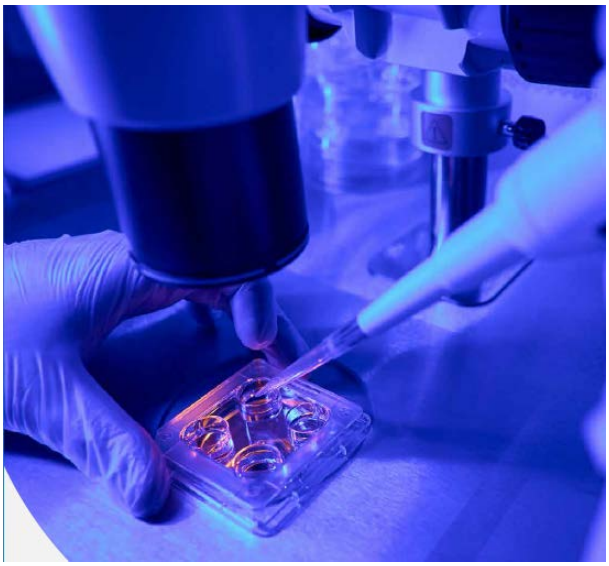

ESHRE recommendations on Good Practice in the IVF laboratory

UPDATE 2026

European Society of Human Reproduction and Embryology

April 2026

ESHRE Good Practice in the IVF lab working group

REVIEW REPORT

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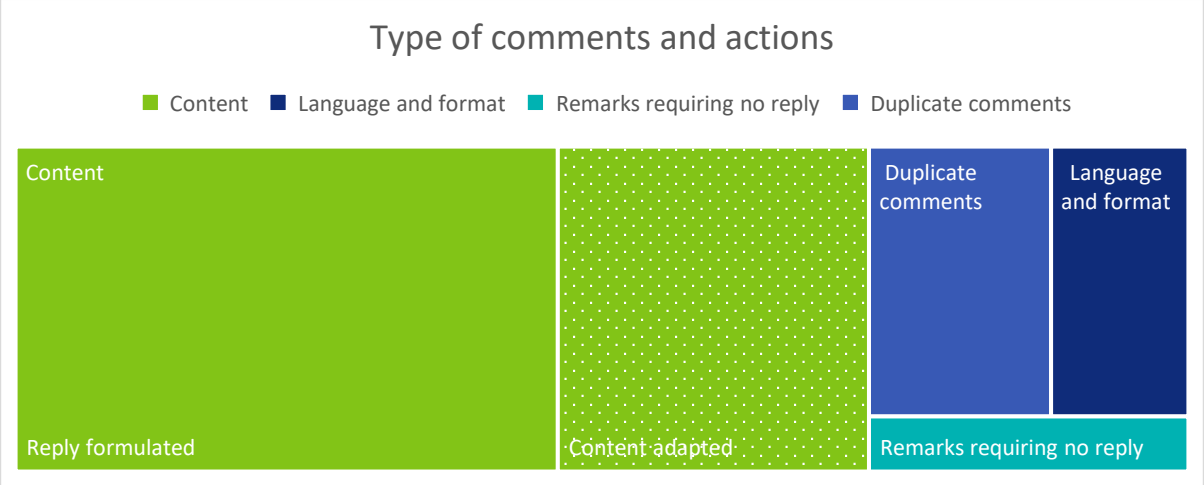
The draft of the ESHRE recommendations on Good Practice in the IVF laboratory was published for public review for 4 weeks, between 3 February and 3 March 2026.

This report summarises all reviewers, their comments and the reply of the working group and is published on the ESHRE website as supporting documentation to the Good Practice Recommendations paper.

During the stakeholder review, a total of 406 comments were received from 36 reviewers.

The comments were focussed on the content of the guideline (356 comments), language and format (40 comments), or were remarks that did not require a reply (12 comments). All comments to the language and format were checked and corrected where relevant.

The comments to the content of the paper (n=299, not taking into account duplicate comments) were assessed by the working group and where relevant, adaptations were made in the paper (n=109; 36.5%). Adaptations included revisions and/or clarifications of the text, and amendments to the recommendations. For a number of comments, the working group considered them outside the scope of the paper or not appropriate/relevant (n=190; 63.5%).



Experts that participated in the stakeholder review

The list of representatives of professional organisation, and of individual experts that provided comments to the Good Practice Recommendations paper are summarised below.

Reviewer	Country	Participation on behalf of (if any)
Lodovico Parmegiani	Italy	Individual
G. Sreekanth	India	Individual
AMM Wetzels	The Netherlands	Individual
Shridhar S Amanchi	India	Individual
Alexia Chatziparasidou	Greece	Individual
Aneta Macur	Poland	Individual
Liliana Ramos	The Netherlands	Individual
Chand Mohammad	India	Individual On behalf of an (inter)national organisation: ISAR consensus On behalf of a company: Rainbow IVF, Agra
Byron Asimakopoulos	Greece	Individual
Karin Rosenstein	Estonia	Individual
Sheryl Homa	UK	Individual
Durga Rao Gedela	India	On behalf of a company: Oasis Fertility
Fikret Gürkan Agircan	Germany	Individual
Alessio Paffoni	Italy	Individual
Alev Özer	Turkey	Individual
Hannah Park	Sweden	Individual
Keerti Singh	Barbados	Individual
Fayezi, Shabnam	Germany	Individual
Shivansh Jaiswal	India	Individual
Esther Baart	The Netherlands	On behalf of a company: Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam
Zuzana Holubcová	Czech Republic	Individual
Susanna Apter	Sweden	On behalf of a company: Vitrolife Group
Stefan Matik	North Macedonia	Individual
Irene Cuevas-Saiz	Spain	Individual
Sheila Mae Poulain	United Arab Emirates	Individual

Maria Filippa	Greece	Individual
Labadi Leila	Algeria	Individual
S.I.RU.	Italy	On behalf of an (inter)national organisation: S.I.RU. (Società Italiana della Riproduzione Umana)
Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)	Germany	On behalf of an (inter)national organisation: Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)
Koen Wouters	Belgium	Individual
Verena Nordhoff	Germany	On behalf of an (inter)national organisation: German Society of Reproductive Biology (AGRBM)
Samantha Wake	Estonia	Individual
Nadia Kazdar	France	Individual
Lars Björndahl	Sweden	Individual
David Morroll	UK	Individual

Reviewer comments and replies

NR	Reviewer	Page	Line	Comment	Action / Reply
Introduction					
7	Liliana Ramos	2	31 57	2 x Typo: (31)after literature, add a comma(“,”). (57)After documents add a comma and remove “and”	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	2	29-31	“What is the currently good practice…” Consider: “What is the **current** good practice in the IVF laboratory, based on the best available evidence in the literature and the expertise of the working group?”	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	2	32-33	“provide recommendations on all activities” Consider: “The updated ESHRE Recommendations on Good Practice for the IVF laboratory **provide recommendations for all activities** in the IVF Laboratory.”	The sentence is correct as is.
26	Sheila Mae Poulain	3	74-80	Use one consistently throughout when referring to the document title. Use **“Good Practice in the IVF Laboratory” instead of “Good Practice in the IVF Laboratories”**	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	3	97-98	Avoid repetition of “published” Consider: “This Good Practice Recommendations paper **was finalized** before the 6th edition of the EDQM guidance **was released**.”	The sentence was adapted.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	3-4	99-101	“The 6th Edition will be aligned with the new regulation and that compliance with this guide will equal compliance with the regulation.” It is unclear what is meant by “the (new) regulation”. Does this refer to the MDR?	This refers to the 6th edition of the EDQM.

NR	Reviewer	Page	Line	Comment	Action / Reply
1. Staffing and direction					
2	G. Sreekanth	/	/	Staff: discipline: It should be a part, and I recommend doing group work for higher success rates.	The working group does not understand the intention of the comment.
4	Shridhar S Amanchi	/	/	More guidance on minimum supervised case numbers for ICSI and embryo biopsy, along with periodic competency assessment, would help maintain consistent quality and patient safety.	We have referenced the requirements for the ESHRE certification programs.
4	Shridhar S Amanchi	/	/	Guidance on workload management and staffing levels would be useful, as heavy workload and fatigue can affect performance and safety in IVF labs.	It has been a deliberate decision by the WG not to give specific numbers of staffing levels. The minimum number depends on many factors, such as educational levels, types of treatments included, equipment, schedules (e.g. workdays/weekends) etc, so can differ widely between clinics. Suggestions on numbers can be found in the references. However, we have reinforced the numbers by Sharisawa by adding: "Such calculations can be used for guidance when calculating the number of staff needed."
19	Fayezi, Shabnam	/	/	Consider adding procedure-specific minimum competency numbers, certification recommendations (e.g., ESHRE pathways), and suggested revalidation intervals to support harmonisation across centres.	We have referenced the requirements for the ESHRE certification programs.
20	Shivansh Jaiswal	/	/	Consider inclusion of benchmarking principles for laboratory workload distribution and structured annual competency review. Even indicative workload guidance may support safer delegation models across centres of varying volume. Clarification of defined organizational structures and documented lines of responsibility would further enhance governance transparency and resilience, particularly in larger or network-based ART centres.	The working group considered this proposal, however, considered this outside the responsibilities of IVF laboratory staff.

NR	Reviewer	Page	Line	Comment	Action / Reply
20	Shivansh Jaiswal	/	/	Clarification of expected procedural exposure documentation through structured logbooks may improve reproducibility of competency assessment. Expanded emphasis on multidisciplinary training, including nursing and technical personnel, would support comprehensive staff development and reinforce a systems-based approach to quality improvement.	The working group considered this proposal, however, considered this outside the scope of this document.
29	S.I.R.U	/	/	We would suggest explicitly including patient counselling among the core competencies of the Clinical Embryologist. In current clinical practice, Embryologists increasingly interact directly with patients during several critical phases of the reproductive pathway (e.g., lab procedures planning, gamete and embryo handling explanations, unexpected outcomes, and treatment failure). Clearly defining counselling responsibilities would better reflect the multidisciplinary role of the Clinical Embryologist, promote consistent professional standards, and support patient-centred care by ensuring that patients receive accurate, transparent, and empathetic information throughout the most sensitive stages of their treatment journey. In addition, the draft guidelines do not appear to provide clear indications regarding the training and supervision of laboratory trainees and thesis students. Given the central role of Clinical Embryologists in academic and clinical laboratory environments, it would be advisable to acknowledge mentorship, teaching, and supervision activities as part of their professional competencies. Explicitly addressing this aspect would help standardize educational responsibilities, ensure high-quality training, and reinforce the role of the laboratory as both a clinical and educational setting.	The working group does not agree with the reviewer. Counselling should be done by health care professionals with training on the topic.
7	Liliana Ramos	4	118	Suggestion: Use “factor” instead of “parts”	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	4	120-122	“Appropriate human and logistic resources should provide an adequate climate” (to use **climate** is slightly vague). Consider: “Appropriate human and logistical resources should provide **conditions** that allow all laboratory tasks to be performed in timely and safe manner, ensuring patient safety and quality of care.”	The working group agrees with the reviewer, the sentence was adapted.

NR	Reviewer	Page	Line	Comment	Action / Reply
26	Sheila Mae Poulain	4	128-130	(**2015**) is unclear, if referencing a guideline then give full citation style.	The citation was adapted.
33	Samantha Wake	4	131	Some clinics have only lab manager (not director)	Then whoever is in charge of the lab has to take on these tasks… And our recommendation is that they should have a director…
26	Sheila Mae Poulain	5	135	Consider: "…a minimum of 6 years of **documented experience in human embryology**…"	The working group agrees with the reviewer, the sentence was adapted.
36	David Morroll	5	136	Though attainment of SCE certification may be desirable, national qualifications (such as FRCPath in the UK) may take precedence. The document should acknowledge this even if only "or other higher professional qualification recognized nationally" or similar. (See also 1.3 Clinical Embryologists 155)	Adapted as suggested by the reviewer.
7	Liliana Ramos	5	138	Suggestion: Add that the participation of a national or international CPD program (if ESHRE certified, they should be an active ESHRE CPD. If the director is not ESHRE certified, then they must participate in the CPD program of their own country)	Adapted as suggested by the reviewer.
25	Irene Cuevas-Saiz	5	142	Define and regularly update laboratory SOPs	Updating SOPs is part of a QMS.
26	Sheila Mae Poulain	5	142	Consider adding the following: • Development of the laboratory's long-term strategic plan aligned with clinic vision and growth. • Evaluation and adoption of emerging technologies (AI embryo scoring, time-lapse systems, microfluidics, automation platforms). • Contribution to clinical policy development (stimulation protocols, embryo transfer strategy, freeze-all policies). • Monitoring and approval of biopsy indications and PGT workflow integrity. • Review of difficult or adverse clinical cases (e.g., total fertilization failure, repeated implantation failure). • Development and approval of SOPs, policies, and document control systems.	Most of these points are already covered in the table and/or part of a QMS. A sentence was added to the paragraph above about promoting staff performance and wellbeing.

NR	Reviewer	Page	Line	Comment	Action / Reply
				• Benchmarking laboratory KPIs against national and international databases. • Publication and conference presentation leadership. • Development of competency assessment frameworks. • Implementation of digital audit trails and electronic witnessing systems. • Promotion of psychological safety and team well-being.	
27	Maria Filippa	5	142	It will be extremely useful to exist supplementary data on all of the Laboratory director responsibilities for reference and confirmation	This will be different in different clinics due to management set-up, regulations, etc..
36	David Morroll	5	142	second bullet point needs amending: Maintenance of, and safe and appropriate laboratory facilities and equipment according to European and/or national regulations.	The bullet point was adapted
36	David Morroll	5	142	fifth bullet point: Sufficient **NUMBERS OF** laboratory staff members with the appropriate skills.	The bullet point was adapted
25	Irene Cuevas-Saiz	6	1.2 1.3	Responsibilities of lab manager and clinical embryologists: In concordance with the rest of the document regarding biosurveillance and traceability, I would add among their resposabilities the communication to the lab director any adverse event that occurred in the lab and actively participate in the investigation (if needed)	This was added to the document.
26	Sheila Mae Poulain	5	147	Consider: "…a minimum of 3 years of **documented experience in human embryology**…"	The working group agrees with the reviewer, the sentence was adapted.
26	Sheila Mae Poulain	6	148	Consider adding the following: • Development and supervision of daily laboratory schedules (oocyte retrievals, ICSI, biopsy, freezing, FET prep). • Allocation of cases according to staff competency level. • Monitoring workload balance to prevent fatigue-related errors. • Oversight of emergency coverage planning and on-call systems. • Ensuring smooth coordination between embryology and andrology. • Daily monitoring of incubator parameters, gas supply, temperature logs, and alarm systems. • Oversight of media preparation, lot validation, and expiry tracking.	"Manager/supervisor" is a additional position, not required in all laboratories, and could have very different tasks in different clinics. Some of the tasks suggested are already in the current table although in a compressed version (i.e.. supervision, allocation, monitoring, coordination, improvement, etc), although sometimes in a different part, and we do not believe that

NR	Reviewer	Page	Line	Comment	Action / Reply
				• Supervision of cryotank mapping, location traceability, import and export of gametes. • Coordination of cryostorage renewals and patient follow-up lists. • Asset tracking and consumable inventory management. • Immediate response to laboratory deviations or near-miss events. • Documentation and preliminary root cause analysis (RCA). • Escalation of critical issues to the Laboratory Director. • Implementation of corrective and preventive actions (CAPA). • Oversight of non-conformance logs. • Providing structured feedback and performance appraisals. • Identifying training gaps and arranging targeted workshops. • Supporting staff during complex or high-risk procedures. • Promoting a safety-first culture. • Encouraging open reporting of errors without fear. • Supporting team morale and psychological safety. • Leading daily briefings or case analysis.	this level of detail would contribute to this document.....
12	Durga Rao Gedela	6	1.3	Periodic Emotional and Cognitive Competency Assessment: Given the high cognitive load and risk-sensitive nature of gamete/embryo handling, the guideline may consider inclusion of periodic **Human Factors and Emotional Competency Evaluation.**	The working group considered this proposal, however, considered this outside the responsibilities of IVF laboratory staff.
25	Irene Cuevas-Saiz	6	155-156	…. for the ESHRE clinical embryologist certification OR SIMILAR.. … for the ESHRE senior clinical embryologist certification OR SIMILAR… (for director and supervisors, "or similar" is included, so it should be included for clinical embryologists too).	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	6	156	Consider the following: • Participation in validation studies (media, equipment, devices). • Contribution to research projects and clinical audits. • Attendance at scientific meetings and workshops. • Engagement in continuous professional education (ESHRE, IVF related webinars, local IVF conferences etc.). • Maintaining procedural competency or in competency re-evaluations.	The educational and competency part are covered in the text. The following points were added: • Participation in validation studies (media, equipment, devices). • Contribution to research projects and clinical audits.

