method of sperm selection. Patients should be given clear instructions regarding the collection of the sperm sample (hygiene, sexual abstinence, timing, etc.). A frozen back-up sample should be requested if sperm collection difficulty on the day of oocyte retrieval is anticipated.

The abstinence suggested for diagnostic analysis is for sample standardisation, but increasing evidence indicates that regular ejaculation and short abstinence (<2 days) leads to sperm being exposed to lower oxidative stress and being of improved quality. Shorter abstinences may therefore have potential to improve outcomes and should be considered for most patients. Some emerging studies even suggest that consecutive ejaculates (i.e. <3h apart) may yield better sperm (Kulkarni et al., 2022), but there is currently insufficient high quality evidence to support an effect on outcome and therefore this as routine practice. Consecutive ejaculates also impact laboratory workflow, patient anxiety and comfort so outside well organised studies may be impractical. A second sample may be requested on the day of oocyte pick-up when

394 unexpected parameters are observed in the first sample.

395 Sperm preparation aims to:
396 - eliminate seminal plasma, debris and contaminants;
397 - concentrate progressively motile sperm;
398 - select against morphologically abnormal sperm.

399 Sperm selection is usually an additional step within, or after the preparation process, that aims
400 to further identify and isolate sperm which are more likely to lead to fertilisation and healthy
401 live birth. Many such suggested techniques exist, though evidence for them is limited. These
402 are currently discussed within the ESHRE Good Practice Recommendations on Add-Ons paper
403 (ESHRE Add-ons working group et al., 2023). Where selection procedures are utilised then the
404 traditional concept of 'yield' of sperm is inappropriate in assessing their utility (Gallagher et al.,
405 2023) (see also 7.9).

7.1	Temperature of heated stages and incubators should be regularly checked, even slight changes (+/- a degree Celsius) can have a considerable effect upon any observed or measured sperm motility parameters.
7.2	Semen samples should be collected into sterile, plastic containers (at least sperm-toxicity tested). The use of spermicidal condoms, creams or lubricants should be avoided. Where men request a lubricant for masturbation, the provision of a small amount of the same oil as used for embryo-culture oil overlay in the embryology laboratory is suggested (as this will have known testing and not introduce an unknown parameter, it is also not miscible with semen so does not complicate handling procedures).
7.3	All containers used throughout the process to hold sperm or semen should be clearly labelled with the patient's identifiers. Records should also be kept of the type of container used and batch. The use of medication, fever during the previous months and completeness of the ejaculate collection should be documented.
7.4	Collection should be preferably performed in a room near the laboratory. After collection, the sample should be delivered to the laboratory as soon as possible, avoiding extreme temperatures (<20°C and >37°C).
7.5	**An identity check should always be performed upon receiving a sample from a patient.** Each laboratory should have in place a protocol to ensure the patient's identification. The patient should verbally confirm their name and date of birth with the person receiving the sample from them and sign or electronically confirm that the sample is theirs.
7.6	All semen or sperm handling steps should include appropriate laboratory witnessing and label checks, this is mandatory for the chain of custody. The time and place of collection and the time interval between collection and

	analysis/preparation should be noted for semen, as should times for each subsequent processing step.	
7.7	Sperm analysis and preparation should start as soon as possible when therapeutic use is intended (within 30 min if possible to perform a ”wet check”) as prolonged sperm exposure to seminal plasma may affect subsequent sperm quality. The seminal plasma osmolality also changes over time making sperm more prone to osmotic shock when being moved to media.	(Holmes et al., 2020)
7.8	The following data on sperm preparation should be documented: • sample origin (ejaculate/epididymal/testicular, donor/partner, fresh/frozen) • preparation method and media used • pre- and post-preparation sperm parameters	
7.9	An appropriate sperm preparation method should be chosen according to the characteristics and origin of individual samples. The swim-up technique and discontinuous density-gradient centrifugation are historically the most frequently used and widely accepted. Any preparation method including alternative methods should be validated by the clinic to determine the optimum sperm parameters required. This will include for achieving fertilisation but later monitoring should also be in place to ensure any method selected also does not negatively impact the later pregnancy, miscarriage or live birth rates (as these are also known to be independently influenced by sperm factors).	(West et al., 2022)
7.10	In case of azoospermia on the day of oocyte retrieval and in the absence of a back-up sample, alternative sperm retrieval procedures or oocyte cryopreservation should be considered.	
7.11	Where the ejaculate is beneath 1.4 ml, or totally absent when ‘dry orgasm’ occurs then (partial) retrograde ejaculation may be suspected. If more sperm are required than available or in case of complete retrograde ejaculation, a method of sperm retrieval with bicarbonate can be used (see Supplementary Data 3). NOTE: Potential treatments are available to reverse the retrograde ejaculation phenotype but are beyond the scope of this guideline (e.g.; *EAU Guidelines. Edn. presented at the EAU Annual Congress, Madrid 2025. ISBN 978-94-92671-29-5*.). NOTE: More complex methods of maintaining urine osmolality as well as pH for improved sperm handling have been suggested but these have not as yet entered routine use or been widely tested. Older techniques involving	(World Health Organization, 2021) (Crich and Jequier, 1978) (Okada et al., 1998, European Association of Urology, 2025) (Aust et al., 2008, Hotchkiss et al., 1954, Jefferys et al., 2012)

	catheterisation of the bladder also lack robust evidence of additional benefit and require more invasive care so are not recommended for routine use.	

406

407 8. Oocyte retrieval and processing

408 Oocyte are very sensitive cells, requiring rapid handling and strict control of culture conditions.
409 Special attention should be given to keeping the appropriate physical parameters during the
410 oocyte retrieval.

	Temperature of heated stages and incubators, CO2 and O2 concentration of incubators should be regularly checked.	
	Before starting the procedure, the embryologist should verify that the patients have signed the corresponding informed consent and that the virological tests are valid.	
8.1	**An identity check before the oocyte retrieval is mandatory**. Each laboratory should have in place a protocol to ensure patient's identification. The embryologist in charge of the procedure should check the patient's name and date of birth in the presence of a witness. The identity check should include the labelling of dishes and tubes in contact with the collected specimens and should be documented. The identity check should also be verified by a witness (double witnessing).	
8.2	**Appropriate equipment should be in place to maintain oocytes close to 37°C**. Flushing medium, collection tubes and dishes for identifying oocytes should be pre-warmed. Media buffered to keep an appropriate pH in air should be used under strict temperature control as temperature changes in buffered media can make pH conditions unstable, with negative consequences on oocytes. Alternatively, the procedure should be performed using a gassed chamber or in media overlaid with oil.	(Agarwal et al., 2022, Gatimel et al., 2020, Sciorio and Rinaudo, 2023)
8.3	Follicular aspirates should be checked for the presence of oocytes using a stereomicroscope and heated stage, usually at 8-60x magnification. Exposure of oocytes to light should be minimised.	(Bódis et al., 2020)
8.4	The time between oocyte retrieval and start of culture should be minimal.	
8.5	**Timing of retrieval, number of collected oocytes, operator and witness should be documented. A record should also be kept of the lot and type of disposables and media used.**	
8.6	The oocyte retrieval rate is the ratio between the number of oocytes recovered over the number of ovarian follicles seen at ultrasound or aspirated. The expected range is 80–95% of follicles measured or aspirated in stimulated cycles. Values outside the expected range could be important to evaluate (ovarian stimulation protocol, follicle assessment, oocyte pick-up technique).	(ESHRE Working Group on Ultrasound in ART et al., 2019, ESHRE Special Interest Group of Embryology and Alpha Scientists in

		Reproductive Medicine, 2017)

411

412 # 9. Insemination of oocytes

413 Oocytes can be inseminated by conventional IVF or by ICSI. The insemination/injection time
414 should be decided based on the number of hours elapsed from ovulation trigger and/or oocyte
415 retrieval, also keeping in mind that fertilisation will need to be checked 16-17h later (Working
416 Group on the update of the ESHRE/ALPHA Istanbul Consensus et al., 2025).

417 There is no evidence regarding the advantages of ICSI for non-male factor infertility in terms
418 of pregnancy outcomes, live birth rates (LBRs), and cumulative LBRs. In addition, ICSI is
419 associated with higher costs compared to conventional IVF treatment. There may be specific
420 treatments where ICSI is indicated, such as for thawed oocytes in pre-implantation genetic
421 testing (PGT) cycles (ESHRE Add-ons working group, et al., 2023).

422 ## 9.1. Conventional IVF

9.1.1	The number of progressively motile sperm used for insemination should be sufficient to optimise the chance of normal fertilisation. Traditionally, a progressively motile sperm concentration ranging between 0.1 and $0.5x10^6$/mL is used. Novel preparation methods such as microfluidic separation, may result in reduced concentration but superior sperm quality. Novel methods should be validated by the clinic to determine the optimum sperm parameters required for fertilisation.	(ESHRE Add-ons working group, et al., 2023)
9.1.2	**An identity check before the oocyte retrieval is mandatory**. Each laboratory should have in place a protocol to ensure the identification of gametes at the time of insemination procedure. This step should also be verified by a witness (double witnessing).	
9.1.3	Records should be kept of the time of insemination, the operator and the concentration of progressively motile sperm used.	
9.1.4	Co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a short co-incubation (2-4h) can be considered following internal validation.	(Fan et al., 2023).

423

424 ## 9.2. ICSI procedure

9.2.1	Preparation of oocytes for ICSI. • When removing cumulus cells from oocytes, hyaluronidase concentration and exposure should be kept to a minimum.	
	• In order to prevent oocyte damage, pipettes with appropriate lumen size should be used and vigorous pipetting avoided.	

- After denudation, oocytes should be thoroughly washed to remove traces of hyaluronidase.
- The maturation stage of the oocytes should be recorded.
- Current evidence does not suggest that denudation should be performed at a specific time between oocyte recovery and ICSI.