NR	Reviewer	Page	Line	Comment	Action / Reply
27	Maria Filippa	6	156	It will be extremely useful to offer the basic SOPs as supplemented material for reference especially for uniform training of Clinical Embryologists	There is no "standard" set of SOPs
26	Sheila Mae Poulain	6	157	Grammar, consider: "All **procedures** should be performed by experience practitioners…"	Adapted as suggested by the reviewer.
17	Hannah Park	6	158	"All procedure**s** should be performed by experienced practitioners.."	Adapted as suggested by the reviewer.
25	Irene Cuevas-Saiz	6	160-162	In my opinion, this paragraph is unnecessary here. The previous phrase (lines 158-159) include micromanipulation and a great overview of the biopsy procedure and skills and the reference to the recommendation ESHRE paper for embryo biopsy is further explained in section 12 (page 28 and following)	The working group agrees with the reviewer, the paragraph was removed.
10	Karin Rosenstein	7	1.4	It is very important that ESHRE provide a recommendation on the minimum number of embryologists required per number of IVF procedures. Unfortunately, there are still hospitals and clinics where only one or two embryologists handle 500–1000 procedures per year (IVF/ICSI/FET combined), often without any witnessing system in place. Clear guidance from ESHRE would help draw national authorities' attention to this issue. The current wording on page 7 (rows 184–189) leaves the issue unresolved and does not provide clear guidance.	It has been a deliberate decision by the WG not to give specific numbers of staffing levels. The minimum number depends on many factors, such as educational levels, types of treatments included, equipment, schedules (e.g. workdays/weekends) etc, so can differ widely between clinics. Suggestions on numbers can be found in the references. However, we have reinforced the numbers by Sharisawa by adding: "Such calculations can be used for guidance when calculating the number of staff needed."
19	Fayezi, Shabnam	6	1.4	Consider providing a simple worked example/template for staffing calculations and clarify whether workload estimates include QMS-related time (QA meetings, incident reporting, audits/inspections, training/CPD).	The document provides references with (estimated) numbers of the totals, and e.g. Veiga *et al.* provides (estimated)numbers per procedure. The reviewer is referred to the provided refs (e.g. ASEBIR) for calculations.

NR	Reviewer	Page	Line	Comment	Action / Reply
30	Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)	6	166	Comment: Since the preparation of ovarian tissue is very time-consuming, it makes sense to include this in the calculation of laboratory staff. Add: Others all complex treatment cycles such as **embryo** biopsies, gamete or embryo donation, **ovarian tissue preparation**, fertility preservation.	Ovarian tissue is part of fertility preservation and therefore already covered in the sentence.
25	Irene Cuevas-Saiz	7	174, 180,181, 182	There is always the concern of the definition of "cycle": oocyte pick-ups and/or cryopreservation cycles. To include the definition here, could led to infinite meetings, but maybe it would be interesting to put in parentheses something like "for the definition of cycles see the biblio: Veiga and Shirasawa). I make this comment because I think embryologists have this concept of splitting type of cycle in mind, but for owners of clinics, personnel management, etc, whenever they read cycles, they make the association to oocyte pick-ups and all we know that nowadays this is far away from reality, and this can influence the number of embryologists (or lab staff) that will be hired by the clinic, based on the numbers mentioned in the paragraph.	The working group has adopted the terminology from the research papers. The reviewers are referred to the references for their definition of "cycle".
27	Maria Filippa	7	180-182	A supplement in the form of a table mentioning the no of staff according to the number and type of each procedure performed would be useful for reference & for proof to ask for more staff	The document provide references with (estimated) numbers of the totals, and e.g. Veiga et al provides (estimated) numbers per procedure
28	Labadi Leila	7	183-186	It is suggested to include a standardized calculation template or a validated spreadsheet link in the appendix to help labs harmonize how they document their specific staffing needs are recommended in these lines	References for calculations are provided, e.g. Alikani and ASEBIR.
26	Sheila Mae Poulain	7	188-189	Citation format (for consistency with journal style, separate text and URL), consider: "for example (Alikani et al., 2014) and **the ASEBIR 'Cassandra' calculator** (https://asebir.com/cassandra-calculadora-de-rrhh/?idioma_cassandra=en)."	Adapted as suggested by the reviewer.
19	Fayezi, Shabnam	7-8	1.5	The training section is strong, but additional specificity would help standardisation. • Consider including minimum competency numbers by procedure	We have referenced the requirements for the ESHRE certification programs.

NR	Reviewer	Page	Line	Comment	Action / Reply
				category, suggested certification pathways (ESHRE), and revalidation/recertification intervals.	
26	Sheila Mae Poulain	7	192	Consider more neutral and typical of guideline tone: "have become **substantially** more complex."	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	7	192-194	Consider adding: ovarian tissue cryopreservation, advanced sperm selection techniques, computer assisted technologies and artificial intelligence software for sperm, oocyte and embryo selection.	The working group does not agree that these are routine techniques in the lab.
26	Sheila Mae Poulain	7	196	Grammar, consider: …the volume of necessary documentation and reporting have increased **substantially**.	The sentence is correct as is.
18	Keerti Singh	7	197	It is vital that an embryologist 'learns' not only	Adapted as suggested by the reviewer.
32	AGRBM	7	197	embryologist learn**s** not	Adapted as suggested by the reviewer.
17	Hannah Park	7	200	Suggestion: "Additional and continuous training, such as participating in workshops and conferences, is of great importance."	The sentence was changed to: including structured workshops and certification programs offered by IVF laboratories, academic institutions, and specialised reproductive medicine organisations.
26	Sheila Mae Poulain	7	200	Consider adding: "Additional and continuous training is of great importance, **including structured workshops and certification programs offered by IVF laboratories, academic institutions, and specialized reproductive medicine organizations**."	The sentence was changed to: including structured workshops and certification programs offered by IVF laboratories, academic institutions, and specialised reproductive medicine organisations.
26	Sheila Mae Poulain	7	201	Consider: "One of the challenges in clinical embryology is the lack of **harmonised** regulation concerning the criteria required to be recognized as a specialist…"	Adapted as suggested by the reviewer.
26	Sheila Mae Poulain	7	204-207	Consider adding: "Trainees should begin with a solid academic background in biological or biomedical sciences, **ideally supported by foundational knowledge in clinical laboratory sciences, followed by a structured, written training plan incorporating observation, supervised practice, and the gradual assumption of progressive responsibility.**	This is already covered in the following sentences (parallel assessments, demonstration of proficiency etc) just in other words.
27	Maria Filippa	7	204-209	A supplement of a detailed logbook describing each procedure observed, practiced supervised and independently practiced would be a great reference	We have referenced the requirements for the ESHRE certification programs.

NR	Reviewer	Page	Line	Comment	Action / Reply
28	Labadi Leila	8	210-211	Beyond the 30-60 supervised cycles, the document should specify minimum success rate KPIs (eg survival or fertilization rates) that a trainee must consistently achieve before being validated for independent practice	Defining success rates is not the scope of this guideline.
36	David Morroll	8	211	Though useful to give an idea of numbers of procedures for training logs, the use of LC-CUSUM to objectively determine acquisition of competence could be added (Dessolle et al, 2009; Durban et al, 2016)	The references were added to the document.
18	Keerti Singh	8	213	Ensuring should be 'ensures'	The sentence is correct as is.
26	Sheila Mae Poulain	8	216-217	Consider adding: **Trainees in the andrology and embryology laboratory should receive structured theoretical instruction from the Senior Embryologists (Head of Education) within the IVF laboratory department. Formal evaluations should be conducted to ensure comprehensive understanding of the concepts and principles underlying the observed procedures and techniques.**	Mentorship is already in the text. A sentence was added to the text: "Formal evaluations should be conducted to ensure comprehensive understanding of the concepts and principles underlying the observed procedures and techniques."
2. Quality management					
4	Shridhar S Amanchi	/	/	The focus on quality management is appreciated. Simple examples of KPIs, audit checklists, and incident reporting formats would help labs implement these recommendations more easily.	There are 2 references of KPI papers in the text. Considering incident reporting: this reporting is country dependent and often templates are provided by competent authorities. Audit checklist are an interesting item, however, outside the scope of this recommendations paper.
12	Durga Rao Gedela	/	/	Chain of IVM/IVF Clinics – Centralized Monitoring and Digital Governance: Multi-site variability is a recognized source of performance heterogeneity. Centralized data governance reduces unwarranted variation and strengthens patient safety.	Thank you for your remark.
19	Fayezi, Shabnam	/	/	Some sections (notably Quality Management and Traceability) are dense and could be easier to scan. • Break long paragraphs into shorter bullet lists. • Add subheadings and 'key takeaways' boxes for critical steps.	The section was divided up into smaller lists.

NR	Reviewer	Page	Line	Comment	Action / Reply
19	Fayezi, Shabnam	/	/	Please consider adding a practical 'starter set' of KPIs (with definitions, suggested monitoring frequency, and example thresholds/control limits) and a brief example dashboard layout.	References are given to ESHRE KPI papers. In addition, there are also numerous sessions on the ESHRE e-learning platform on KPI
20	Shivansh Jaiswal	/	/	The QMS section is robust and well-articulated. It may benefit from encouraging structured categorization of non-conformances and centralized tracking systems to facilitate trend analysis. Explicit linkage between incident documentation, corrective and preventive action workflows, and management review cycles would reinforce the dynamic nature of the quality system. Additional clarification of document control principles, including unique identification, version control, revision tracking, and approval authority, may enhance audit readiness.	The section was divided up into smaller lists.
33	Samantha Wake	8	218	ISO 15189 is a recommended accreditation	ISO 15189 accreditation is not something that ESHRE can recommend.
26	Sheila Mae Poulain	8	219-220	Clearly reference SoHO regulation and European Committee on Organ Transplantation. Consider: "According to the **SoHO Regulation (European Parliament, 2024) and guidance from the European Committee on Organ Transplantation (EDQM, Council of Europe)…"**	The URL was added to the text.
18	Keerti Singh	8	222	After QMS, replace so called quality managers with 'as quality managers'.	The sentence was adapted
30	Deutsche Gesellschaft für Reproduktions medizin (DGRM)	8	225	The QMS should be documented and designed to assure quality, safety and effectiveness of **all** processes.	The sentence was adapted.
27	Maria Filippa	8	226-228	A supplement addressing risk management principles and suggested written procedures of all critical processes as a reference would be extremely valuable	This information can be found in EDQM guide. In addition, there is information on the e-learning platform of ESHRE.

NR	Reviewer	Page	Line	Comment	Action / Reply
18	Keerti Singh	8	227	After 'in light of new knowledge' put a full stop. Remove the word 'and' and start a new sentence with the word 'Significant'	The sentence was adapted
27	Maria Filippa	8	232	A supplement of the QMS describing all it incorporates and addresses to solve with effectiveness and safety would be a valuable tool	This information can be found in EDQM guide. In addition, there is information on the e-learning platform of ESHRE.
18	Keerti Singh	8	2.1	Reference is not provided here. Suggest Wirka et. al., 2022, "Taking a closer look at the key performance indicators in ART lab"	We are already referring to the ESHRE KPI documents for both the clinic and the IVF laboratory.
34	Nadia Kazdar	8	2.1	Add :A procedure for managing identity discrepancies and cases of patients with identical names (homonyms) must be established, documented, and communicated to all laboratory staff.	This is a very interesting remark on a very specific detailed procedures. There are many more in the IVF lab. It was decided not to go into detailed procedures.
27	Maria Filippa	9	2.7 2.8	Supplementary Data on a documented validation of all critical processes & a documented risk assessment strategy will be valuable	This would be an interesting topic for a different recommendations paper, however, outside the scope of this recommendations paper.
12	Durga Rao Gedela	9	2.8	Periodic Quality Audits and Review Meetings: The guideline may strengthen emphasis on **structured periodic internal audits** beyond annual review.	A sentence was added.
19	Fayezi, Shabnam	9	2.8	A short comparative guide/decision tree on when to use HACCP vs FMEA vs RCA (etc.), and what minimum outputs are expected (risk register, CAPA linkage, review interval), would improve usability.	There is more information on these tools in the EDQM guide.
3. Laboratory safety					
4	Shridhar S Amanchi	/	/	Clear suggestions on gas calibration frequency, acceptable recovery time after door opening, and how long embryos can remain outside the incubator would be helpful.	A sentence was added to the embryo culture section.
7	Liliana Ramos	/	/	Protective measurements: With the upcoming "green IVF lab policy", allow to use less gloves after a risk assessment of the infection risks at some procedures. The use of gloves and PPE during some of the procedures which are not proved to really prevent infections, should be revised. Encourage the "green IVF initiative"	The working group did not think it possible to recommended less use of gloves. It is also not possible to do a risk-assessment on not using gloves.

NR	Reviewer	Page	Line	Comment	Action / Reply
20	Shivansh Jaiswal	/	/	Further clarification of expectations regarding environmental monitoring frequency and documentation of filtration validation may improve implementation consistency. Where infrastructure permits, stronger supportive language for reduced oxygen culture systems may be considered, acknowledging evidence trends supporting physiological oxygen tension in embryo culture.	The reviewer is referred to the Cairo consensus document for further information.
22	Zuzana Holubcová	10	236	The wording "minimal distances" is somewhat ambiguous. The key objective appears to be minimising the time that gametes and embryos are handled outside controlled incubation conditions, rather than simply reducing physical walking distance. Consider rephrasing to: "Laboratory design should minimise the time that gametes and embryos are handled outside controlled environmental conditions.	The sentence was adapted.
26	Sheila Mae Poulain	10	247	The statement maybe misinterpreted, consider: "…located **outside the main laboratory working area but in close proximity."** Or consider: "…located **in a separate room from the main laboratory working area but in close proximity."**	The sentence was adapted.
8	Chand Mohamad	10	248	Second point is off-gassing always before use & prior a day of Procedure.	Off-gassing was referring to newly built rooms not to plastic packaging. For packaging, this falls under "avoidance of VOCs".
22	Zuzana Holubcová	10	248	In addition to newly constructed or renovated laboratories, it would be helpful to explicitly mention painting, refurnishing, flooring replacement, and other refurbishment activities. Off-gassing from paints, adhesives, furniture, and flooring materials may significantly affect VOC levels and embryo culture conditions. Consider broadening this statement to include all refurbishment activities.	Painting, refurnishing, flooring replacement, and other refurbishment activities are all covered under "renovation". The reviewer is referred to the Cairo consensus for more details.
26	Sheila Mae Poulain	10	248	Consider placing a **comma (,)** after "…or renovated laboratories, sufficient time…	The sentence was adapted.
27	Maria Filippa	10	252-254	Supplementary Data on advice on workplace ergonomics & operator confort & schedule options to avoid human errors would be vital	The working group considers this outside the scope of this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
26	Sheila Mae Poulain	10	257-258	Consider: "**Appropriate** environmental lighting and air-conditioning with controlled humidity and temperature are **recommended**."	The sentence was adapted.
26	Sheila Mae Poulain	10	258-259	Consider adding: "An ante room should be incorporated into IVF laboratory design to function as a controlled transition zone, enabling gowning, hand hygiene, and material transfer while preserving air pressure gradients and minimizing particulate and VOC ingress into critical laboratory areas."	This is covered in section 3.1.3 to 3.1.5.
26	Sheila Mae Poulain	10	258-259	Consider adding: "The laboratory commonly incorporates pass-through hatches to facilitate the movement of specimens (e.g., from an adjacent operating room) without opening the main laboratory doors, thereby preserving air pressure differentials."	A hatch does also change pressure, so it is not really better than a door. That's why we suggested overpressure
34	Nadia Kazdar	10	3.1	It is preferable to implement a pass-through hatch system between the operating room, the embryo transfer rooms, and the sperm collection room, designed so that both doors cannot be opened simultaneously, in order to maintain optimal air quality and environmental control.	A hatch does also change pressure, so it is not really better than a door. That's why we suggested overpressure
12	Durga Rao Gedela	10	3.1	Waterproof and Earthquake-Resistant Cryostorage Units: The guideline may consider structural resilience specifications for cryostorage facilities, particularly in high-risk geographies.	The working group considers this outside the scope of this document.
18	Keerti Singh	10	3.1.1	Reference - Anagnostopoulou C, Maldonado Rosas I, Gugnani N, Desai D, Manoharan M, Singh N, Leonardi Diaz SI, Singh K, Wirka KA, Gupta S, Darbandi S, Chockalingam A, Darbandi M, Boitrelle F, Finelli R, Sallam HN, Agarwal A. An expert commentary on essential equipment, supplies and culture media in the assisted reproductive technology laboratory. Panminerva Med. 2022 Jun;64(2):140-155. doi: 10.23736/S0031-0808.22.04671-7. Epub 2022 Feb 11. PMID: 35146990. - Page 146, left column para 3. Line 4.	The proposed reference can be included.
13	Fikret Gürkan Agircan	11	3.1.8	Please consider revising the restriction on private cell phones. The original publications do not stipulate that phone must be removed und completely removing phones is not feasible for staff members who are parents or caregivers and must remain reachable for schools,	The working group does not agree with the proposed modification, as this would become very complicated. Internal SOPs will regulate this on a case per case basis.

NR	Reviewer	Page	Line	Comment	Action / Reply
				kindergartens, or medical emergencies. Proposition: "Private cell phones should not be used for casual purposes in the laboratory to avoid distraction; however, they may be kept nearby strictly to receive emergency or urgent family communications."	
32	AGRBM	11	3.1.8	Removing might be difficult; this may be the only way to be reachable. Proposed change: should not be used	The sentence was adapted.
4	Shridhar S Amanchi	11	3.2	Guidance on minimum frequency for VOC and particle monitoring would help labs that have limited resources but want to maintain safety.	A sentence was added to the text.
27	Maria Filippa	11	3.2	Supplementary Data mentioning the range of optimal values in a table concerning positive pressure, the number of fresh air changes, VOC levels, air cleanliness at rest & in operation and microbial contamination level at which surfaces and equipment would be great	The reviewer is referred to the Cairo consensus for details.
36	David Morroll	11	260	The Cairo consensus (Mortimer et al, 2018) tends to highlight the benefits of positive pressure (PP) in maintaining lab conditions (see sections on lab design philosophy and physical isolation), such that overpressure is a consensus point for basic design criteria. Should the use of PP be more strongly promoted and advised rather than simply “recommended”? I would advocate for this. Note also that 3.2.2 sates “Air conditioning should ensure a sufficient number of fresh air changes” but should this refer to air filtration or air supply rather than air conditioning?	Recommend is the correct wording. The sentence on air changes was adapted for better understanding.
13	Fikret Gürkan Agircan	11	3.2.1	The more detailed information regarding the air quality is given EDQM und GMP Annex 1. It would be nice to direct the readers to those sections.	The reference was added.
18	Keerti Singh	11	3.2.1	Reference - Anagnostopoulou C, Maldonado Rosas I, Gugnani N, Desai D, Manoharan M, Singh N, Leonardi Diaz SI, Singh K, Wirka KA, Gupta S, Darbandi S, Chockalingam A, Darbandi M, Boitrelle F, Finelli R, Sallam HN, Agarwal A. An expert commentary on essential equipment, supplies and culture media in the assisted reproductive technology laboratory. Panminerva Med. 2022 Jun;64(2):140-155.	The content in the reference is too minimal to be included here.