9.2.2 Preparation of oocytes for ICSI.

- **An identity check before the oocyte retrieval is mandatory**. Each laboratory should have in place a protocol to ensure the identification of gametes at the time of insemination procedure. This step should also be verified by a witness (double witnessing).
- Records should be kept of the injection time (start and end of the procedure) and the performing operator.
- The duration of sperm identification and immobilisation followed by injection should be minimised.
- The number of oocytes transferred to the injection dish should relate to operator's skills and sperm quality.
- During ICSI, the following points are important:
    - Only mature oocytes should be injected.
    - Oocyte morphology should be recorded. Oocyte dysmorphisms such as large perivitelline space, localised granularity and smooth endoplasmic reticulum aggregates are associated with diminished clinical success. Giant oocytes should not be injected. (Working Group on the update of the ESHRE/ALPHA Istanbul Consensus, et al., 2025)
    - Morphologically normal, motile sperm should be selected.
    - Tail membrane breakage should be posterior to the midpiece, and performed immediately before the injection of each individual oocyte.
    - Polar body should be away from the injection site.
    - Oolemma rupture should be assured prior to sperm injection.
- Appropriate temperature and pH should be maintained during injection. Viscous substances such as PVP can be used to facilitate sperm manipulation. In case of only immotile sperm cells, a non-invasive vitality test can be used to select viable sperm for injection.
- After injection, oocytes should be washed prior to culture.

9.2.3 Artificial oocyte activation is currently not recommended for routine clinical use. It is however recommended for complete activation failure (0% 2 pronuclei (PN)), very low fertilization (<30%), or globozoospermia (ESHRE Add-ons working group, et al., 2023)

425

10. Scoring for fertilisation

10.1	Fertilisation assessment should be performed under high magnification (at least 200x), using an inverted microscope equipped with Hoffman or equivalent optics (or a suitable time-lapse microscopy device), in order to verify pronucleus (PN) number and morphology.	
10.2	All inseminated or injected oocytes should be examined for the presence of PN and polar bodies at 16-17h post insemination (time suggested in case of static observation) in both conventional IVF and ICSI cases. For conventional IVF, cumulus cells should be removed and fertilised oocytes transferred into new dishes containing pre-equilibrated culture medium. The use of 2PN zygotes is the standard of practice. However, as reported below (10.3 and 10.4) from the Istanbul consensus Update, the use of specific categories of atypically fertilised zygotes may be considered.	(Working Group on the update of the ESHRE/ALPHA Istanbul Consensus, et al., 2025)
10.3	By static observation, pronuclei may not be seen at fertilisation check, and yet normal embryo development can occur. This may be explained by time-lapse technology data, which show that a significant proportion of 2PN zygotes undergo PN breakdown at earlier times than the above-recommended fertilization check interval. In such cases, the presence of the second polar body should accompany 2PN fertilisation and therefore be used as a scoring criterion. While these zygotes may be incorrectly categorized as 0PN, if cultured, they may produce normal laboratory and clinical outcomes. Therefore, the term unfertilized or '0PN' should not be used in these cases. Instead, 'PN not observed' may be a more suitable alternative for zygotes undergoing normal development without confirmation of fertilisation.	(Working Group on the update of the ESHRE/ALPHA Istanbul Consensus, et al., 2025)
10.4	Preliminary PGT-A data suggest that a significant proportion of 1PN zygotes may be biparental diploid. In addition, a growing number of studies have reported normal live births from 1PN zygotes derived from both ICSI and IVF cycles. Collectively, this evidence supports cautious clinical use of 1PN zygotes, combining blastocyst culture and, if available, PGT-A technology appropriate for biparental diploidy assessment. 2PN zygotes with one extra micropronucleus (2.1PN) are relatively rare. However, they also may have a diploid genotype and lead to apparently normal live births. Their clinical use may be considered, especially if associated with PGT-A technology. In general, the possible clinical use of 1PN and 2.1PN zygotes should be discussed with the clinical team and the patient and governed by an internally approved policy.	(Working Group on the update of the ESHRE/ALPHA Istanbul Capalbo et al., 2024, Consensus, et al., 2025)

Pronuclear pattern	Laboratory recommendations	Clinical use without PGT-A technology appropriate for biparental diploidy assessment

PN not observed but presence of a second polar body **(often incorrectly defined as "0PN")**	Culture to blastocyst stage to discriminate from rare non-fertilized oocytes undergoing early abortive cleavage (Coticchio et al., 2025).	Yes
1PN	Culture to blastocyst stage and, if available, PGT-A technology appropriate for biparental diploidy assessment	Yes, with caution, after discussion with the clinical team and the patients and governed by an internally approved policy.
2.1PN (2PN with a small micropronucleus)	Culture to blastocyst stage **AND** PGT-A technology appropriate for biparental diploidy assessment (Girardi et al., 2024).	Yes, with caution, after discussion with the clinical team and the patients and governed by an internally approved policy.
3PN	Culture to blastocyst stage **AND** PGT-A technology appropriate for biparental diploidy assessment	Not recommended as a routine practice, but admissible in pilot clinical studies

11. Embryo culture

In order to optimise embryo development, fluctuations of culture conditions should be minimised. Precautions should be taken to maintain adequate conditions of temperature, pH and osmolality during culture dish preparation to protect embryo homeostasis during culture and handling (Korakaki et al., 2020, Sciorio and Rinaudo, 2023, Wale and Gardner, 2016). Owing to our current inability to assess the in vivo reproductive tract environment in real time, the ideal levels of these variables have been arbitrarily set (Ng et al., 2018). Nevertheless, monitoring of adequate culture circumstances such as temperature, gas concentrations in the incubator during embryo culture is good practice and should be systematically performed (Gatimel, et al., 2020, Mestres et al., 2022).