NR	Reviewer	Page	Line	Comment	Action / Reply
				doi: 10.23736/S0031-0808.22.04671-7. Epub 2022 Feb 11. PMID: 35146990. - Page 146, left column para 3. Lines 7-10	
15	Alev Özer	11	3.2.1-3.2.4	Recommendations appropriately emphasise HEPA filtration and VOC control; however, the text remains largely qualitative. Given increasing evidence that even low-level VOC variability can influence embryo developmental parameters, the guideline would be strengthened by recommending quantitative VOC monitoring (continuous or periodic), trend analysis, and explicit CAPA triggers. Proposed text addition : Centres should implement quantitative monitoring of volatile organic compounds (VOCs) (continuous or periodic) and define centre-specific alert thresholds based on risk assessment. VOC trend analysis should be integrated into the QMS, with predefined corrective and preventive action (CAPA) pathways (e.g., source investigation, filter replacement, material quarantine, and re-commissioning checks) when sustained deviations are detected." Full-text supporting literature • Sciorio R. Air quality in the clinical embryology laboratory: a mini-review. 2021. https://pmc.ncbi.nlm.nih.gov/articles/PMC7882750 • Ying Y. et al. Insights from volatile organic compounds in the IVF laboratory environment. 2024. https://www.fertstert.org/article/S0015-0282%2824%2901285-8/fulltext • Pontes BS. et al. Air quality control on in vitro fertilization outcomes… 2018.https://www.humanreproductionarchives.com/article/10.4322/hra.000617/pdf/hra-32-3-e000617.pdf	A sentence was added to 3.2.1
34	Nadia Kazdar	11	3.2.2	A positive pressure cascade between the airlock, the laboratory, and the external environment is preferable.	That is indeed what the recommendation indicates.
21	Laboratory for Reproductive Medicine,	11	3.2.4	It would be helpful if some recommendations are made concerning what "regular intervals" means. Is once every year enough or should this be	The sentence was adapted to clarify the recommendation.

NR	Reviewer	Page	Line	Comment	Action / Reply
	Erasmus MC, University Medical Center Rotterdam			more or less frequent? And what should be considered to make a decision?	
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	11	3.2.5	What exactly is meant by the "microbial contamination level of the laboratory"? For me, this requires further explanation	The sentence was adapted.
7	Liliana Ramos	11	266 (3.3.2)	Suggestion: table incubators should be suggested as these are designed so each patient has its own chamber and avoids opening the door and disturbing other patient's incubation	An explanatory sentence was added.
25	Irene Cuevas-Saiz	11	3.3.2	… should be based on the number of cycles, embryo culture duration AND TYPE OF INCUBATOR (benchtop, box). … distributed across incubators to minimize ENVIRONMENTAL FLUCTUATIONS. I think that "minimize door openings" is something inherited to the use of big box incubators, but nowadays with the use of benchtop incubators with independent compartments it´s more accurate to talk about fluctuations of physical parameters rather than door openings	The sentence was adapted.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	12	3.3.5	I think the EDQM is more specific about the use of equipment with a CE mark (mandatory if available)	The sentence was adapted.
36	David Morroll	12	3.3.5	I would suggest this be stronger "Equipment should be CE-marked for the intended use, where this option is available."	The sentence was adapted.
17	Hannah Park	12	3.3.6	"Gas cylinders should be located outside **of** the laboratory."	The sentence was adapted.

NR	Reviewer	Page	Line	Comment	Action / Reply
18	Keerti Singh	12	3.3.12	Reference - Anagnostopoulou C, Maldonado Rosas I, Gugnani N, Desai D, Manoharan M, Singh N, Leonardi Diaz SI, Singh K, Wirka KA, Gupta S, Darbandi S, Chockalingam A, Darbandi M, Boitrelle F, Finelli R, Sallam HN, Agarwal A. An expert commentary on essential equipment, supplies and culture media in the assisted reproductive technology laboratory. Panminerva Med. 2022 Jun;64(2):140-155. doi: 10.23736/S0031-0808.22.04671-7. Epub 2022 Feb 11. PMID: 35146990. Page 146, left column para 4. Lines 2-5.	The proposed reference can be included.
13	Fikret Gürkan Agircan	12	3.3.13	It would be nice to add a new section stating that: "All personnel must receive proper training prior to operating the equipment, and all training activities shall be officially documented."	Back up power system switches on automatically. In-house technicians will do training and maintenance. As a general rule, no lab person should touch a power supply system.
26	Sheila Mae Poulain	11-12	262-263	Consider adding the following: 3.3.14 A documented risk assessment should be performed to classify equipment according to criticality, defining contingency plans and recovery timelines in case of malfunction. 3.3.15 A lifecycle management plan should be implemented, including acquisition evaluation, commissioning, calibration schedules, service contracts, performance review, and end-of-life replacement planning. 3.3.16 All measuring instruments (e.g., temperature probes, CO_2/O_2 sensors, balances, pH meters) should undergo scheduled calibration traceable to national or international standards. Calibration certificates should be maintained and readily accessible. 3.3.17 Continuous electronic data logging systems should be used for incubators, cryostorage tanks, and environmental monitoring. Trend analysis should be performed regularly to identify early deviations before critical failure. 3.3.18 Electronic witnessing systems or equivalent validated double-check procedures should be integrated with laboratory equipment to enhance traceability and reduce the risk of gamete or embryo misidentification.	These are all topic covered in other sections of the Good Practice Recommendations paper.

NR	Reviewer	Page	Line	Comment	Action / Reply
				3.3.19 Critical equipment such as micromanipulators and incubators should be positioned to minimize exposure to vibration, electromagnetic interference, and temperature fluctuations that may affect performance. 3.3.20 Written procedures should define approved cleaning agents compatible with laboratory materials; and oocyte, embryo and sperm safety, minimizing VOC exposure and residue accumulation. 3.3.21 An inventory of essential spare parts (e.g., incubator sensors, HEPA filters, alarm batteries) should be maintained to reduce downtime during equipment failure. 3.3.22 Routine integrity checks, including vacuum integrity testing and LN_2 consumption trend monitoring, should be performed to ensure cryostorage safety. 3.3.23 Where time-lapse incubators or AI-assisted systems are used, contingency plans should ensure uninterrupted embryo culture in case of system failure. 3.3.24 Equipment layout and workstation height should support ergonomic practice to reduce operator fatigue and procedural errors.	
18	Keerti Singh	12	3.4	Reference - Nagy, Z. P., Varghese, A. C., & Agarwal, A. (Eds.). (2024). Cryopreservation in Assisted Reproduction : A Practitioner's Guide to Methods, Management and Organization (1st ed. 2024.). Springer International Publishing. https://doi.org/10.1007/978-3-031-58214-1	There are many books on this topic.
36	David Morroll	12	3.4.2	I would require audible and visible alarms at each point of entry to the cryostorage room. In addition, forced ventilation should be linked to the low oxygen alarms.	A sentence was added.
26	Sheila Mae Poulain	12	263	Grammatical refinement, consider: "3.4.6 All liquid nitrogen **should** preferably be clinical grade."	The sentence was adapted.
27	Maria Filippa	12	264	Supplementary Data on separating the storage liquid nitrogen tanks according to viral patient load for Hep B, Hep C etc and the essential role of vapor cryopreservation and of quarantine tank should be mentioned and explained, as well as nitrogen sterilization and disinfection of	The working group considers this outside the scope of this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
				cryopreservation equipment and consumables, as explained clinical grade nitrogen	
26	Sheila Mae Poulain	12	264-265	Consider adding the following: 3.4.7 Additional backup cryostorage tanks should be available, fully validated, and maintained in a ready-to-use state to ensure immediate transfer of specimens in the event of equipment failure or emergency. 3.4.8 Each cryostorage tank, canister, goblet, and storage position should have a documented and validated mapping system to ensure precise traceability and minimize retrieval errors. 3.4.9 Written emergency procedures should be established for: • Tank failure • Rapid LN_2 depletion • Alarm malfunction • Power outage • Natural disasters Staff should undergo periodic drills to ensure preparedness. 3.4.10 A contingency plan for uninterrupted LN_2 supply should be in place, including agreements with multiple suppliers and emergency delivery protocols. 3.4.11 Where applicable, validated closed-system vitrification devices should be considered to reduce potential cross-contamination risks in liquid nitrogen storage. Furthermore, a dedicated cryostorage tank should be allocated for specimens from patients with known infectious diseases to minimize the risk of cross-contamination. 3.4.12 Cryotanks should undergo scheduled vacuum integrity checks and monitoring of LN_2 consumption trends to detect early signs of insulation failure. 3.4.13 Access to cryostorage facilities should be restricted to authorized personnel. All sample retrieval and placement activities should be documented in a traceable audit trail system. 3.4.14 A system should be in place for periodic review of storage duration, patient consent validity, and legal compliance for long-term	Points 3.4.7 and 3.4.13 were included in the document. The others are considered out of scope for this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
				cryopreserved materials. 3.4.15 Cryolabels and storage devices should be validated for LN_2 compatibility, long-term durability, and resistance to fading or detachment under cryogenic conditions. 3.4.16 Validated procedures should be established for the safe transport of cryopreserved material, including dry shippers, temperature monitoring, and chain-of-custody documentation.	
13	Fikret Gürkan Agircan	18	3.5.1	It would be nice to add a new bulletpoint:" After opening, reagents should be stored according to the manufacturer's guidelines, and the maximum usage duration must not be exceeded. To maintain sterility, bottles should be opened under a Class A laminar flow cabinet using appropriate hygiene measures."	The proposed sentence was added.
27	Maria Filippa	13	3.5.1	Supplementary Data should exist with European regulations for control infection- prevention tips and corrective actions for reference and confirmation of good practice and safety	The working group considers this outside the scope of this document.
36	David Morroll	13	3.5.2	The wording requirement for dedicated areas and equipment for virally positive patients is too prescriptive. Real-world demands mean temporal and spatial separation, plus adequate cleaning, are more common approaches. The key is risk assessment.	The sentence was adapted.
27	Maria Filippa	13	270	Supplementary Data on European guidelines for fate of supernumerary gametes & embryos should be listed concerning donation issues or research and therapy options or destruction of genetic material as reference	Fate of supernumerary embryos is included in section 14 on cryopreservation. However, the fate of supernumerary embryos is often covered under national legislation.
33	Samantha Wake	13	271	Eye washing stations should be installed in ivf / Andro labs	Eye washes should be part of every first aid kit. This information is too detailed for the document.
22	Zuzana Holubcová	13	273	The phrase “where appropriate” is unclear. It would be helpful to define under which circumstances masks are required (e.g., during open handling of gametes and embryos outside closed systems, during micromanipulation, etc.). Clear criteria would avoid variability in interpretation between laboratories.	The working group discussed this, but decided not to include it. No scientific evidence of an effect on embryo quality. Most hospitals have a policy already.

NR	Reviewer	Page	Line	Comment	Action / Reply
				While avoidance of perfumes and cosmetics is mentioned, consideration could also be given to jewellery (rings, bracelets), watches, and eyewear handling practices, as these may contribute to particle shedding, contamination risk or compromise aseptic technique during manipulation with biological material and critical consumables..	
24	Stefan Matik	13	273	Maybe here it could be added that patients coming in for embryo transfer should be advised to minimize the use of cosmetics and avoid the use of perfumes on the day of the embryo transfer, as well as should be provided with a hairnet.	The working group does not agree with the reviewer.
33	Samantha Wake	14	273	Shoes specific to only IVF lab should be worn (changed into and out of). Hand washing should be without scent/chemicals	The sentence was adapted.
26	Sheila Mae Poulain	13-14	273-274	Consider adding the following: • Staff experiencing respiratory infections, gastrointestinal illness, or other communicable conditions should report symptoms and may be restricted from direct handling of gametes and embryos to minimize contamination risk. • A defined gowning sequence (e.g., shoe covers, head covering, mask, gloves) should be followed consistently to maintain cleanroom integrity. • Hand washing with approved non-fragranced soap followed by proper drying should be mandatory prior to gowning and glove use. Gloves should be changed between procedures and whenever contamination is suspected. • Conversation should be minimized during gamete and embryo handling to reduce particulate and microbial dispersion. •Unnecessary movement within the laboratory should be avoided to maintain airflow stability and reduce particle disturbance. • Rings, bracelets, watches, and other jewellery should not be worn during laboratory work to reduce contamination and glove perforation risk. • Artificial nails and nail polish should be prohibited, as they may harbor microorganisms and compromise aseptic technique. • Regular competency-based training and re-evaluation in aseptic technique should be conducted and documented.	Most of these points are either already covered in the document or considered out of scope. With regards to jewellery and nail polish, the working group discussed this, but decided not to include it. No scientific evidence of an effect on embryo quality. Most hospitals have a policy already.

NR	Reviewer	Page	Line	Comment	Action / Reply
				• Staff should be trained in understanding the impact of VOCs, temperature fluctuations, and air quality on embryo viability. • Laboratory-approved electronic devices only should be permitted; personal devices should remain outside the laboratory environment. • A non-punitive system should be in place for reporting breaches in aseptic practice or contamination events. • Staff should refrain from smoking during break periods immediately prior to re-entering the laboratory, as residual volatile organic compounds (VOCs) and particulate contaminants may compromise air quality and embryo safety.	
4. Identification for patients and traceability of their reproductive cells					
4	Shridhar S Amanchi	/	/	Clear guidance on documentation standards and electronic witnessing systems would help improve traceability and reduce human error.	Each electronic system has its own risk to consider and therefore a guidance with detailed steps cannot be listed in the present document. However a explanatory paragraph of deviations was added.
19	Fayezi, Shabnam	/	/	Some sections (notably Quality Management and Traceability) are dense and could be easier to scan. • Break long paragraphs into shorter bullet lists. • Add subheadings and 'key takeaways' boxes for critical steps.	The section was divided up into smaller lists.
19	Fayezi, Shabnam	/	/	A concise summary table of traceability requirements (including any SoHO-driven elements) plus a 'minimum vs ideal' implementation description (manual vs electronic witnessing) would improve clarity.	Working group decided not to comply, such lists would require regular updating. Each laboratory is encouraged to make their own.
19	Fayezi, Shabnam	/	/	Consider specifying a minimum set of critical steps that must be witnessed, and add brief guidance on validation/audit frequency and exception handling (downtime procedures) for electronic witnessing systems.	The working group had a long discussion about this, and decided to include the witnessing steps in each section, rather than making one table.
20	Shivansh Jaiswal	/	/	The traceability section is comprehensive. The document may consider endorsing electronic witnessing systems where feasible as part of an	The section was reviewed and some additional information was added.

NR	Reviewer	Page	Line	Comment	Action / Reply
				integrated safety culture framework. Emphasis on structured, blame-free incident reporting could further strengthen patient safety governance.	
27	Maria Filippa	14-15	276-277 & 305-309	Supplementary Data of the basic issues of the new EU Regulation on standards of quality & safety of SoHO and their traceability should be outlined, with a uniform form of SEC for each patient for reference	The working group considers this outside the scope for this document.
26	Sheila Mae Poulain	14	278-280	Consider to rephrase it as: "should **comply**, among others, **with key traceability provisions1**, including having systems to enable tracking of SoHO from the donor to the recipient and vice versa."	The sentence was adapted
26	Sheila Mae Poulain	14	289-290	Revise wording, and consider: "Moreover, **reporting and communication of serious adverse reactions** and events between SoHO entities and or regulatory bodies are facilitated."	The sentence was adapted.
7	Liliana Ramos	14	293	Health monitoring of children should be defined, as it is not always possible to do this for the long term.	There is no a defined time for monitoring but we can say to cover overs up to birth and neonatal outcome whenever possible .
3	AMM Wetzels	15 20	298 371	Although this is a recommendation, 'should' has to be replaced by 'shall', e.g. in lines 298 and 371 and in the numbered recommendations.	The sentence is correct as is.
28	Labadi Leila	15	308-311	For within relationship sample excluded from the SEC, it would be helpful to clarify the minimum required internal coding standards to ensure they remain compatible for potential future cross border exchange	The reference to SoHO was added.
32	AGRBM	15	310	This sentence might need some addition: If gametes or embryos for within-relationship use are moved between centres, use of the SEC is not mandatory, but a code needs to be applied that is unique within the EU, is machine-readable as far as this is possible, and does not reveal the identity of the person from whom the SoHO were collected. Centres may choose to use the SEC for this purpose.	The reference to SoHO was added.
27	Maria Filippa	15	311	Supplementary Data on the prerequisites age, medical history, limitations, mental-psychological profile, genetic, microbiological, immunological and viral testing for sperm and oocyte donors should ideally be outlined for reference and confirmation	The working group considers this outside the scope for this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
30	Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)	16	4.2	The implementation **of** EWS audits for design flaws, unclear alerts,	This was adapted
30	Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)	16	4.2	Comment: Test alert during the implementation of a EWS verifies the correct functioning of processes and notifications Add: The implementation EWS audits for design flaws, unclear alerts, **test alerts**, or normalization of deviation	The suggestions were included.
15	Alev Özer	16	317	The draft correctly frames manual/electronic witnessing as best practice and promotes a blame-free reporting culture. To make implementation more robust, the guideline should explicitly require structured EWS governance: audit of mismatch/near-miss typology, false-positive rates, workflow interruption points, and alarm-fatigue mitigation—integrated into QMS review cycles. Proposed text addition : "For laboratories using EWS, governance should include scheduled audits (e.g., quarterly) of: (i) mismatch/near-miss typology; (ii) false-positive alert rates; (iii) workflow interruption points; and (iv) staff response consistency. Alarm-fatigue mitigation strategies and competency refreshers should be documented within the QMS, and audit findings should feed into CAPA." Full-text supporting literature: •Holmes R. et al. Comparison of electronic versus manual witnessing… 2021. https://pmc.ncbi.nlm.nih.gov/articles/PMC8267391/ •Ifenatuoha CW. et al. Errors in IVF laboratories: risk assessments and mitigations. 2023. https://link.springer.com/article/10.1186/s43043-023-00130-0	The paragraph was added to the document.