11.1 Different approaches or culture systems can be used in order to optimise embryo development.

11.1.1	A culture medium designed for embryo development should be used. There is currently insufficient evidence to recommend either sequential or single-step media as being superior for the culture of embryos to the blastocyst stage, both in terms of ongoing pregnancy rate, live birth rate and neonatal outcome.	(Dieamant et al., 2017, Sacha et al., 2022, Sfontouris et al., 2016, Sonigo et al., 2024)
11.1.2	Oil overlay minimises changes to temperature, pH and osmolality. Its use reduces, but not eliminates, evaporation and consequent changes in osmolality, or fluctuations in temperature and pH. These changes depend on several factors, including microdrop size, type of culture dishes, incubator atmosphere and intrinsic oil properties. There are no data confirming that the use of a high-viscosity oil confers better protection against evaporation than light oil, even when the rate of blastocyst formation is evaluated.	(Bossi et al., 2023, Korakaki, et al., 2020, Mestres et al., 2021, Scarica et al., 2022) (Murray et al., 2025, Watanabe et al., 2025)

11.1.3	The type and number of incubators should be appropriate to the workload.	
11.1.4	For traceability purposes, single embryo culture is advisable. If group culture is used, the size of the droplet and the number of embryos per droplet should be considered and validated.	
11.1.5	To limit the damage caused by oxidative stress, low oxygen concentration should be used.	(Gardner, 2016, Herbemont et al., 2021, Slatinšek et al., 2025)

11.2 Embryo quality assessment

Embryo quality assessment records should include the operator(s), date and time of assessment and embryo morphological characteristics. (For more detailed information, please see (Working Group on the update of the ESHRE/ALPHA Istanbul Consensus, et al., 2025)).

12. Biopsy procedure

PGT cycles currently represent 6.9% of initiated IVF + ICSI and frozen embryo transfer (FET) cycles (EIM for ESHRE et al., 2023) translating in a progressively increasing workload involving embryo biopsy in the embryology laboratory. Embryo biopsy is a technically demanding procedure, requiring a high degree of operator skill and laboratory organisation.

Technical recommendations for embryo biopsy and tubing are covered in detail in the ESHRE PGT Consortium and SIG Embryology Good Practice Recommendations for polar body and embryo biopsy for PGT (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, et al., 2020).

12.1. Method of fertilisation

ICSI is the preferred method for PGT, as it reduces the risk of contamination from both maternal and paternal sources—specifically, residual cumulus cells and excess sperm adhering to the zona pellucida (ZP). To minimize maternal contamination in biopsy samples, it is essential to thoroughly remove cumulus cells during denudation and rinse oocytes before ICSI.

12.2. Embryo culture

Usual embryo culture principles apply, while paying extra attention to avoid inadvertent exogenous contamination during embryo handling. Culture in a time-lapse incubator will limit the embryo exposure to sub-optimal conditions and will facilitate the decision on the optimal time of biopsy for each embryo.

Following biopsy, embryos should be placed individually in multiple-well dishes or droplets in separate dishes, to prevent mixing of embryos due to accidental movement during handling (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, et al., 2020).

12.3. Embryonic stage of biopsy

The blastocyst biopsy is the current standard practice. The blastocyst represents a more efficient stage for biopsy because it provides more cells for genetic analysis, it is less sensitive to possible damage as the inner cell mass (ICM) is unaffected, and extended culture excludes through deselection embryos of low viability unable to form a blastocyst. It is recommended that approximately 5-10 trophectoderm (TE) cells are biopsied, according to the stage of expansion and number of cells of the TE layer (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, et al., 2020). A retrospective study comparing the size of TE biopsies from the PGT program with the size of TE biopsies from donated embryos whose cells were fixed to count them, showed that removal of approximately 10 TE cells is associated with lower implantation rates compared to removal of 2-6 TE cells (Guzman et al., 2019).

12.4. Method of zona opening

The use of a guided non-contact laser beam is the preferred method of ZP opening, compared to mechanical and chemical zona drilling methods. The laser can be adjusted to accurately create a ZP opening of the desired size, avoiding the damage of embryonic cells.

Blastocyst biopsy strategies entail ZP opening either on Day 3/Day 4, artificial hatching at the blastocyst stage, or simultaneous ZP opening and TE biopsy (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, 2020).

Simultaneous ZP opening and TE biopsy was associated with higher live birth rates per euploid single embryo transfer (SET) compared to Day 3 hatching, while miscarriage rates were similar (Cimadomo et al., 2023).