NR	Reviewer	Page	Line	Comment	Action / Reply
28	Labadi Leila	16	317-319	Further guidance is recommended on how to maintain best practice witnessing during solo shifts or out of hours work specially when second physical witness is not available in the lab	Witnessing should be done in the same manner after hours, during the weekend and on holidays, as during hours.
26	Sheila Mae Poulain	16	321-322	Consider adding the following: •Each laboratory should formally define and periodically review all critical control points requiring witnessing (e.g., gamete identification, insemination, ICSI, embryo transfer, biopsy, cryopreservation, warming). • When manual witnessing is used, it should involve two qualified staff members (double eyewitness) performing independent verification rather than passive confirmation. • Where applicable, a standardized verbal confirmation protocol (e.g., read-back technique) should be implemented to reduce confirmation bias. • Any witnessing discrepancy should trigger a predefined escalation protocol, including immediate procedure halt, documentation, and supervisory review. • All witnessing events, mismatches, overrides, and corrective actions should be documented in a traceable audit system, whether manual or electronic. • Work scheduling should minimize fatigue during high-risk procedures (e.g., ICSI, embryo transfer), as cognitive overload increases error probability. • Override events in EWS should be monitored and periodically reviewed to detect patterns suggestive of system misuse or normalization of deviation. • Laboratories using EWS should maintain a validated manual witnessing backup protocol in case of technical failure. • Regular witnessing simulations or mock mismatch drills should be conducted to reinforce team response and preparedness. • All mismatches and near misses should undergo structured root cause analysis to identify system-level vulnerabilities rather than individual fault. • Electronic witnessing systems should comply with data security	A couple of suggestions were added to the document.

NR	Reviewer	Page	Line	Comment	Action / Reply
				standards, ensuring protection against unauthorized access, data loss, or manipulation. • Key performance indicators (KPIs) related to witnessing (e.g., mismatch rates, near-miss frequency, override frequency) should be monitored and reviewed in quality meetings.	
22	Zuzana Holubcová	16	328	The acronyms MEA and HSSA are not defined at first use. Please provide the full terms (e.g., Mouse Embryo Assay, Human Sperm Survival Assay) upon first mention for clarity and consistency with guideline standards.	This was adapted.
5. Consumables					
4	Shridhar S Amanchi	/	/	Simple advice on media equilibration time and handling after opening bottles would help maintain consistency and reduce variability.	Compliance with the manufacturer's instructions is included in the MDR.
5	Alexia Chatziparasidou	/	/	Management of the plastic/glass consumables and resource use: Establish and implement best practices for efficient use of plastic/glass consumables (minimum stock, rational use and minimum waste). Develop an eco-friendly management plan for expired consumables, and packaging materials. Maintain a record of the lab's annual electricity/water and paper consumption and develop good practices for ongoing monitoring. Set measurable sustainability goals to reduce the laboratory's environmental footprint and develop more eco-friendly IVF laboratory system.	A sentence was added to the document.
20	Shivansh Jaiswal	/	/	Reinforcement of validated batch testing procedures and supplier qualification documentation may enhance procurement governance and traceability integrity. Clear documentation of expiry tracking and material verification processes would further strengthen quality assurance.	Some modifications were made to the section to accommodate the comments.
34	Nadia Kazdar	17	5.1	"Compliance with the manufacturer's instructions is recommended.	The sentence was added to the document.
27	Maria Filippa	17	328	Supplementary Data would be extremely useful to explain the meaning of CE/ Medical Device Regulation (MDR)(EU) 2017/745 for medical devices, consumables and culture media with their corresponding confirmation tested by bioassay for sterility and compatibility for in vitro culture in a clinical setting	Working group decided not to comply, such lists would require regular updating.
7	Liliana Ramos	17	330	Not only PVP but include also their alternatives. Actually it is **all** media or products that get in direct contact with the gametes or embryos. Include	The sentence was adapted.

NR	Reviewer	Page	Line	Comment	Action / Reply
				also CE marked oil even it does not get in direct contact with embryos or gametes	
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	17	330-	The EU MDCG Guidance on classification of medical devices, 2021 states that IVF cell media with or without human albumin fall in class III, whereas here it is stated that they are classified as class IIa. Is this correct?	The paragraph was corrected.
30	Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)	17	330	IVF culture **and handlin**g media,	The sentence was adapted.
23	Susanna Apter*	17	330-335	Most IVF media are classified as risk class III based on rules 3, 14 and 18 in MDR. Most oils, dishes and tubes are classified as class IIa based on rule 2. For more details, please refer to MDCG 2021/24, Guidance on classification of medical devices.	The paragraph was corrected.
27	Maria Filippa	17	330-334	Supplementary Data on the classification of culture and handling gamete and embryo media would be of value of what it entails the MD Class IIa(low to medium risk) & the consumables MD Class I (low risk) in order to recognize and confirm its status	Working group decided not to comply, such lists would require regular updating.
8	Chand Mohamad	17	343	I want to add few lines here or includes Scenario of group of packing of tubes/dishes , What to do when you required only one-two dishes/tubes only and there is Few left in that packing. Instead of repacking, we can put them into the dry incubator and that Disposable should be used within a day's like next day.	The working group understands the comment, however, they cannot go into this level of detail in this document.
8	Chand Mohamad	17	343	Do not do- repacking by tape, staple etc. Repeated reopening and repacking can cause dish/tube contamination. And That can lead to hamper culture environments which can be Potential impairing embryo development/sperm survival.	The working group understands the comment, however, they cannot go into this level of detail in this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
18	Keerti Singh	17	5.3	Before line 344, please include: "as per ESHRE guidelines, all consumables should be of embryo culture grade quality, preferably CE marked" (Ref- Anagnostopoulou C, Maldonado Rosas I, Gugnani N, Desai D, Manoharan M, Singh N, Leonardi Diaz SI, Singh K, Wirka KA, Gupta S, Darbandi S, Chockalingam A, Darbandi M, Boitrelle F, Finelli R, Sallam HN, Agarwal A. An expert commentary on essential equipment, supplies and culture media in the assisted reproductive technology laboratory. Panminerva Med. 2022 Jun;64(2):140-155. doi: 10.23736/S0031-0808.22.04671-7. Epub 2022 Feb 11. PMID: 35146990. - Page 147, left column under Disposable supplies, para 2, lines 2-4.	This is already covered in the section on laboratory safety.
33	Samantha Wake	18	351	Aliquotting of media is not recommended	Aliquoting is not recommended according to manufacturer's instruction
26	Sheila Mae Poulain	18	351-352	Consider adding the following: • All reagents, media, and consumables should be labeled with the date of receipt and, where applicable, the date of opening. Products with limited post-opening stability should have clearly indicated in-use expiry dates. • A First-Expiry–First-Out (FEFO) inventory system should be implemented to ensure materials with the shortest remaining shelf life are prioritized for use. • New batches of culture media or critical consumables should be quarantined upon receipt and released only after verification of documentation (e.g., certificate of analysis, lot validation). • Where applicable, laboratories should perform internal validation or quality control testing when introducing new media or reagent lots to detect potential performance variability. • Lot numbers of critical materials (e.g., media, oil, pipettes used during embryo handling) should be traceable to specific patient cycles for audit and incident investigation purposes. • A written policy should define acceptable in-use durations for opened	The points were added to the document.

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				media bottles, oil overlays, prepared dishes, and pre-equilibrated media. • Expired or compromised materials should be clearly labeled as "out-of-use" and removed promptly. Disposal should be documented according to laboratory waste management procedures.	
27	Maria Filippa	18	355	It would be of value to state the range of Room temperatures for storage and for freezing consumables as well as the Lab temperatures, VOC levels and particle measurements to have an ideal working environment for the operators and the specimen	This is already addressed in section 5.7.
26	Sheila Mae Poulain	18	355-356	Consider adding: • Storage conditions (e.g., refrigerated, frozen, room temperature) should be continuously monitored and documented. Products exposed to temperature excursions should undergo documented risk assessment before use.	The sentence was added to the document.
26	Sheila Mae Poulain	18-19	357-367	Consider adding the following: • A minimum stock threshold should be defined for critical consumables to prevent last-minute use of near-expiry materials due to supply shortages. • Where feasible, digital inventory systems should be used to automate expiry alerts and reduce human oversight errors.	The points were added to the document.
18	Keerti Singh	19	366	Bullet point 1 Record data …Ref Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7. - Page 147, left column under Disposable supplies, para 2, lines 5-7.	The intention of the comment is unclear.
6. Handling of biological material					
4	Shridhar S Amanchi	/	/	Guidance on maximum handling time for oocytes and embryos outside controlled conditions would help maintain stable culture environment.	This entirely depends on the culture conditions in the lab. The working group cannot attribute values to this.
6	Aneta Macur	/	/	Could you please consider changing the fragment: "6.5 Where observations take longer than 2 minutes, MOSP or HEPES (or simularly buffered solution) should be used (e.g. denudation or ICSI)." First of all, many experienced embryologists worldwide perform ICSI using bicarbonate-based media under oil, changing the ICSI dishes every	The sentence was adapted.

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				5-10 minutes. Moreover, we cannot state with certainty that after 2 minutes the changes in pH and osmolarity are always unacceptable when bicarbonate is used. The parameters depend on many factors, such as drop size, oil type and volume. None of the cites articles conform this 2-minute threshold. According to the cited studies, HEPES may be detrimental to oocytes, and the influx of MOPS and especially HEPES into the oocyte during the first 2 minutes afer ICSI may influence the transcriptome. The conclusion of the study by Mendola et al. (2024) is: "These findings further support the utilitzation of bicarbonate buffer as the oocyte-holding medium during ICSI." Magli M.C. et al (2023) reported that pre-equilibration in 6.5% CO2/5.0% O2 and the use of a heavy oi overlay allowed the pH to remain within acceptable limits throughout the entire duration of ICSI under these conditions (0, 5, and 10 minutes). The plate should be outside the incubator for as short time as possible - it is not questionable. However, the exact time depends on many factors. **(references were provided)**	
16	Manuela Puchner	20	368	„Warming of these consumables could increase the risk of release of VOCs" This is a very strong statement and actually sparsely documented in literature. Please provide a proper reference. As it stands, it is a little hear say (certainly it´ll depend on the time catheter is on warming plate). Alternatively, one could suggest to remove this phrase. Reduced temperature during embryo transfer is also a negative feature, it is T vs (theoretical VOC) please see doi: 10.1016/j.rbmo.2021.05.014.	The working group thinks the wording is mild enough - 'could'.
27	Maria Filippa	19	368	Supplementary Data is essential to state the range of the optimal values for pH, temperature, room & equipment humidity, oxygen in culture & osmolarity with the appropriate time intervals of handling the specimen ie timing for fertilization after oocyte retrieval, time left for ICSI, time for denudation before ICSI or after IVF etc	This is considered out of scope for this document.
23	Susanna Apter*	19	6.3	Original text: Buffered media (HEPES, MOPS or similar) should be kept in atmospheric air, whereas bicarbonate-buffered media should be kept in 5-7% CO2.	This is actually already covered under 6.2, where it says to maintain the appropriate pH.

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				Suggestion: Prior to use, bicarbonate-buffered media should be equilibrated to the equivalent of 5-7 % CO2 at sea level. Altitude affects atmospheric pressure and requires increased concentrations for a similar effect. Media containing HEPES or MOPS should be used according to the manufacturer's specification and, depending on the HEPES/MOPS to bicarbonate ratio media, may not be incubated in a CO2 incubator.	
25	Irene Cuevas-Saiz	19	6.3	… should be kept in THE % OF CO2 (USUALLY 5-7%) THAT ENSURES A pH OF THE CULTURE MEDIA 7.2-7.4. (In my opinion,the recommendation should be the final goal 😉)	This is covered under 6.2, where it says to maintain the appropriate pH.
23	Susanna Apter*	19	6.5	Original text: 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. Suggestion: 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). Recent evidence shows that an influx of surrounding culture buffer into the oocyte occurs during injection with MOPS/HEPES which significantly changes cytoskeletal transcripts in the oocyte (Mendola et al 2024). Selection of an appropriately buffered medium (bicarbonate, HEPES or MOPS) should be based on the expected time taken for manipulation or culture as well as gas availability and laboratory setup.	The sentence was adapted.
18	Keerti Singh	20	6.7	Reference - Ref Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7. - Page 151, left column, para 1, lines 12-14	The working group does not understand the intention of the comment.

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7	Liliana Ramos	20	R6.10	Suggestion: mouth pipetting should never be allowed! (not recommended is not strong enough)	"not recommended" is the correct terminology here.
36	David Morroll	20	6.10	Mouth pipetting is not acceptable.	"not recommended" is the correct terminology here.
26	Sheila Mae Poulain	20	369	Consider adding the following: 6.6 Handling time outside controlled incubation conditions should be minimized. Standardized time limits for procedures (e.g., denudation, ICSI, assessment) should be defined and monitored where feasible. 6.7 Heated stages, warming blocks, and work surfaces should be regularly validated to ensure temperature stability at 37°C ± defined tolerance. Independent temperature verification should be performed periodically. 6.8 Culture media and oil overlays should be adequately pre-equilibrated under appropriate gas conditions prior to use, according to manufacturer recommendations and internal validation protocols. 6.9 Pipetting techniques should minimize shear stress, excessive turbulence, and repetitive aspiration/expulsion cycles that may compromise gamete or embryo integrity. 6.10 Embryo assessment should be conducted under low-intensity, filtered lighting conditions where possible to minimize photo-oxidative stress. 6.11 Movement of gametes and embryos between rooms (e.g., operating room to laboratory) should be conducted using validated, temperature-controlled transport systems. 6.12 Preparation of culture dishes should follow validated protocols for oil overlay volume, drop size, and labeling to ensure reproducibility and minimize variability. 6.13 When incubators are opened, procedures should be performed efficiently to reduce gas fluctuation and re-equilibration time. 6.14 Separate pipettes and sterile consumables should be used per patient case to prevent cross-contamination. Agree to add in - discuss at online meeting. 6.15 Any deviation from defined handling parameters (temperature, pH,	6.8 was added to the document, the other points are either already covered in other parts of the document or considered out of scope.

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				gas, time) should be documented and risk assessed. 6.16 Personnel performing complex procedures (e.g., ICSI, biopsy) should undergo documented competency assessment and periodic re-evaluation. 6.17 Handling should occur in areas protected from volatile organic compound exposure, and cleaning activities should not occur concurrently with embryo manipulation. 6.11 Aspiration and expulsion pressure during pipetting should be controlled and consistent to avoid cellular deformation or zona pellucida damage. 6.12 Repeated transfer of dishes in and out of incubators should be minimized to prevent cumulative temperature and pH stress. 6.13 Mineral oil used for overlay should be validated for embryo safety, sterility, peroxide levels, and VOC content prior to clinical use. 6.14 Air bubble formation during pipetting and dish preparation should be minimized, as it may cause mechanical trauma or localized pH instability. 6.15 Handling steps should follow a predefined workflow to reduce unnecessary manipulation time and variability between operators. 6.16 Pipettes should be routinely inspected for smooth edges, internal defects, or irregular bore size that may increase shear forces. 6.17 Procedures should be conducted efficiently to minimize exposure to atmospheric oxygen levels that may increase oxidative stress in gametes and embryos.	
7. General andrology procedures					
7	Liliana Ramos	/	/	I miss the recommendations for sperm preparation for IUI, most IVF labs also prepare sperm for insemination.	We cover this in 7.9. It was the decision of the working group not to add detail by methods.
11	Sheryl Homa	/	/	A separate section on best practice for handling testicular sperm should be included, as handling of testicular sperm is a specialised skill that is not comparable to handling of sperm from ejaculates. Testicular sperm retrieval is an extremely important and often neglected area of practice. I have not included a detailed methodology for isolation	After discussion in the working group, it was decided to take the sentence out, however, a reference was provided.

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				and handling of testicular sperm but am happy to do so if it is considered appropriate. Handling of testicular sperm is a very labour-intensive technique, requiring a considerable amount of expertise, dedication and time to correctly identify sperm in amongst other cellular material such as immature sperm cells, blood cells and other cells of the seminiferous tubule epithelium (Shin and Turek, 2013; Esteves and Varghese 2012; Ramasamy et al, 2011). A high detection rate for sperm retrieval is dependent on the skill of the Andrology staff (Shin and Turek, 2013; Ishikawa et al, 2010). Motility of sperm within the seminiferous tubules is negligible, with spermatozoa only acquiring the ability to move progressively during epididymal transit (Gervasi and Visconti, 2017). For this reason, it is essential that methods for detecting testicular sperm viability are implemented in clinics using testicular sperm for ICSI, as in many cases, no motile sperm will be found. 1. Embryologists handling testicular sperm must demonstrate proficiency in processing testicular sperm samples, identifying sperm and selecting sperm for ICSI. They should have a specified training period under supervision and demonstrate competence before they are permitted to practice as a testicular sperm specialist. 2. Testicular sperm handling should be conducted using minimum volumes of culture medium to prevent over dilution of negligible numbers of sperm. Avoid sample evaporation, leading to detrimental changes in osmolarity and pH. Centrifugation should also be avoided to preserve the integrity of the sperm. Additionally, small numbers of sperm may be obscured by debris in a centrifuged pellet. 3. Embryologists who handle testicular sperm should be appropriately trained in freeze-thawing TESE sperm and should be confident with detecting sperm in test thawed samples. 4. Embryologists performing ICSI with testicular sperm must be proficient in using the hypo-osmotic swelling (HOS) test, sperm tail flexibility test,	

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				laser-assisted immotile sperm selection (LAISS) or chemical stimulants such as phosphodiesterase inhibitors e.g. theophylline, for sperm selection when they are immotile (Mohamad et al 2024; Esteves and Varghese 2012). 5. The IVF laboratory handling testicular samples for ICSI must be able to: a. provide a fully trained member(s) of staff for identifying sperm in fresh or thawed TESE samples (Ishikawa et al. 2010) b. provide the necessary time to search through the sample to find sperm. A minimum of 2 hours should be set aside, although some cases may require more than 4 hrs (Ramasamy et al. 2011). c. have a quality control process to monitor key performance indicators (KPIs) such as: i. sperm detection rate ii. recovery rate after freezing iii. pregnancy rate References Esteves, S. C., & Varghese, A. C. (2012). Laboratory handling of epididymal and testicular spermatozoa: what can be done to improve sperm injections outcome. Journal of Human Reproductive Sciences, 5(3), 233-243. Gervasi, M. G., & Visconti, P. E. (2017). Molecular changes and signaling events occurring in spermatozoa during epididymal maturation. Andrology, 5(2), 204–218. https://doi.org/10.1111/andr.12320 Ishikawa, T., Nose, R., Yamaguchi, K., Chiba, K., & Fujisawa, M. (2010). Learning curves of microdissection testicular sperm extraction for nonobstructive azoospermia. Fertility and sterility, 94(3), 1008–1011. https://doi.org/10.1016/j.fertnstert.2009.03.108 Mohamad, M. A., Assaf, H. A., & Mohamed, M. (2024). Methods for assessment of testicular sperm viability: a mini-review Running title:	

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				Assessment of testicular sperm viability. Sohag Medical Journal, 28(3), 83-91. Ramasamy, R., Fisher, E. S., Ricci, J. A., Leung, R. A., & Schlegel, P. N. (2011). Duration of microdissection testicular sperm extraction procedures: relationship to sperm retrieval success. The Journal of urology, 185(4), 1394–1397. https://doi.org/10.1016/j.juro.2010.11.074 Shin, D. H., & Turek, P. J. (2013). Sperm retrieval techniques. Nature Reviews Urology, 10(12), 723-730.	
16	Manuela Puchner	/	/	What to do in the case of leucospermia?	The working group considers this outside the scope of the current document.
20	Shivansh Jaiswal	/	/	Clarification of recommended abstinence intervals and structured viability assessment for immotile sperm prior to ICSI may support procedural consistency and decision-making transparency.	Abstinence intervals are already covered in the document. The reviewer is referred to the ESHRE Add-ons paper for further information on sperm selection.
3	AMM Wetzels	15 20	298 371	Although this is a recommendation, 'should' has to be replaced by 'shall', e.g. in lines 298 and 371 and in the numbered recommendations.	The sentence is correct as is.
27	Maria Filippa	20-22	370-405	Supplementary Data on Suggested SOPs examining and processing sperm step by step according to its initial potential either for testicular or epididymal biopsy, cryopreservation, IUI, IVF or ICSI and some accepted Add ons would be beneficial for reference like hyaluran usage and microfluidics Especially the algorithm of dealing with oligoasthenospermia and azoospermia would be useful to be outlined with its testing by endocrinology markers, imaging, genetics, microbiology and functional tests like DFI	The working group considers this outside the scope of the current document.
3	AMM Wetzels	20	373	There is an international standard (ISO 23162) on semen analysis as well. Why do you not use or include this standard?	The sentence was adapted to include the international standard.
35	Lars Björndahl	20	373	In the first paragraph it would be advisable to also state the WHO recommendation for basic semen examination does not include the use of Makler chamber or CASA…	The sentence was adapted.

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31	Koen Wouters	20	374	Treatment of infertility does not exist	Some causes of infertility can be treated.
3	AMM Wetzels	20	376	As WHO warns for a dichotomous categorization as reference value: what WHO reference ranges should we use? I suggests basically the 5th centile of table 8.3, page 213 in the 6th edition of the manual.	The WHO 5th centile was added to the sentence.
32	AGRBM	20	378	A second semen analysis is not only necessary when sperm count or motility parameters are out of WHO reference ranges in case of **febrile illness**. Also other problems or diseases might have an impact on sperm parameters and a repetition of a semen analysis might be indicated or advisable. Proposed change: A repeated semen analysis might be necessary when sperm count or motility parameters are out of WHO reference ranges. Moreover, a literature citation for the febrile illness and its relation to sperm parameters should be included.	The sentence was adapted.
24	Stefan Matik	20	378-379	Here a recommendation should be added that repeat semen analysis is mandatory also in cases where azoospermia is diagnosed in the first analysis (irrespective of presence or absence of previous febrile illness). Also, an andrologist should be included in the work-up of male infertility.	The sentence was adapted.
7	Liliana Ramos	20	379	Repeated semen analysis is necessary at any case it is not in the normal ranges, not only after an episode of illness.	The sentence was adapted.
11	Sheryl Homa	20	379	Morphology is also affected by febrile illness: History of febrile illness and variation in semen quality Carlsen, E., Andersson, A. M., Petersen, J. H., & Skakkebæk, N. E. (2003). History of febrile illness and variation in semen quality. Human Reproduction, 18(10), 2089-2092.	We have removed the febrile reference in other adjustments so now not relevant.
36	David Morroll	20	380	No citation. Without any strong evidence to support this, the statement that a test sperm prep is advisable is questionable and should be removed or downgraded to may be suggested or similar.	"May also be advisable" is not a recommendation as such and others asked for reinforcement of this so current is a balance.
7	Liliana Ramos	20	381	Add to IVF/ICSI also IUI, as sometimes a test preparation might indicate the possibility to apply IUI before undergoing IVF.	IUI was added to the sentence.

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30	Deutsche Gesellschaft für Reproduktions medizin (DGRM)	20	381	In addition, where assisted conception is planned, a test sperm preparation my also be advisable in order to con firm the most adequate insemination technique (IVF/ICSI **or IUI**)	The sentence was adapted.
33	Samantha Wake	20	385	Maximum 7 days abstinence period	The working group considers this outside the scope of the current document.
7	Liliana Ramos	20	386	Short abstinence is ≤2 days (not <2days)	The sentence was adapted.
31	Koen Wouters	20	386	Still confusing as everywhere is asked 2-7 days abstinence.	That is why we clarify in this document.
3	AMM Wetzels	20	388	I propose to complete this sentence: …. should be considered for most patients **during MAR and on indication for routine semen analysis, e.g. if motility parameters are low.**	The sentence was adapted.
32	AGRBM	20	390	Are there probably some words missing? Proposed change: "..but there is currently insufficient high quality evidence to support an effect on outcome and therefore **this is not a** routine practice. Consecutive ejaculates also impact laboratory workflow, patient anxiety and comfort, **so** outside well organised studies **it** may be impractical."	The sentence was adapted.
7	Liliana Ramos	20	391	"… and therefore this as routine practice": I guess you mean "this is **not** a routine practice"	The sentence was adapted.
3	AMM Wetzels	21	394	Do you mean unexpected LOW parameters or HIGH as well?	Unexpected is fine - usually this will be low, but no need to confine/restrict.
30	Deutsche Gesellschaft für Reproduktions medizin (DGRM)	21	395	Comment: Prostaglandins in IUI: can trigger uterine contractions, promote inflammation and affect sperm function. Add: Eliminate seminal plasma, debris, **prostaglandin** and contaminants.	"prostaglandins" was added to the sentence.
7	Liliana Ramos	21	403-404	This sentence is not clear	The working group considers the sentence clear.

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21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	21	403-405	Consider revising this sentence, as it is unclear to what "their utility" refers to, to "the sperm" or "the selection procedure"	Yield of the sperm cannot be used as an outcome parameter to judge the efficacy of a sperm selection method.
24	Stefan Matik	21	405	Here to be added at the end that 'The use of spermicidal condoms, creams or lubricants, as well as coitus interruptus should be avoided.'	This was added to the sentence.
26	Sheila Mae Poulain	21	405	Consider adding with 7.4: If semen collection is performed outside the clinic premises, a written informed consent should be obtained acknowledging the potential risks associated with transport, timing, and environmental exposure. The patient should be instructed to deliver the sample to the laboratory within 30–60 minutes of collection, ensuring that it is maintained close to body temperature (e.g., transported in an inside pocket) and protected from extreme temperatures. The time of collection and time of receipt must be documented	A sentence was added.
33	Samantha Wake	22	405	Centrifugation can generate ROS	The working group considers this outside the scope of the current document.
7	Liliana Ramos	21	R7.1	Use "one degree Celsius" and not "a degree"	The sentence was adapted.
13	Fikret Gürkan Agircan	22	7.1	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. I think the most appropriate is ""In case of suspicion of azoospermia, prior to starting stimulation, patients must be informed of the risk of having insufficient sperm on the day of oocyte retrieval, which may necessitate freezing the oocytes."	The sentence was adapted in response to another comment.
31	Koen Wouters	21	7.1	one degree is quiet high as range. 38˚C is in my opinion fever-ish.	This is person-specific.

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13	Fikret Gürkan Agircan	21	405/ 7.2	I think it is better to use what WHO Semen Analysis… suggest: "Lubricants should be avoided, since they may contaminate the ejaculate and change its properties. If absolutely necessary, validated non-spermatotoxic lubricants must be used " (commercially available) instead of suggestion giving Paraffin Oil to patients.	After discussion in the working group, it was decided to take the sentence out, however, a reference was provided.
23	Susanna Apter*	21	7.2	Overlay oils for IVF are not intended nor tested for use as a lubricant. This suggestion may lead to increased off-label use of these medical devices.	After discussion in the working group, it was decided to take the sentence out, however, a reference was provided.
30	Deutsche Gesellschaft für Reproduktions medizin (DGRM)	21	7.2	Comment: The use of the oil for embryo-culture as lubricant should be doubt: the oils for embryo-culture are usually MEA-tested and not HSSA-tested. Moreover, lubricants can be water-based or also oil-based but they don´t contain paraffin (dermatological reactions to paraffin include itching and redness).	After discussion in the working group, it was decided to take the sentence out, however, a reference was provided.
32	AGRBM	21	7.2	Is there any evidence or a publication for the use of oil in this context?	After discussion in the working group, it was decided to take the sentence out, however, a reference was provided.
32	AGRBM	21	7.3	The use of drugs and smoking (cigarettes and vapes) might also be worth to document.	This will not change therapeutic handing.
17	Hannah Park	21	7.4	"Collection should **preferably be** performed…"	The sentence was adapted.
7	Liliana Ramos	22	R7.6	Why should the time of each consequence processing step be noted?	This is considered best practice.
26	Sheila Mae Poulain	22	7.6	Consider adding with 7.6: The duration of sexual abstinence prior to semen collection must be documented, as it may influence semen parameters and subsequent laboratory outcomes.	This was added to 7.3.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	22	7.7	It is unclear what is meant with: "within 30 min if possible to perform a "wet check" What is a wet check? And should it be: within 30 min if possible, to perform a "wet check" or: within 30 min, if possible to perform a "wet check"	The sentence was adapted.

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31	Koen Wouters	22	7.7	Everywhere is mentioned within 1 hour. Confusing?	The recommendation states "if possible".
32	AGRBM	22	7.7	After 30 min (wait for liquefaction, see WHO manual), not within	Liquefaction is not necessary for processing, this is not about accurate sperm count.
7	Liliana Ramos	22	R7.9	The sentence "This will include for achieving…" is not clear	The sentence was clarified.
32	AGRBM	22	7.9	Are there some words missing? Proposed change: …, this will include a validation for achieving fertilisation. A post-monitoring should also be in place to ensure that any method selected does not negatively impact the later pregnancy and may lead to a miscarriage or a lower live birth rate (as these are also known to be independently influenced by sperm factors).	The sentence was adapted.
7	Liliana Ramos	22	R7.10	Add that in case of azoospermia or no possibility to produce an ejaculate. Alternatives for the last one might be the use of a vibro-ejaculator as well	A sentence was added.
7	Liliana Ramos	22	R7.11	Evaluation of a possible retrograde ejaculation should be done during the diagnostic procedures, not at the time of treatment. A low volume at the time of treatment without previous history of low volume might have other causes and in that case, a second ejaculate should be asked to proceed treatment. It is important to mention that retrograde ejaculation in bicarbonate is normally not suitable for IUI or IVF procedures, so a diagnostic sperm preparation test is advisable.	The sentence was adjusted to clarify.
17	Hannah Park	22	7.11	"Where the ejaculate is **below** 1.4 ml,.."	The sentence was adapted.
24	Stefan Matik	22	7.11	This should be redefined as: Where the ejaculate is beneath 1.4 mL, and has normal pH value (> 7.2) (to distinguish it from a case where the volume is below 1.4 mL, but pH is acidic (<7.0) which would suggest absence or blockage of the seminal vesicles, congenital (bilateral) absence of vas deferens, or prostatic midline/utricle cyst, ejaculatory duct obstruction…)	The sentence was adapted.
32	AGRBM	22	7.11	Ejaculate can also be below 1.4ml without being the result of a retrograde ejaculation. If seminal vesicles are not working properly the amount can be naturally lower, of if this is a second ejaculation on that day. If a retrograde ejaculation is assumed, urine can be collected and sperm can be retrieved from there. Then the processing has to be done	Thank you for your comment.

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				immediately to omit harmful exposure to the sperm. Is the term “dry orgasm” an officially used diagnose?	
3	AMM Wetzels	22	7.12	It is important that monomorphic abnormalities of spermatozoa are recognized during semen analysis as they can interfere with the effectivity of MAR and might be related to co-morbidities. I think this must be added as 7.12. Ref: Gatimel et al, Andrology, 2025; 0:1-15	This goes against WHO guideline with no foundational data that has correct EQA. We have specified the SFA method is ISO/WHO and leave there.
26	Sheila Mae Poulain	21-22	405-406	Consider adding the following: 7.12 Centrifugation force (g-force), duration, and number of spins should be standardized and validated, as excessive centrifugation may increase reactive oxygen species (ROS) production and DNA fragmentation. 7.13 Preparation techniques should aim to reduce exposure to atmospheric oxygen and minimize prolonged incubation in seminal plasma, particularly in samples with known high leukocyte concentration. 7.14 Where clinically indicated, evaluation for leukocytospermia should be performed, and appropriate processing adjustments made to reduce inflammatory or oxidative damage. 7.15 All media, density gradients, microfluidic devices, and consumables used in sperm preparation should be validated for sperm toxicity and clinical performance. 7.16 When sperm samples are cryopreserved, documentation should include semen analysis report prior freezing, cryoprotectant type, freezing method, freezing date, storage date, storage location, and post-thaw survival assessment. 7.17 Samples from patients with known infectious diseases should be processed using designated equipment and, where applicable, segregated storage protocols. 7.18 Where there is known risk of collection failure (e.g., severe oligozoospermia), a pre-planned backup strategy such as sperm cryopreservation prior to oocyte retrieval should be discussed and documented. 7.19 Personnel performing semen analysis and preparation should undergo periodic competency assessments and participate in internal and	The working group considers this outside the scope of the current document.

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				or external quality control schemes. 7.20 Care should be taken during pipetting and centrifugation to minimize aerosol generation and cross-contamination between samples. 7.21 When alternative sperm selection technologies (e.g., microfluidic sorting, magnetic-activated cell sorting) are used, they should be internally validated and clinically monitored for fertilization and downstream outcomes.	
8. Oocyte retrieval and processing					
7	Liliana Ramos	/	/	Some countries can offer the possibility of oocyte retrieval in one clinic and transport of the oocytes to another for fertilization and culture. This alternative has been proven to work well but it is not mentioned in this document	The working group agrees with the reviewer. A sentence was added to the document.
27	Maria Filippa	23	407	Supplementary Data on the Clinical features of the oocyte retrieval should be incorporated and outlined concerning the maternal age and the cause of infertility, the stimulation protocol used and the dosage of gonadotrophins, with or without antagonist / agonist, the number of days of stimulation, the triggering mode, the expected follicles and the collected oocytes at which pressure at aspiration (optimal range) and time to perform with or without adverse effects. Maybe a clinical SOP of oocyte retrieval followed by an embryological SOP of oocyte retrieval	This is a medical issue. The suggested information regarding patient's characteristics as well as the stimulation protocol scheme should already be listed in the patient's file.
17	Hannah Park	23	408	"Oocyte**s** are very sensitive cells,.."	This has been corrected.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	23	408	Replace Oocyte with Oocytes	Adapted.
17	Hannah Park	23	409	"Special attention should be given to **maintaining** the appropriate physical parameters during oocyte retrieval."	The sentence has been adapted.