12.5. Technique of cell removal

For trophectoderm biopsy, aspiration and excision with a laser can be used, or aspiration in combination with mechanical detachment of the TE cells (aka flicking) (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, et al., 2020).

12.6. Tubing

Tubing should be performed in a separate area within the embryology laboratory or in a different tubing laboratory, with strict measures to minimise contamination and sample loss.

Personnel should wear protective clothing, including a clean surgical gown, a hair cover or hat, a face mask covering both nose and mouth, and preferably shoe covers or dedicated footwear. Gloves should be worn at all times and replaced frequently.

Detailed recommendations on tubing are outlined in the ESHRE Good Practice Recommendation paper (ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group, et al., 2020).

12.7. Rebiopsy and refreeze

Inconclusive diagnosis due to DNA amplification failure or low-quality results may occur in 2-6% of TE biopsies. In these cases, an additional round of warming, biopsy, and cryopreservation is required to obtain a genetic diagnosis (Li Piani et al., 2025). Double cryopreservation with

single biopsy and double biopsy with double cryopreservation were both associated with lower live birth rates, lower clinical pregnancy rates and higher miscarriage rates compared to single biopsy and single cryopreservation (Bartolacci et al., 2025, Guarneri et al., 2024, Li Piani, et al., 2025, Vireque et al., 2025, Yang et al., 2025). However, despite the reported detrimental effect, double biopsy and double cryopreservation when necessary may increase the total number of euploid blastocysts available for transfer. In addition, neonatal outcomes and the health of babies born so far, albeit in small numbers, did not indicate any adverse effects (Li Piani, et al., 2025).

13. Embryo transfer

Based on the ESHRE Guideline on the Number of Embryos to Transfer, single embryo transfer is recommended to avoid multiple pregnancies. The decision on the number of embryos to transfer should be based on embryo quality and stage of development, female age, ovarian response and rank of treatment. Single embryo transfer is recommended in most cases. However, it is advisable not to transfer more than two embryos (ESHRE Guideline Group on the Number of Embryos to Transfer et al., 2024).

13.1	For the transfer procedure, the patient records should include: • Date and time of embryo transfer. • Name of the operator. • Name of the practitioner performing the transfer. • Number, developmental stage and quality of embryo(s) at the time of transfer. • Type of catheter used. • Fate of supernumerary embryos. • Details about the procedure, e.g. presence of blood, retained embryo(s).	
13.2	It is recommended that the room in which the embryo transfer is performed, is in close proximity to the laboratory. If the laboratory is some distance from the embryo transfer room, arrangements should be made to maintain temperature and pH whilst transporting embryos.	(Gurner et al., 2024, Macklon et al., 2021)
13.3	A double identity check of the patient, the patient file and the culture dish(es) is mandatory immediately before the transfer. This patient identity check should be documented.	
13.4	Catheter loading and technical aspects of the ET are described in D'Angelo et al., 2022. In summary: There is insufficient evidence to suggest that flushing the loading catheter before transfer eliminates potential toxic agents benefiting clinical outcomes.	(D'Angelo et al., 2022) (Maldonado Rosas et al., 2022)

	There are few references in favour to perform an afterload (double step) versus direct (single step) catheter techniques.	(Cirillo et al., 2023)
	There are few references in favour to the softness as well as in relation to the material of the catheter used.	(2017, Ebner et al., 2001)
	There is insufficient evidence to suggest the superiority of the air-fluid or fluid-only methods during embryo loading, and echogenic catheters available nowadays are easily visible by echography.	(Abou-Setta et al., 2007, D'Angelo, et al., 2022, Ebner, et al., 2001)
	Loading the catheter directly from the culture media under oil versus loading from a transfer dish without oil presents no differences in pregnancy rates.	(Halvaei et al., 2013)
	The use of specific transfer media enriched with adherence compound as hyaluronic acid has demonstrated to be beneficial to the LBR in fresh transfers, despite no effect has been demonstrated in frozen embryo transfer (FET). Due to the high variability of the studies and the increased multiple PR analysed, the Good Practice Recommendations on add-ons in reproductive medicine recommends their use and the need of further studies.	(ESHRE Add-ons working group, et al., 2023, Heymann et al., 2020, Yung et al., 2021)
	The use of small volume of medium (10-20 µl) is recommended despite other studies increased the transfer volume (up to 35-45 µl) with controversial results in clinical outcomes.	(Ebner, et al., 2001, Montag et al., 2002, Omidi et al., 2015, Sigalos et al., 2018)
13.5	When ET is completed, the catheter has to be checked for possible retained embryo(s). The embryo should be reinjected immediately, preferably with a new catheter, after embryo retention.	(2017, D'Angelo, et al., 2022)

14. Cryopreservation

Cryopreservation can be performed for gametes, embryos and tissues.

14.1	Different cryopreservation approaches, including slow freezing and vitrification, can be used according to the type of biological material.	
14.1.1	For sperm, slow freezing (passive or controlled) is still the method of choice.	
14.1.2	For oocytes, pronuclear and cleavage-stage embryos and blastocysts vitrification is the more effective method and is recommended.	(Golakov et al., 2018, Hajek et al., 2021, Rienzi et al., 2017b)

14.1.3	For ovarian tissues, slow freezing and vitrification have shown similar results.	(Antonouli et al., 2023, Cariati et al., 2023, Hončová, 2023, Kong et al., 2025, Sugishita et al., 2021)
14.1.4	For testicular tissue, not expected to contain sperm, slow freezing is recommended (passive or controlled). Where tissue potentially may contain sperm, it is recommended to analyse the testicular sample to determine if sperm is present. If sperm are identified, a protocol for sperm cryopreservation should be favoured over testicular tissue cryopreservation. If there are no sperm present, testicular tissue cryopreservation should be favoured. Alternatively, part of the tissue could be cryopreserved using a protocol aimed at preserving spermatogonia and the other part to preserve sperm.	(ESHRE FP for Boys Working Group et al., 2025)
14.1.5	(Semi-)Automated vitrification has been shown to have similar survival, embryo development and pregnancy results as manual vitrification, but yet needs to be further evaluated in (large) randomised studies.	(Gatimel et al., 2021, Hajek, et al., 2021, Miwa et al., 2020)
14.1.6	Supernumerary embryos may be cryopreserved individually, donated to research or discarded, according to their quality, patient wishes and national legislation.	
14.2	In order to minimise any risk of transmission of infection via LN2:	
14.2.1	Contamination of the external surface of cryo-devices should be avoided when loading them with samples.	
14.2.2	Safety issues have been raised regarding direct contact of the biological material with the LN2; however, the risk of contamination from LN2 or from other biological samples is considered to be negligible. Closed devices have shown to have similar cryosurvival, embryo utilization and pregnancy rates as open devices. Laboratories should make decisions based upon risk analysis and regulations in place.	(Cai et al., 2018) (De Munck et al., 2016, Porcu et al., 2021, Sugishita, et al., 2021)
14.2.3	Specimens from sero-positive patients should be stored in high-security closed devices. Dedicated vapour phase tanks are recommended.	
14.3	At cryopreservation, documentation on biological material should include: • Patient consent form;	(European Committee on Organ Transplantation, 2026)

	• Labelling of devices;	
	• Cryopreservation method;	
	• Date and time of cryopreservation;	
	• Operator;	
	• Sperm quality;	
	• Embryo quality and stage of development;	
	• Number of oocytes or embryos per device;	
	• Number of devices stored per patient;	
	• Location of stored samples (tank, canister);	
	• Unique code, or SEC codes when applicable.	
14.4	Cryo-devices should be clearly and permanently labelled with reference to patient details, treatment number and/or a unique identification code.	
14.5	A periodic inventory of the contents of the cryobank is recommended, including cross-referencing contents with storage records. In some EU countries, national laws regulate a maximum legal storage period for gametes and/or embryos.	
14.6	At thawing (warming), documentation on biological material should include:	
	• Thawing method;	
	• Date and time of thawing;	
	• Operator;	
	• Post-thawing sample survival and quality.	
14.7	A double-check of patient identity and embryo number is recommended in the following steps: transfer of samples into labelled cryo-dish, loading of the labelled device, deposition in the cryobank, removal from the cryobank. This patient identity check should be documented.	
14.8	During storage, handling and transport of cryopreserved material, care should be taken to maintain adequate and safe conditions. Temperatures should never rise above -140°C. During storage and transport, temperature loggers or indicators should be used.	(European Committee on Organ Transplantation, 2026)
14.9	In case of transport of cryopreserved SoHO materials, it needs to be clearly documented which centre is responsible from release to receipt. The distribution to another establishment should be authorised by a responsible person and restricted to authorised entities. Written agreements should be in place. Reports should be established, and include: • Name and identification of distributing and receiving parties; • Name and identification of transporting party; • Type of sample and packaging; • Time and date of distribution and delivery; • Patient identification codes;	(European Committee on Organ Transplantation, 2026)

- Any possible incidents occurring during the transport.

15. Contingency and Emergency plan

As a part of the clinic's general backup plan, all IVF laboratories should develop and implement a contingency (Cairo Consensus and Alpha Scientists In Reproductive, 2025) and emergency plan (2021, Goldman et al., 2022) with specific procedures in order to safeguard patients' reproductive material as well as the laboratory staff in case of unexpected events. Contingency planning is a proactive strategy to ensure continuity of IVF procedures when unforeseen and abrupt circumstances occur. It will establish alternative ways to maintain service quality. Emergency planning on the other hand is a reactive protocol for immediate response to high risk treats and real disasters. Periodic revision of these plans is necessary together with emergency preparedness drills for the staff.

Collaboration with neighbouring clinics should be sought in both contingency and emergency planning. Plans are therefore discussed and protocols written down in service level agreements with these back-up centers.