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17	Hannah Park	23	before 8.1	"Temperature of heated stages and incubators, CO**2** and O**2** concentration of incubators should be regularly checked."	This has been corrected.
13	Fikret Gürkan Agircan	23	410	Before starting the procedure, the embryologist should verify that the patients have signed the corresponding informed consent and that the virological tests are valid. I strongly suggest revising the requirement that the embryologist must verify the informed consent and virological tests before starting the procedure. Clinically and legally, the responsibility for patient counseling and verifying consent lies with the attending physician and the doctor performing the oocyte retrieval. "Before the oocyte retrieval begins, a standardized departmental mechanism (e.g., a preoperative checklist) must be utilized to confirm that valid virological tests and signed informed consents are present in the patient's file. The clinical team is responsible for ensuring these requirements are met prior to handing over the procedure to the laboratory."	We agree that the responsibility for patient counselling and verifying consent lies with the clinical team. Nevertheless, embryologists should be aware, for their own safety, about the virological tests of the patients as well as of their consent to the treatment.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	23	8.0	In the Netherlands, it is not common practice to ask for informed consent for an IVF treatment. There is, however, a mandatory embryo cryostorage agreement	This is a general recommendation paper and therefore consideration of specific legal and regulatory issues in different countries is understandably not possible.
7	Liliana Ramos	23	R8.6	Define the diameter of ovarian follicles seen at ultrasound (small follicles are seen at ultrasound but these will not be mature at the time of OPU).	This is a medical issue. According to the Maribor Consensus Document (Hum Repr Open, 2021), "Clinicians may choose the follicle size upon which final oocyte maturation is triggered on a case-by-case basis. Most often, final oocyte maturation is triggered when

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					several of the leading follicles are between 16 and 22 mm".
31	Koen Wouters	23	8.6	I would suggest only aspirated as ultrasounds may vary and are not always correct, is also mostly of two days before.	The intention of the comment is unclear.
26	Sheila Mae Poulain	23-24	410-411	Consider adding the following: 8.7 Aspiration pressure and flow rate during follicular puncture should be standardized and monitored, as excessive negative pressure may increase mechanical stress and compromise oocyte integrity. 8.8 Handling time outside controlled temperature and gas conditions should be minimized, with clearly defined internal time targets for transfer from retrieval to culture. 8.9 Validated, temperature-controlled transport systems should be used when transferring follicular aspirates from operating room to laboratory to prevent thermal fluctuation. 8.10 If follicular flushing is performed, the number of flushes and media used should be documented, and the clinical benefit of flushing should be periodically reviewed. 8.11 Exposure of retrieved oocytes to atmospheric oxygen levels should be minimized; where feasible, handling should occur in reduced oxygen environments consistent with culture conditions. 8.12 The degree of blood contamination in aspirates should be noted, as excessive contamination may affect oocyte identification and quality assessment. 8.13 Needles, tubing, aspiration sets, and collection tubes should be lot-traceable and validated for embryo safety. 8.14 The type of anesthesia used during retrieval should be documented, as certain agents may theoretically influence oocyte competence. 8.15 Quality Indicator Monitoring In addition to retrieval rate, laboratories should monitor: • Mature (MII) oocyte rate • Degeneration rate • Empty follicle rate	The majority of the suggested comments are already covered throughout the document.

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				• Oocyte–follicle size correlation These indicators may assist in evaluating procedural efficiency and stimulation protocols. 8.16 Contingency procedures should be established for: • Power interruption during retrieval • Incubator malfunction • Witnessing mismatch during dish preparation 8.17 Oocyte retrieval outcomes should be periodically reviewed by operator to identify variation in technique or training needs. 8.18 Clear communication between clinician and embryologist during retrieval should be maintained to confirm follicle count, suspected empty follicles, and procedural anomalies.	
9. Insemination of oocytes					
7	Liliana Ramos	/	/	I miss the possibility of the use half-half inseminations with IVF+ICSI: as many countries are performing only ICSI to prevent TFF, it might be a good alternative to use IVF+ICSI inseminations to overcome the absolute use of ICSI only	Outside of scope for this recommendations paper and it is dependent on local policy.
25	Irene Cuevas-Saiz	24-25	/	I´m missing some reference to "timing". I know in some cases it is difficult to perform cIVF or ICSI in specific timing (workflow, workload,…), but nowadays there is enough bibliography that supports that insemination (either IVF or ICSI) should be performed, if possible, around 40 hours post trigger and no longer than 42 hours later in order to improve clinical outcomes. As this document is a "good practice" one, I think this should be at least mentioned as a recommendation. Clinical outcomes can be influenced by timing as other issues listed here, and it´s important to keep in mind the concept of oocyte ageing in culture. Here you can find some references that can be used: - L T M Vandenberghe, S Santos-Ribeiro, N De Munck, B Desmet, W Meul, A De Vos, H Van de Velde, A Racca, H Tournaye, G Verheyen, Expanding the time interval between ovulation triggering and oocyte injection: does it affect the embryological and clinical outcome?, Human Reproduction, Volume 36, Issue 3, March 2021, Pages 614-623,	The literature was reviewed by the working group and it was not strong enough evidence to be included for recommendations.

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				https://doi.org/10.1093/humrep/deaa338 - M Carvalho, F Leal, S Mota, A Aguiar, S Sousa, J Nunes, C Calhaz-Jorge, The effect of denudation and injection timing in the reproductive outcomes of ICSI cycles: new insights into the risk of in vitro oocyte ageing, Human Reproduction, Volume 35, Issue 10, October 2020, Pages 2226–2236, https://doi.org/10.1093/humrep/deaa211 - Wang, X., Xiao, Y., Sun, Z. et al. Effect of the time interval between oocyte retrieval and ICSI on embryo development and reproductive outcomes: a systematic review. Reprod Biol Endocrinol 19, 34 (2021). https://doi.org/10.1186/s12958-021-00717-0 - Novo S, Yeste M, Martiñá L, Boninu F, Rovira S, Moffa F, Antich M. Examining the impact of denudation and ICSI timing on embryological and clinical outcomes in oocyte donation cycles. J Assist Reprod Genet. 2025 Dec 8. doi: 10.1007/s10815-025-03770-5. Epub ahead of print. PMID: 41359227.	
24	Stefan Matik	24	419-420	Here the indications should be thawed oocytes and preimplantation genetic testing (PGT) cycles. And not thawed oocytes in preimplantation genetic testing (PGT) cycles.	The sentence was adapted.
14	Alessio Paffoni	24	420	"There may be specific treatments where ICSI is indicated, such as for thawed oocytes in pre-implantation genetic testing (PGT) cycles (ESHRE Add-ons working group, et al., 2023).". The sentence is unclear. It probably means thawed oocytes OR pre-implantation geentic testing". However, as in my previous point, I believe that regarding PGT, this statement is not fully supported by current evidences.	The sentence was adapted.
31	Koen Wouters	24	420	typo, should be AND	The sentence was adapted.
9	Byron Asimakopoulos	24	9.1	To my opinion, Conventional IVF (cIVF) is a poorly standardized method and this working group has a nice opportunity to clarify/standardize at least some points concerning the application of cIVF. 1. cIVF in tubes or plates? Initially, tubes were used for conventional IVF, but these were gradually replaced by plates of various types that were more practical. However, even in this issue, a standardization is needed.	IVF is indeed done differently across the industry and many methods work effectively. Local procedures need to be validated using KPIs.

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				2. Cumulus oocyte complexes as they recovered or after a reduction of the mass of cumulus cells? Oocyte stripping is not recommended in conventional IVF. However, many colleagues reduce the mass of cumulus cells by cutting off a part of it before fertilization. It is reasonable to assume that in this way the oocyte and sperm are “facilitated” as the competition for vital sources of energy substrates is limited within the culture drop, which has a limited volume. However, the relevant studies are lacking. 3. What is the optimum volume of the culture medium? The volume of the culture medium in which the oocyte and sperm will be placed is also an issue that requires clarification-standardization. This becomes particularly important when more than one cumulus-oocyte complexes are placed. A very large volume of culture medium may reduce the chances of fertilization, especially if the spermatozoa do not have good motility. On the other hand, a very small volume of culture medium may cause a lack of energy substrates and accumulation of metabolic by products, especially if the time that the oocytes remain together with the sperm is prolonged. 4. How many cumulus oocyte complexes per culture drop? The number of cumulus-oocyte complexes is related to the volume of the culture medium and the total number of spermatozoa placed in it. Actually in this matter, every clinical embryologist has a different practice. 5. How many spermatozoa per cumulus oocyte complex? This issue has been of considerable concern to embryologists. It is important on the one hand for the successful fertilization of oocytes, and on the other hand for the avoidance of polyspermy. The relevant studies showed that relatively small sperm concentrations have better fertilization rates, while at the same time they showed that the optimal concentrations also depend on the overall quality of the sperm samples. However, it should be noted that the number of sperm with progressive motility also depends on the volume of the culture medium drop and the number of cumulus-oocyte complexes. In cases of “crowded” culture medium drops	

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				with many cumulus oocyte complexes and a large number of spermatozoa, there is a profound risk of accumulation of metabolic and degenerative by-products (e.g. reactive oxygen species). Here, it is important to make a recommendation not for the sperm concentration in the processed sperm but for the number of spermatozoa per cumulus oocyte complex. 6. What is the optimal duration of co-culture? According to traditional practice, oocytes and sperm should remain in the same culture drop for 16 hours. Their separation is done when assessing the presence of pronuclei. This practice is still used today. However, many laboratories prefer a short co-culture of one, two, four or six hours. In short co-culture, the oocytes that have released a second polar body are considered fertilized. Those that do not have a second polar body are subjected to rescue ICSI. This practice seems to be advantageous in terms of the timely application of rescue ICSI as well as in that any destructive effects of hyaluronidase released by sperm on the oocytes are avoided. The adverse change in parameters of the culture medium from the prolonged coculture of oocytes and sperm is also avoided and such changes are more likely to occur when the culture drop is very small and the number of sperm and oocytes is large. In the above sentences I simply raise controversial issues and do not suggest solutions or cite relevant bibliography. If you agree that further standardization is needed, then I can come back with specific suggestions. At this moment, the only citation I would like to take into consideration is the recently published very good review by Carullo et al (2026). Carullo et al (2026). Short conventional IVF, big impact: navigating protocol variations towards standardization. RBMO, 52 (1): 105214	
27	Maria Filippa	24	9.1 9.2	Supplementary Data on suggested step by step SOPs for IVF and ICSI describing different culture set ups with different types of dishes and culture media, describing the consumables being used stating their material, their sizes, their packaging would be useful	The working group considers this out of scope for this Good Practice Recommendations paper.

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13	Fikret Gürkan Agircan	24	422/ 9.1.1	Caution must be taken when using microfluidic sperm sorting devices (such as ZyMōt) for conventional IVF although it was marketed for it as well. With earlier protocols, some clinics observed higher rates of total fertilization failure and lower overall fertilization rates, prompting them to discontinue its use for this procedure. To address this, recent updates to the manufacturer's instructions now require an additional centrifugation step after the microfluidic sorting process. Please see this publication as well: PMID: 39723883	Thank you for your remark.
7	Liliana Ramos	24	R9.1.2	I suppose you mean "oocyte insemination" and not "oocyte retrieval" here	The sentence was adapted.
13	Fikret Gürkan Agircan	24	422/ 9.1.2	I think the aim was to say to check everything once more before **insemination**: "An identity check before the oocyte insemination is mandatory."	The sentence was adapted.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	24	9.1.2	"An identity check before the oocyte retrieval is mandatory" Should this be: An identity check before oocyte insemination is mandatory?	The sentence was adapted.
32	AGRBM	24	9.1.2	An identity check before **oocyte insemination** is mandatory. Or An identity check before oocyte retrieval **from the incubator** is mandatory.	The sentence was adapted.
36	David Morroll	24	9.1.2	Refers to oocyte retrieval – needs amendment (see also 9.2.2)	The sentence was adapted.
26	Sheila Mae Poulain	24	422-423	Consider adding the following: 9.1.5 The sperm–oocyte ratio should be standardized and periodically reviewed based on fertilization outcomes and polyspermy rates, particularly in cases of high sperm concentration or high oocyte yield.. 9.1.6 Laboratories should monitor rates of polyspermy and adjust insemination parameters (e.g., sperm concentration, co-incubation duration) accordingly. 9.1.7 Co-incubation should preferably occur under reduced oxygen	The points were reviewed and were either already included in other sections of the document or considered out of scope.

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				conditions (5–6% O_2) where available, as elevated oxygen concentrations may increase oxidative stress. 9.1.8 Following insemination, culture dishes should not be unnecessarily disturbed to prevent disruption of sperm–oocyte interaction. 9.1.9 The timing of fertilization check (e.g., 16–18 hours post-insemination) should be standardized to ensure accurate assessment of normal fertilization (2PN) and minimize misclassification. 9.1.10 In addition to concentration, total number of motile sperm inseminated per oocyte or per dish should be recorded to enhance reproducibility and auditability. 9.1.11 A predefined strategy should be in place for total fertilization failure (TFF), including documentation, counseling considerations, and potential use of rescue ICSI (if validated and permitted). 9.1.12 If short co-incubation protocols are used, internal validation should demonstrate non-inferiority in fertilization, embryo development, and clinical outcomes compared to standard overnight exposure. 9.1.13 Lot numbers of insemination media and oil overlay should be documented to ensure traceability in case of abnormal fertilization outcomes. 9.1.14 Personnel performing insemination procedures should undergo documented competency assessment and periodic performance review based on fertilization rates. 9.1.15 Monitoring of Key Performance Indicators (KPIs) Laboratories should regularly monitor: • Normal fertilization rate (2PN) • Polyspermy rate • Total fertilization failure rate • Cleavage rate following IVF • Clinical pregnancy rate per IVF cycle	
25	Irene Cuevas-Saiz	25	9.2.1	After EXPOSITION TO HYALURONIDASE, oocytes should be… (because of the mechanical part of denudation)	The sentence was adapted.

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18	Keerti Singh	25	9.2.1	Bullet point 3, use of Good's buffers can help minimize changes due to temperature, concentration, salt concentration, etc. especially for long duration procedures outside the incubators like oocyte collection, sperm preparation, denudation, ICSI, and cryopreservation,94 although it does not eliminate 100% of the effects	This is covered in the handling section.
32	AGRBM	24	9.2.1	In order to prevent oocyte damage, pipettes with appropriate lumen size should be used and vigorous pipetting **should be** avoided.	The sentence is correct as is.
7	Liliana Ramos	25	R9.2.2	I suppose your mean "oocyte injection" and not "oocyte retrieval"	The sentence was adapted.
13	Fikret Gürkan Agircan	25	424/ 9.2.2	I think the aim was to say to check everything once more before **insemination**: "An identity check before the oocyte insemination is mandatory."	The sentence was adapted.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	25	9.2.2	"An identity check before the oocyte retrieval is mandatory" Should this be: An identity check before the oocyte injection is mandatory?	The sentence was adapted.
32	AGRBM	25	9.2.2	An identity check before oocyte **injection** is mandatory. Or An identity check before oocyte retrieval **from the incubator** is mandatory.	The sentence was adapted.
17	Hannah Park	25	9.2.2	"**The** polar body should be away from the injection site."	The sentence was adapted.
33	Samantha Wake	25	424	Injection with polar body at 12 or 6 oclock position	The working group does not agree with the proposed modification.
17	Hannah Park	25	9.2.2	"Oocyte dysmorphisms such as large perivitelline space, localised granularity and smooth endoplasmic reticulum aggregates **may be** associated with diminished clinical success."	The sentence was adapted.
30	Deutsche Gesellschaft für Reproduktions	25	9.2.2	Add: Oolemma rupture should be assured prior to sperm injection **and the volume of PVP or media into the oocyte should be reduced to a minimum during ICSI**.	A sentence was added to the Good Practice Recommendations document.

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	medizin (DGRM)				
22	Zuzana Holubcová	25	424	The terminology describing oocyte dysmorphisms would benefit from clarification and harmonisation with commonly used embryology consensus terminology. The term “localised granularity” may be ambiguous; consideration could be given to specifying “central cytoplasmic granularity” or providing a definition. In addition, smooth endoplasmic reticulum aggregates/discs terminology should be clearly defined. Consider also addressing other frequently encountered dysmorphisms such as cytoplasmic vacuoles, which are commonly recorded in clinical practice and may influence decision-making. Greater precision would support consistency in training and inter-laboratory interpretation.	This wording is taken from the updated Istanbul consensus and aligns with industry terminology.
18	Keerti Singh	25	9.2.2	Can include this - Preparation of oocytes for ICSI – role of plasticware being extensively used in IVF labs for oocyte retrieval, BPA embryo toxic component commonly present in plastics used for ART. All plastic material used in ART Labs must be approved by FDA and EEA. - Oocyte retrieval needles (single or double lumen and 17/18 gauge) and embryo transfer catheters (soft & firm). Soft catheters preferred, can be used with a malleable stylet. Reference : Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7. - Page 150 left column last para lines 6-12. - Page 148 Rt column para 2 Page 147 (left column last para), Page 147 (Rt column para 1),	The working group considers this out of scope for this Good Practice Recommendations paper.
18	Keerti Singh	24	9.2.2	Bullet point 2, after pipettes with appropriate lumen size please include detailed information regarding the inner and outer diameters of the holding and injecting pipettes “The two different micropipettes used for gamete micromanipulation during ICSI are holding pipettes (inner diameter: 18-25 µm; outer diameter: 80-150 µm) to fix and position the oocytes, and the injecting pipettes (inner diameter: 4.8-5.6 µm; outer diameter:	The working group considers this out of scope for this Good Practice Recommendations paper.

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				6.8-7.8 µm) to immobilize, aspirate and inject the sperm" Reference: Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7. - Page 148 left column last para lines 13-18.	
36	David Morroll	25	9.2.2	Fourth bullet point: number of oocytes should be related to operator skill and sperm quality in the context of the time period in which control of conditions (pH and temperature) is deemed satisfactory	This is covered in the handling section.
7	Liliana Ramos	25	R9.2.2 bullet 5. Sub 3	Morphological normal, motile sperm should be injected: this is for ejaculated sperm, as TESE samples the criteria might differ	Language is 'should' this infers that it's where possible. No change was made to the sentence.
36	David Morroll	25	9.2.2	"Morphologically normal, motile sperm should be selected." This does not account for relatively rare but inevitable cases with apparently 0% normal forms (but, say, lots of borderline morphology) or immotile sperm using HOS test (as mentioned below bullet point)	The wording used is appropriate.
32	AGRBM	25	9.2.2	The duration of sperm identification and immobilisation followed by injection should be minimised. Add: In case of low sperm count the retrieval of single sperm has to be done before placing the oocytes into the dish.	This is covered in the handling section.
32	AGRBM	25	9.2.2	The evidence for smooth endoplasmic reticulum aggregates as cause for diminished success is questionable (see Mateizel et al. 2013).	This wording is taken from the updated Istanbul consensus.
32	AGRBM	25	9.2.2	Morphologically normal, motile sperm should **primarily** be selected.	The working group does not agree with the proposed modification.
17	Hannah Park	25	9.2.3	"It is however recommended for complete activation failure (0% 2 pronuclei (PN)), very low fertilization (<30%), or globozoospermia**.**"	The sentence was adapted.
32	AGRBM	25	9.2.3	It is however recommended **in cases of previous** complete activation failure	The sentence was adapted.
26	Sheila Mae Poulain	24-25	424-425	Consider adding the following: 9.2.X Where feasible, consideration should be given to minimizing spindle disturbance during injection. Excessive oocyte rotation or prolonged manipulation should be avoided.	The points were reviewed and were either already included in other sections of the document or considered out of scope.