15.1 Contingency plan

A contingency plan or continuity plan is essentially a plan 'B' and it entails a predefined set of actions to take when unexpected event disrupts normal operations. This plan's purpose is to minimize damage and restore laboratory processes quickly. Events that would trigger the contingency plan could be technological failures in the laboratory (short time power outage, sudden tank failure, ..), supply chain disruptions, IT disruption or even sudden absence of critical staff.

A contingency plan for the IVF laboratory contains at least the following items (please find a comprehensive list in the Cairo Consensus): (Cairo Consensus and Alpha Scientists In Reproductive, 2025)

15.1.1 Alarm system

The laboratory should have an alarm system to notify staff of malfunctioning or failure of critical equipment or occurrence of adverse environmental conditions in the laboratory.

15.1.2 Facilities

- Electricity: loss of electrical power should be compensated by generators or uninterrupted power supply (UPS) systems. It could be useful to have a separate UPS unit specifically for the lab in case the hospital system fails or needs this power to keep operating theatres running and live support for patients.
- LN2: in case of failure of automatic supply lines, tanks should be filled manually. A reserve LN2 tank should be available and knowledge on how low tanks can keep their temperature without being filled.

	• Gasses: have a strategy to minimize disruption in gas supply and have if it happens, a backup plan.	
15.1.3	Equipment • In case of power failure, critical equipment should be prioritised. • A second item of critical equipment should be available if the first item fails. All reserve equipment should be fully validated and ready for use. • Freezer (-20°C) and refrigerator: back-up cooled freezers and refrigerators should be available.	
15.1.4	Contingency culture / freezing protocols Based on the remaining facilities in the laboratory, it could be necessary to prioritize vitrification of material to safeguard all the reproductive material.	(Sharma et al., 2024)
15.1.5	Medical records: Records to identify the ownership of human tissue should be kept on a secure web server.	
15.1.6	Staff Have sufficient and well-trained staff and have backups for key personnel.	
15.1.7	Consumables Assess the amount of consumables needed to be able to execute contingency and emergency laboratory protocols. Evaluate the possibility to reach out to other suppliers if necessary.	

Aside from several technical and laboratory specific items, the contingency plan describes the strategy of the critical asset protection of reproductive material, it contains a communication protocol and staff roles in case of unexpected events. The resumption of laboratory operation is part of the plan and several examples of scenarios can make the theoretical aspects better understandable. Flowcharts showing 'event-> response-> recovery' steps can be insightful.

15.2 Emergency plan

In light of global climate change, natural disasters like flood or earthquakes can happen in more geographic regions in the world than was the case before. Aside from these, other non-natural disasters, like fire caused by electrical faults, chemical reactions of gas leaks – which can also be a result of natural disasters- can have a huge impact on the general contingency plan, as compliance to fire safety protocols will require a complete shutdown of power. Such Emergency Power Off (EPO) is specifically designed to shut down electrical equipment, including UPS systems during a fire. This is a safety measure to prevent electrical shock risks when a fire suppression system, like sprinklers, or use of fire extinguishers by fire men, are activated. In such a scenario, all electrical circuits, equipment and backup generators will cease to operate until the fire is under control. In such scenario, there is very little time to act and safeguard embryos. It is important to know how long incubators can last without any power whatsoever and how to prioritise moving or freezing samples if there is time to do this and if it is safe

considering the safety of the staff. Not only a complete loss of all electricity but also the toxicities of burning material in proximity of an IVF laboratory will have an impact on the environment in which embryos reside and staff operates (Kornfield et al., 2024). An immediate reaction to such a crisis is the well-being of staff in the IVF laboratory and to evacuate the lab immediately. In a scenario where the laboratory is still reachable and provided that your emergency crisis manager gives green light to enter the lab, an emergency move of embryos in culture or emergency vitrification of reproductive material could be the only remaining option (Song et al., 2023).

An emergency plan for the IVF laboratory contains at least:
• Guidance on evacuation procedures.
• Communication channels and clarity of chain of command.
• Emergency contact information.
• Decision making on treatment continuation or cessation of patients and/or off-site treatments of patients in neighbouring centers.
• Critical actions for the lab if entering is deemed safe by chain of command including moving embryos in culture to other incubators on a secure location and/or emergency vitrification protocol with details on the labelling of straws / vials and record keeping of them.
• Critical actions for the cryostorage if entering is deemed safe by chain of command including evacuation of tanks or temporary on-site retaining for a pre-defined period.
• What to do the day after.
• What to communicate the day after.
• Strategy for startup of the IVF laboratory (partial or complete).

In the likelihood of a temporary shutdown of the lab, it is necessary to have an agreement with other laboratories in the area to transfer reproductive material and medical records if necessary.

Discussion

The updated ESHRE Recommendations on Good Practice in IVF laboratory serve as a comprehensive guide to all procedures performed within the IVF laboratory, aiming to promote the highest standards of safety, quality, and effectiveness in MAR. The recommendations in this Good Practice Recommendations paper are supported by data from the literature, if available, and the expertise of the working group. Since the 2015 version of the Good Practice in the IVF labs guideline was published, ESHRE has developed a standardised methodology for the development of Recommendations for Good Practice document (Vermeulen, et al., 2019). This updated Recommendations on Good Practice in IVF Laboratories document was therefore developed using this methodology, rather than adhering to the evidence-based guideline format, which is less suited.