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				9.2.X Injection pressure and aspiration force should be standardized to minimize cytoplasmic turbulence and reduce oocyte degeneration rates. 9.2.X The duration of oocyte exposure outside controlled incubation conditions during micromanipulation should be minimized and internally audited. 9.2.X Laboratories should adopt a consistent orientation policy (e.g., polar body at 6 or 12 o'clock) based on validated internal protocol. 9.2.X If PVP or alternative viscous agents are used, lot validation and toxicity testing should be documented. 9.2.X When using high-magnification selection (IMSI), microfluidics, or other advanced sperm selection techniques, these methods should be internally validated for fertilization and downstream clinical outcomes. 9.2.X In addition to morphology and motility, sperm origin (ejaculate, epididymal, testicular, fresh, frozen-thawed) should be recorded for traceability and outcome monitoring. 9.2.X Micromanipulators, injectors, and heated stages should undergo routine performance verification to ensure mechanical precision and temperature stability. 9.2.X In cases of total fertilization failure or other unexpected outcomes, a predefined clinical and laboratory review procedure should be initiated and formally documented. 9.2.X Personnel performing ICSI should undergo periodic competency evaluation based on: • Fertilization rate • Degeneration rate • Abnormal fertilization rate • Clinical outcomes	
36	David Morroll	25	9.2	There is no mention of the role of rescue ICSI which is gaining in popularity.	The working group discussed this, however, there is not enough evidence, and the procedure is not standardised. Therefore, it was not included in this document.

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10. Scoring for fertilisation					
36	David Morroll	26	10	Scoring for fertilization: again, no mention of checking for appearance of 2nd PB for potential rescue ICSI.	The original text says: "All inseminated or injected oocytes should be examined for the presence of PN and polar bodies". So it mentions checking/annotation of the second polar body. However, for more clarity, "(first and second)" was added.
7	Liliana Ramos	26	R10.2	… and fertilized oocytes transferred into new dishes **or new clean dropplets** (some dishes are designed to hold COC and culture wells without the need to use a new dish.	The requested modification refers to a situation that was implicit in the original text. A small relevant note was added
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	26	10.3	If no PNs are observed, there is uncertainty if it could have been a 1PN, 2.1PN or 3PN that underwent NEBD. So the same caution should apply to this category as at least the 1PN category As the first polar body often divides in human embryos, having 2 polar bodies is not a very reliable parameter for fertilization	This text was adapted from the Istanbul consensus. The reader is referred to this document for more extensive information.
28	Labadi Leila	26	10.3	It is suggested to add a recommendation on how to report PN not observed in medical records or to patients to prevent confusion regarding the biological status of embryos showing normal development	Added sentence in response to the comment "The clinical use of zygotes with atypical pronuclear patterns should be fully traceable."
26	Sheila Mae Poulain	26-27	426-428	10.X The exact time post-insemination or post-ICSI at which fertilisation assessment is performed should be documented to ensure consistency and reproducibility of scoring. 10.X Abnormal fertilisation patterns (e.g., 3PN, ≥4PN, multinucleation at zygote stage) should be recorded and monitored as part of laboratory quality indicators. 10.X Pronuclear morphology (alignment, size symmetry, nucleolar precursor body distribution) may be recorded where validated internal scoring systems are used. 10.X Laboratories should periodically review correlations between fertilisation category (2PN, 1PN, 2.1PN, PN not observed) and subsequent	All such comments are correct: however, they focus on aspects that are in fact covered (e.g. measures accompanying the clinical use of zygotes with atypical pronuclear pattern) or relevant to other documents (e.g. comments on KPIs, whose recommendations may be found in the ESHRE/Alpha The Vienna Consensus; comments on NPB, whose recommendations may be found in the revised Istanbul Consensus)

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				blastocyst development, euploidy rates (if applicable), and clinical outcomes. 10.X Oocyte degeneration observed at fertilisation check should be recorded and monitored as a KPI. 10.X Zygotes exhibiting clearly abnormal fertilisation (e.g., 3PN) should be segregated from normally fertilised embryos according to laboratory policy and regulatory requirements. 10.X Embryos should not be prematurely classified as unfertilised without confirmation of polar body status and appropriate observation timing. 10.X The clinical use of 1PN, 2.1PN, or PN not observed embryos should follow a written, ethically approved, and multidisciplinary policy framework. 10.X Laboratories should monitor: • Normal fertilisation rate (2PN per MII) • Total fertilisation failure rate • Abnormal fertilisation rate (≥3PN) • Oocyte degeneration rate Operator-specific analysis may be performed in ICSI cycles.	
11. Embryo culture					
4	Shridhar S Amanchi	/	/	More practical guidance on droplet volume, number of embryos per dish, and oil quality checking would help standardize culture conditions across labs.	The present GPR is based on the available evidence in the scientific literature. There is no agreement on the ideal droplet volume or in the number of embryos per dish. Nevertheless, we recommend that this information is recorded (point 11.1.4).
4	Shridhar S Amanchi	/	/	Clear suggestions on gas calibration frequency, acceptable recovery time after door opening, and how long embryos can remain outside the incubator would be helpful.	A sentence was added to the embryo culture section.
4	Shridhar S Amanchi	/	/	Sometimes embryos reach early blastocyst stage but are not fully expanded at the planned biopsy time. Practical suggestions on whether to wait, proceed, or reassess later may be helpful.	Indications on blastocyst biopsy are given in the ESHRE PGT Consortium and SIG-Embryology Biopsy Working Group document, 2020, as written in point 12.

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20	Shivansh Jaiswal	/	/	Where infrastructure allows, consideration may be given to stronger supportive language for reduced oxygen culture systems in light of consistent evidence trends demonstrating improved blastocyst development.	The working group reviewed the evidence and found it lacking to base recommendations on.
16	Manuela Puchner	/	/	No information is provided what to do if there is a contamination in the embryo culture (yeast, bacteria etc.). Should we wash, can we transfer thereafter. or should the embryos be discarded?	A sentence was added to the document.
36	David Morroll	27	432	This really should include Swain J et al. Reprod Biomed Online 2012; 24 142-147.	The paper by Swain et al., 2012 is indeed included in the review by Wale and Gardner, 2016.
27	Maria Filippa	27	436-438	Optimal values in incubators for temperature, gas concentrations, with or without humidity, embryo culture in a table form according to different culture media would be informative for reference	The requested values are strictly related to the type of media used as well as to the type of culture system. The given recommendation is to follow the manufacturer's recommendations (see 11.1.1 with the current correction).
33	Samantha Wake	28	440	Specifically 5% oxygen	The working group agrees with the reviewer. An addition to the recommendation was made.
36	David Morroll	27	438	Gatimel cites the need for monitoring of pH rather than gas concentrations – suggest adding pH to the parameters listed	The working group agrees with the reviewer, pH was added to the parameters listed.
23	Susanna Apter*	27	11.1.1	A comment on ensuring that media change is performed according to the manufacturer's recommendations could be added.	The working group agrees with the reviewer.
36	David Morroll	27	11.1	It would be preferable to give some indication of what is considered optimal pH for gametes (noting that sperm prefer higher pH0 and embryos.	As written in section 11, the pH as well as the other variables have been arbitrarily set. It is important to follow the manufacturer's recommendations.
18	Keerti Singh	27	11.1.2	Can include this - The type of oil used during embryo culture oil overlay influences embryo development; mineral oil can also be embryo toxic. Reference : Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7.	The working group agrees with the reviewer. A sentence was added to the document.

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				- Page 151 left column, para 2 lines 4-7. - Page 148 Rt column para 1 lines 2-5.	
23	Susanna Apter*	27	11.1.2	Original text: 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. Suggestion: Testing of oils with different viscosities indicates differences in protection against changes in osmolality or pH depending on the oil viscosity (Mestres et al). There is however no solid evidence that this also results in better protection leading to differences in laboratory KPIs as well as clinical outcomes	The sentence was added to the document.
18	Keerti Singh	28	11.1.3	Can include - Since the incubators are crucial for creating optimal culture conditions. the choice of the most appropriate culture system depends on lab design and the type of incubators, it would be recommended to have at least two different kinds of incubators to start off with (small benchtop models (portable)/ large incubators (holding or storage). The use of single-step media combined with time-lapse technology allows uninterrupted culture from pronuclear to blastocyst stage, minimizing the changes in temperature and pH throughout in-vitro culture. Reference: Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7. - Page 152 left column, para 1 & 2. - Page 152 Rt column 'Conclusions" lines 4-14.	The decision to have two different types of incubators is quite subjective, and cannot be considered a recommendation. The use of single-step media is commented in point 11.1.1.
25	Irene Cuevas-Saiz	28	11.1.5	It would be interesting to define "low". 2 to 7%? Below 10%?	The working group agrees with the reviewer. An addition to the recommendation was made.
26	Sheila Mae Poulain	27-28	440-441	11.1.X Appropriate preparation and handling of culture media are critical to ensure optimal physicochemical stability and embryo homeostasis. All procedures related to media preparation should be standardised, documented, validated, and subject to regular quality control. 11.1. X Media preparation shall be performed under validated aseptic	The working group reviewed the suggestions, most are already covered in other sections or considered out of scope for this document.

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				conditions within an appropriate clean-air environment (e.g., Class II biological safety cabinet or laminar airflow workstation). 11.1.X Surface disinfection procedures shall be standardised, including defined disinfectants, concentrations, and contact times. 11.1.X Only embryo-tested sterile consumables shall be used for preparation. 11.1.X Exposure of opened media containers to ambient laboratory air shall be minimised. 11.1.X Culture media shall be warmed gradually and in a controlled manner prior to use. Repeated warming and cooling cycles of unopened or opened bottles shall be avoided unless validated. 11.1.X Vigorous agitation, vortexing, or foaming of media shall be avoided to minimise gas loss and pH instability. 11.1.X Droplet volume, number of droplets per dish, and oil overlay volume shall be standardised and documented. 11.1.X Prepared culture dishes shall be clearly labelled with: • Media name • Preparation date and time 11.1.X Prepared culture dishes shall be pre-equilibrated in incubators under validated temperature and gas conditions prior to clinical use. Minimum equilibration times shall be defined and validated according to incubator type and culture system. 11.1. X Overloading incubators with excessive numbers of dishes during equilibration should be avoided unless validated. 11.1.X Verification of pH and or osmolality of prepared culture systems should be performed periodically, particularly: • When changing media or oil batches • When modifying droplet size or equilibration time • After incubator maintenance • Following unexplained shifts in clinical KPIs 11.1.X Recovery kinetics of temperature and gas concentrations following incubator door opening should be validated and documented.	

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				11.1.x Culture media and prepared dishes should be protected from excessive light exposure during preparation and handling. 11.1.X Any change in media brand, formulation, oil type, dish type, droplet size, incubator type, or gas settings shall undergo documented validation prior to implementation. 11.1.X Clinical and laboratory KPIs shall be monitored following any change to confirm maintained performance.	
12. Biopsy procedure					
4	Shridhar S Amanchi	/	/	Practical guidance on zona opening (laser hole size) would be very useful. Too small → difficult TE aspiration and repeated pulling Too large → risk of blastocyst collapse or cell loss A suggested range based on expansion stage would help standardization. Some practical clarification on commonly used opening size ranges or decision-making based on blastocyst expansion stage may help standardize biopsy practice.	The reviewer is referred to the ESHRE recommendation paper for embryo biopsy for further details.
4	Shridhar S Amanchi	/	/	Guidance on ideal number of cells to remove depending on blastocyst quality would be helpful, as lower-quality embryos may not tolerate removal of many cells.	The reviewer is referred to the ESHRE recommendation paper for embryo biopsy for further details.
4	Shridhar S Amanchi	/	/	Simple advice on when re-biopsy should be considered and when it should be avoided would support decision-making.	This is already adequately described in the existing text.
4	Shridhar S Amanchi	/	/	Some embryos collapse after zona opening. Practical guidance on how long to wait before biopsy would help embryologists.	The working group considers this outside the scope of the current document.
4	Shridhar S Amanchi	/	/	Strong emphasis on double witnessing, correct labelling, and careful handling during tubing and shipment is important to avoid sample mix-ups.	Witnessing and its importance are adequately covered in the document in section 4.
4	Shridhar S Amanchi	/	/	In daily practice, embryologists frequently face borderline-quality blastocysts where biopsy decisions are challenging. Brief practical considerations for selecting such embryos for biopsy may support consistent decision-making.	The working group considers this outside the scope of the current document.
20	Shivansh Jaiswal	/	/	Clarification of recommended trophectoderm sampling ranges may assist in balancing diagnostic reliability with embryo safety within contemporary PGT practice.	The reviewer is referred to the ESHRE PGT papers for further information.

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33	Samantha Wake	28	447	ICSI for PGTM. For PGTA the genetics lab accepts IVF insemination. No longer risk for contamination.	The sentence states “preferred method” not “recommended method”.
36	David Morroll	28	12.1	Depending on amplification methods, conventional IVF can be used	The sentence states “preferred method” not “recommended method”.
14	Alessio Paffoni	28	458	"ICSI is the preferred method for PGT, as it reduces the risk of contamination from both maternal and paternal sources". My comment is that conventional IVF can be used in cases of IVF with preimplantation genetic testing for aneuploidy in the absence of male factor infertility, as it is safe, effective, and not inferior to ICSI according to current evidence and guidelines. Multiple large cohort studies, randomized trials, and recent US guidelines suggest that conventional IVF and ICSI yield comparable rates of euploid embryos, embryos suitable for transfer, and live birth rates in PGT-A cycles with non-male factor infertility. The theoretical risk of genetic contamination with conventional IVF has not been substantiated using current next-generation sequencing platforms, and the American Society for Reproductive Medicine and the American College of Obstetricians and Gynecologists both state that ICSI is not routinely required for PGT-A except in rare cases where contamination could affect test accuracy or when PGT-M is performed. The maternal contamination through cumulus cells is also debatable since both ICSI and cIVF include the removal of those cell well before blastocyst development. Supporting papers: - De Munck N, El Khatib I, Abdala A, et al. Intracytoplasmic sperm injection is not superior to conventional IVF in couples with non-male factor infertility and PGT-A. Hum Reprod. 2020. - Tozour JN, Arnott A, Akerman M, et al. Comparison of outcomes between ICSI and IVF with PGT-A: analysis of SART CORS data. Fertil Steril. 2024. - Li X, Li Q, Chang Y, et al. Conventional IVF enhances PGT-A outcomes in couples with non-male factor infertility. Sci Rep. 2025. - Practice Committee. The use of PGT-A: a committee opinion. Fertil Steril.	The sentence states “preferred method” not “recommended method”.

NR	Reviewer	Page	Line	Comment	Action / Reply
				2024. - Iwamoto A, Van Voorhis BJ, Summers KM, Sparks A, Mancuso AC. ICSI vs conventional IVF in non-male factor infertility. Fertil Steril. 2022. - Zhang S, Xie P, Lan F, et al. Conventional IVF is feasible in PGT-A. J Assist Reprod Genet. 2023. My proposal: "ICSI is often used in PGT cycles to help minimize the theoretical risk of DNA contamination (residual cumulus cells and sperm attached to the zona pellucida). However, for PGT-A this contamination risk has not been clearly demonstrated with current biopsy and testing workflows; therefore, the choice between ICSI and conventional IVF should be guided by laboratory procedures and patient-specific factors rather than assumed clinical superiority. Meticulous denudation and washing steps remain essential to minimize maternal contamination using conventional IVF or ICSI."	
31	Koen Wouters	28	464	There are also some difficulties with time-lapse culture for PGT: blastocysts that grow out of the well, embryos that jump out of place (we sometimes see this when changing the medium), numbering is limited to 16, when more embryos are available, you need a scale with the same numbering from 1-16, this creates a risk of taking the wrong embryo, higher caution is needed!	A new sentence added: "Accurate tracking (numbering) of embryos should be ensured, especially when multiple culture dishes are used."
33	Samantha Wake	29	470	It is advised to perform biopsy on fresh embryos. Rather than thawing blastocyst or thawing from cleavage stage, ensuring freezing occurs only once where possible.	This is already adequately described in the existing text.
28	Labadi Leila	29	478-480	To support the recommendation of removing 2- 6 TE Cells, the addition of visual reference guide or representative images would be highly beneficial for training embryologists to avoid excessive biopsy	The working group considers this outside the scope of the current document.
27	Maria Filippa	28-29	12.4 12.5 12.6	SOPs of embryo biopsy as supplementary data would be of use to state the optimal type & size of the biopsy and holding biopsy to be used as well as the optimal power in msecs for the laser shot to either open the ZP or to cut the TE cells biopsied and tips & tricks on tubing would be highly appreciated for reference	The reviewer is referred to the ESHRE recommendation paper for embryo biopsy for further details.