The working group reviewed the 2015 version of the Good Practice in the IVF labs guideline, identifying knowledge gaps and chapters in need of a more elaborate guidance. Hereto, the section on embryo culture and transfer was split into two separate chapters, the section on sperm preparation was expanded to cover all general andrological procedures and a new section on biopsy procedure was introduced.

Even though the 6th edition of the EDQM guidance was not published yet at the time of publication of this Good Practice Recommendations document, the working group tried to take expected changes in EDQM guidance into account, based on the stakeholder version of the 6th edition. The new SoHO regulation, recently published, describes a required QMS. Personnel, organisation, materials and documentation are defined within an accurate control system. Moreover, measures to enhance traceability throughout the lifecycle of SoHO are reinforced. MAR centres must comply with traceability requirements of SoHO from the donor to the recipient and vice versa.

Important amendments were made to the staffing section of the guidance document. The working group wanted to convey the message that the work load of an embryologist encompasses more than handling embryos and that it is important that these other tasks are taken into account when determining the number of staff needed in the IVF laboratory. It is however not possible to define an ideal number of staff, as this is highly dependent on the set-up of staff and different logistics and workflow of the laboratory. Still, the working group highlighted some attention points and published calculation tools.

The most significant innovation in this document can probably be found in the chapter on scoring for fertilisation, based on the newly released updated Istanbul Consensus (Working group on the update of the ESHRE/ALPHA Istanbul Consensus, et al., 2025).

In conclusion, the ESHRE IVF labs working group updated the recommendations on the general organisation of an IVF laboratory (staffing and direction, quality management, laboratory safety), and on the specific aspects of the procedures performed in IVF laboratories (Identification of patients and traceability of their reproductive cells, consumables, handling of biological material, oocyte retrieval, sperm preparation, insemination of oocytes, scoring for fertilisation, embryo culture and transfer, cryopreservation and emergency procedures), based on the best evidence available. It is anticipated that the recommendations will undergo further revisions as more evidence becomes available or any other approaches are established.

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Supplementary information

- S1: Abbreviations
- S2: List of participants to the stakeholder review
- S3: Sperm retrieval for retrograde ejaculation

Supplementary Data 1: Abbreviations

	Abbreviation
ART	Assisted Reproductive Technology
CAPA	Corrective and/or Preventive Actions
CPD	Continuing Professional Development
DOPS	Direct Observation, of Procedural Skills
EPO	Emergency Power Off
EQC	External Quality Control
EU	European Union
EWS	Electronic Witnessing System
FET	Frozen Embryo Transfer
HEPA	High Efficiency Particulate Air
ICM	Inner cell mass
ICSI	Intracytoplasmic sperm injection
IQC	Internal Quality Control
IVF	In Vitro Fertilisation
KPI	Key Performance Indicator
LBR	Live birth Rate
LN	Liquid Nitrogen
MAR	Medically assisted reproduction
MDR	Medical Device Regulation
PGT	Pre-implantation Genetic Testing
PI	Performance Indicator
PN	Pronucleus
PVP	Polyvinylpyrrolidone
QMS	Quality Management System
SEC	Single European Code
SET	Single Embryo Transfer
SoHO	Substances of Human Origin
SOP	Standard Operating Procedure
TE	Trophectoderm
TL	Time-Lapse
UPS	Uninterrupted Power Supply
VOC	Volatile Organic Compounds
ZP	Zona Pellucida

Supplementary Data 3: Sperm retrieval for retrograde ejaculation

Where the ejaculate is beneath 1.4 ml (World Health Organization, 2021), or totally absent when 'dry orgasm' occurs then (partial) retrograde ejaculation may be suspected. If more sperm are required than available or in case of retrograde ejaculation, a method of sperm retrieval with bicarbonate can be used.

Hereto, the patient is asked to be well hydrated, so that their urine is almost colourless (i.e. not a strong yellow colour – this may reduce osmolality of the urine sperm mix with but also reduces other deleterious effects of urine). It would be aimed to achieve this approximately 1h before orgasm / sample production. At this point (1h) the patient should take 4-5g sodium bicarbonate in 1 litre of water and 10-30 min prior to planned ejaculation time can urinate emptying bladder and test pH with the aim being to have alkalinised the urine to a more neutral pH. The patient can then have further liquids to drink in ratio of 200 ml / 2g bicarbonate drink if they so wish. There is some limited evidence that a full bladder may help increase any antegrade ejaculate component, but presuming processing of a retrograde sample urine neutralisation should be prioritised. The patient should be provided a minimum of two containers, one for any semen ejaculated and a second to fill immediately after ejaculation with 20-30 ml first pass urine. Further urine can be collected in more pots, but dependent upon intended treatment modality may not be required. The urine should be immediately processed by the laboratory via mixing with an equal volume of gamete handling media (containing serum) and centrifugation at 300 g for 10 min. Any semen can be processed by routine semen methods.