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23	Susanna Apter*	29	12.5	The flicking technique also requires the use of a laser to create a clean cut between TE cells. From the ESHRE PGT Consortium and SIG Embryology good practice recommendations for polar body and embryo biopsy for PGT (2020): "Laser pulses are directed at the junctions between cells to either excise the aspirated cells from the blastocyst or to minimise cell damage while detaching TE cells mechanically via a quick flicking movement of the biopsy pipette against the holding pipette. It is recommended to fire as few laser shots as possible." The pushing technique is not mentioned. This should be applied when assisted hatching is not performed before embryos reach the blastocyst stage.	The pushing technique is a method to collapse the blastocyst, not to remove TE cells.
25	Irene Cuevas-Saiz	30	513	… the total number of TRANSFERABLE blastocysts (this change will include mosaic or normal embryos after PGT-M, PGT-SR; euploid blastocysts is more in line with a PGT-A approach, but the recommendation is for all PGT types).	The sentence already reads: "…the total number of euploid blastocysts available for transfer…"
13. Embryo transfer					
15	Alev Özer	30-31	/	Comment: Where evidence is inconsistent (catheter loading nuances, flushing, volumes, retained embryo management), the guideline accurately notes uncertainty but could better support clinical quality by recommending centre-level standardisation and audit. This is particularly relevant because ET is a high-impact, operator-dependent step with measurable non-conformances (e.g., retained embryo events). Proposed text addition :"In technical areas where evidence is inconsistent, centres should implement an internally standardised ET protocol defining: catheter selection criteria, loading method, acceptable transfer volume range, retained embryo management algorithm (including mandatory post-transfer catheter inspection and documented re-transfer procedure), and periodic audit of key ET indicators (retained embryo rate, difficult transfer rate, blood/mucus contamination rate, and implantation outcomes)."	Thanks for your comment. After discussion, this is not a clinical guideline. And eco-guidance concerns to the clinical technique.

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				Full-text supporting literature • D'Angelo A. et al. Evidence and consensus on technical aspects of embryo transfer. 2022. https://academic.oup.com/hropen/article/2022/4/hoac038/6692715 https://www.researchgate.net/publication/363330843_Evidence_and_consensus_on_technical_aspects_of_embryo_transfer/fulltext/631ba737071ea12e361f1582/Evidence-and-consensus-on-technical-aspects-of-embryo-transfer.pdf • Brown J. et al. Ultrasound versus 'clinical touch' for catheter guidance during embryo transfer. Cochrane 2016. https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD006107.pub4/pdf/full • Matitashvili T. et al. Effect of embryo catheter loading technique on pregnancy outcomes. 2022 https://pmc.ncbi.nlm.nih.gov/articles/PMC9175598	
27	Maria Filippa	30	13	Clinical & lab ET SOPs as demo for guidance especially describing the criteria for ET grading in a table would be highly beneficial	This is covered in the embryo culture section, where we are referring to the updated ESHRE and Alpha Istanbul Consensus.
33	Samantha Wake	31	522	Warmed flush media can be given to doctors to clean the cervix, before inserting the outer catheter	This concerns the clinical transfer, which is outside the scope of this document.
33	Samantha Wake	31	522	Both outer and inner catheters should be checked by embryologist for retention of embryo	The sentence was adapted.
7	Liliana Ramos	30	R13.1 bullet 6	Sometimes at the time of Et, the fate of supernumerary embryos is not known yet (eg, transfer on day 3 and cryo on day 5 and 6). At the end of the total culture period, the fate of each embryo should be registered (but not necessary at the time of ET)	Thank you for your comment. We will add your suggestion in general specifications.
7	Liliana Ramos	30	13.4	Eliminate "are described in DAngelo 2022" as the reference is mentioned in the next column	The reference was adapted.
7	Liliana Ramos	31	13.4	This recommendation is not clear and therefore, it should not be placed here (there are a few references in favour to perform an afterload…)	Thanks for your comment: the points of 13.4 follow the same order than previous ESHRE paper

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7	Liliana Ramos	31	13.4	Paragraph about the use of specific transfer media: it is not clear if this is recommended or not; in the Adds-on guideline it stated to be used in study setting	This text is from the Good Practice Recommendations on Add-ons paper.
7	Liliana Ramos	31	13.4	Paragraph about the volume of media for Et is not clear formulated	The sentence was adapted to clarify the recommendation.
17	Hannah Park	31	13.4	"The use of **a** small volume of medium (10-20 µl) is recommended.."	The sentence was adapted.
18	Keerti Singh	31	13.4	-There are few references in favour to perform an afterload (double step) Vs direct (single step) techniques. " the afterloading technique for embryo transfer catheter transfer is a refined method designed to minimize uterine trauma and improve implantation rates". Reference - The impact of difficulties encountered during embryo transfer on the outcome of IVF and ICSI Sallam, Hassan Nooman et al. Fertility and Sterility, Volume 78, S231. - There are few references in favour to the softness as well as in relation to the material of the catheter used. Consider following points which are missing: Soft vs. Hard Catheters Studies including meta-analyses, confirm that using soft ET catheters results in a significantly higher clinical pregnancy rate compared to using firm or rigid catheters. - Comparison of soft catheters (such as Cook K-Jet vs Wallace) showed no significant difference in clinical pregnancy rates (approx. 29-30%). - use of air transfer catheter for positioning embryos does not affect IVF cycle success. - severe blood on the catheter tip post transfer associated with reduced implantation and clinical pregnancy rates, whereas presence of mucus does not affect outcomes. - Evidence-informed practice indicates ultrasound-guided transfers, performing a "dummy" transfer first, and using a soft catheter improves success. Reference: Sallam HN, Gelbaya TA, Maldonado Rosas I, Anagnostopoulou C, Sallam N, Agarwal A. Clinical aspects of oocyte retrieval and embryo transfer: tips and tricks for the novice and the expert. Panminerva Med	Some aspects of the transfer technique are considered clinical instead of embryological. We appreciate your comments and we will add the reference : Sallam HN, Gelbaya TA, Maldonado Rosas I, Anagnostopoulou C, Sallam N, Agarwal A. Clinical aspects of oocyte retrieval and embryo transfer: tips and tricks for the novice and the expert. Panminerva Med 2022;64:185-99. DOI: 10.23736/S0031-

NR	Reviewer	Page	Line	Comment	Action / Reply
				2022;64:185-99. DOI: 10.23736/S0031-0808.22.04679-1	
36	David Morroll	31	13.4	The recommendation of 10-20ul for transfer is not well supported and should be balanced with a call for more data.	Less than 10 µl is detrimental. The text already states that using more than 20 µl had controversial results in clinical outcomes.
30	Deutsche Gesellschaft für Reproduktionsmedizin (DGRM)	31	13.5	The embryo should be **re-transferred** immediately, preferably with a new catheter, after embryo retention.	The sentence was adapted.
32	AGRBM	31	13.5	The name in the citation is missing: There are few references in favour to the softness as well as in relation to the material of the catheter used. (XXX et al. 2017, Ebner et al., 2001)	The citation was corrected.
32	AGRBM	31	13.5	The embryo should be **re-transferred** ~~injected~~ immediately, preferably with a new catheter, after embryo retention.	The sentence was adapted.
14. Cryopreservation					
4	Shridhar S Amanchi	/	/	Mentioning expected survival rates after warming and suggested LN_2 tank inspection frequency would help labs monitor their performance.	Survival/success rates is not within the scope of this GPR. These can be found in the ESHRE and Alpha Vienna Consensus.
33	Samantha Wake	31	525	Blastocysts should be frozen 1 per device to reduce amount of double embryo transfers and or need to refreeze in the event patient does not want both transferred.	The sentence was adapted.
25	Irene Cuevas-Saiz	31	14.1.1	Slow freezing for sperm is the widest used technique, but not necessarily the most effective. Several groups are working on the improvement of sperm and testicular tissue vitrification and in my opinion should at least be mentioned. Here you can find a good review that could be included as a reference: Schulz M, Risopatrón J, Uribe P, Isachenko E, Isachenko V, Sánchez R. Human sperm vitrification: A scientific report. Andrology. 2020 Nov;8(6):1642-1650. doi: 10.1111/andr.12847. Epub 2020 Jul 16. PMID: 32598551.	The recommendation was adapted.

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13	Fikret Gürkan Agircan	32	14.1.3	There is a line on s: For ovarian tissue**s**	This was corrected.
30	Deutsche Gesellschaft für Reproduktions medizin (DGRM)	32	14.1.3	Comment: Ovarian tissue is mentioned here for the first and only time. Like oocytes, sperm, and testicular tissue, it is also a substance of human origin (SoHO). Therefore, it should either receive more attention, like preparation technique, in this guide or be removed from this section and add a reference to the EHSRE Guideline "Female fertility preservation" (2020)	This was discussed by the working group. Preparation of ovarian and testicular tissues are covered in other ESHRE guidance documents, however, the working group wanted to include them in the cryopreservation part.
31	Koen Wouters	32	14.1.3	Goes against your own guidelines from 2020. Should be better written, references are not correct, guideline for onco is not mentioned.	For ovarian tissues, both slow freezing and vitrification are used. Recent studies indicate comparable results regarding follicular viability and proportion of intact primordial follicles. However, cryopreservation protocols differ widely, and comparative data on results after re-transplantation are lacking. Vitrification of ovarian tissue may therefore still be considered innovative.
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	32	14.1.4	This description is very unclear to me. Is prepubertal tissue meant by "testicular tissue, not expected to contain sperm"? And it is unclear what "a protocol for sperm cryopreservation" refers to. Does that include sperm processing before freezing?	This text is the recommendation from the Good Practice Recommendations paper on fertility preservation in prepubertal boys. The reviewer is referred to that document for further details.
31	Koen Wouters	32	14.1.4	Not clear what is mentioned here, what is then the source of the sample?	The text states it concerns testicular tissue, obtained by testicular biopsy.
32	AGRBM	32	14.1.4	This paragraph is not clear. The rationale of freezing tissue without sperm is not clear. Or should tissue regardless if there are sperm or no sperm, be frozen? If the whole testicular tissue for sperm retrieval is cryopreserved, the medium should always contain glycerol. Then the sperm are kept intact.	This is exactly the point, for testicular tissue containing spermatogonial cells but no sperm, a different freezing protocol is recommended than for testicular tissue containing sperm.

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				If the samples are processed, the sperm containing product can also be frozen, also then with glycerol. The protocols are normally the same. There is only one exception: if the testicular tissue only contains spermatogonial stem cells, like e.g. in prepubertal boys, the samples should not be frozen only with glycerol, but instead with e.g. alcohol and DMSO to keep the stem cells intact. Of course there are different handling protocols: - the samples are taken to the lab directly after the operation and each sample is mazerated; if sperm are found, the samples are cryopreserved using glycerol – either one by one or as a mixture of all solutions (if sperm are present in equal amounts) - alternatively, the samples are taken to the lab, each piece is digested enzymatically; if sperm are found, the samples are frozen using glycerol – either one by one or as a mixture of all solutions (if sperm are present in equal amounts). - next option: the samples are taken to the lab, one piece is mazerated or enzymatically treated to detect sperm, the other samples are left intact and are cryopreserved with glycerol. In this scenario the lab assumes that all pieces are of the same quality. - lastly, the samples are taken to the lab and only a small piece of each tissue is taken away and mazerated or enzymatically treated to detect sperm, the larger piece is cryopreserved with glycerol.	
7	Liliana Ramos	32	14.1.6	Suggest to **always** cryopreserve supernumerary embryos individually as it is in line of the SET transfer policy. Also, add after "cryopreserved individually for **own use**, donated to research…" as most embryos after cryo are used by the couple and not necessary for research of donation.	The sentence was adapted.
25	Irene Cuevas-Saiz	32	14.1.6	…donated to research, TRAINING or discarded…. (in some countries, like UK, training purposes is one option for donated embryos)	The sentence was adapted.
7	Liliana Ramos	32	14.1.14	What do you mean by "cryopreservation of testicular samples not expected to contain sperm": do you mean cryopreservation of stem cells in prepuberal boys? Specify better as it is not clear.	This is the recommendation from the Good Practice Recommendations paper in prepubertal boys. A different freezing

NR	Reviewer	Page	Line	Comment	Action / Reply
					protocol is to be used when the tissue may contain sperm vs when it is not expected to contain sperm.
31	Koen Wouters	32	14.2.1	Not really clear what is mentioned here. Do you suggest to work with gloves?	The sentence was removed as this is the same for the external surface of all consumables.
1	Lodovico Parmegiani	32	14.2.2	The negligible risk of contamination was initially hypothesized by Pomeroy (Fertility and Sterility, 2009) but was partially contradicted by the same author in 2020 (Pomeroy and Schiewe, JARG 2020), who suggested precautionary measures for the safe use of liquid nitrogen. Specifically, they recommended that LN_2 should not be shared among patients for vitrification and warming and that LN_2 baths should be disinfected between uses. Therefore, I propose removing the following sentence ("however, the risk of contamination from LN_2 or from other biological samples is considered to be negligible"), as there is a lack of updated supporting references. In contrast, multiple recent studies have highlighted potential contamination risks mediated by liquid nitrogen, particularly considering the high number of vitrification and warming procedures performed daily using polystyrene boxes (De Santis et al., JARG 2021; Scarica et al., JARG 2021; Vajta et al., Human Reproduction, 2022). This concern is further supported by a documented clinical case of cross-contamination via liquid nitrogen in a field closely related to IVF (Tedder et al., The Lancet, 1995). Furthermore, this statement is inconsistent with point 14.2.1 and point 3.4.6 of the guidelines (recommendation for the use of clinical-grade liquid nitrogen). Instead, I would recommend emphasizing the sterilization (and registration of sterilization) of vitrification trays/boxes (preferably CE MDR–certified devices), which aligns with the SoHO recommendations:	We agree that this section can be updated and that current "opinion" argues for safer handling.

NR	Reviewer	Page	Line	Comment	Action / Reply
				"SoHO activities must be carried out in such a way as to prevent SoHO contamination or cross-contamination between SoHO."	
15	Alev Özer	31	14.2.2	Comment: The statement that LN2-related contamination risk is "negligible" may be interpreted differently across jurisdictions and inspection frameworks. A more precise, evidence-consistent phrasing ("low risk with validated protocols") plus explicit requirement for documented validation and risk assessment would improve regulatory robustness without changing the underlying message. Proposed text modification : Replace: "the risk… is considered to be negligible" with: "Available evidence indicates a low contamination risk when validated protocols are applied; however, laboratories should perform and document a structured risk assessment in line with local regulations and include device- and process-validation (including storage strategy for sero-positive samples) within the QMS." Full-text supporting literature :• Cai H. et al. Open versus closed vitrification system of human oocytes and embryos: systematic review and meta-analysis. 2018. https://pmc.ncbi.nlm.nih.gov/articles/PMC6284284/ •Pantos K. et al. The Effect of Open and Closed Oocyte Vitrification Systems… (network meta-analysis). 2024. https://pmc.ncbi.nlm.nih.gov/articles/PMC11084263	We agree that this section can be updated and that current "opinion" argues for safer handling.
20	Shivansh Jaiswal	32	14.2.2	Clarification of the positioning of closed versus open vitrification systems may enhance biosafety governance while acknowledging regulatory diversity across jurisdictions.	The use of open vs closed vitrification systems may depend upon handling and overall environment situation.
32	AGRBM	32	14.2.2	The EDQM guide states that open and closed devices can be used. Is a risk analysis really necessary?	EDQM just states that both these types are being used. Can be used is not the same as having equal risk. May depend upon handling and overall environment situation.

NR	Reviewer	Page	Line	Comment	Action / Reply
7	Liliana Ramos	33	14.2.3	There is a huge difference between cryopreservation of semen (large volumes) of embryos /oocytes (very low volumes): there is sufficient data that the very low volume for oocytes/embryos in closed system have no risk for cross contamination, so no dedicated separate tanks are necessary. Of course, for semen is closed system and vapor recommended but not necessary dedicated tanks for seropositive cases.	The sentence was adapted.
25	Irene Cuevas-Saiz	32	14.2.3	It would be interesting to recommend specific tanks for seropositive patients	This is indeed what we recommend.
18	Keerti Singh	32	14.2.3	Please include point 14.2.4 as: Currently, microfluidic systems have gorwn as viable alternative solution for the precise manipulation of fluids with micro-scale flow features. Microfluidic systems could boost ART pregnancy rates by providing continuous and gradual cryoprotectant agent (CPA) concentration increase, enabling simple and direct embryo loading/retrieval. Quantum dots and magnetic nanoparticles show clinical promise. References: • Anagnostopoulou et al., 2022, doi: 10.23736/S0031-0808.22.04671-7. - Page 148 left column, para 3, lines 1-7 • Ajayi, A.F., Jegede, A.J., David, U.E., Soetan, O.A., & Okeleji, L.O. (2026). The role of nanotechnology in male fertility assessment. Next Nanotechnology. - Page 1, highlights, bullet point 3.	We do not agree that current data shows boosting of pregnancy rates. More research is needed before any recommendations might be possible.
7	Liliana Ramos	33	14.3	Documentation should include “sperm quality **or** embryo quality **or** number of oocytes per devise” (in the way it is mentioned here, it seems it needs all this, but in reality it depends on the material)	Agree in principle, but writing it all in one sentence becomes rather extensive. We believe that the recommendations as they stand now are understandable
13	Fikret Gürkan Agircan	32	14.4.6	Supernumerary embryos may be cryopreserved individually. I think it should: Supernumerary embryos **should** be cryopreserved individually (to promote SET).	The sentence was adapted.

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17	Hannah Park	33	14.7	"…transfer of samples into labelled cryo-dish**es**"	The sentence was adapted.
12	Durga Rao Gedela	33	14.8	Annual Cryostorage Audit and Digital Cryolocation: The guideline may enhance traceability standards by recommending structured cryotank mapping and system-generated cryolocation management.	This is already covered in section 14 on cryopreservation.
26	Sheila Mae Poulain	31-34	525-526	14.X Cryoprotectant solutions shall be prepared, stored, and handled according to manufacturer instructions and validated laboratory SOPs. 14.X Exposure times to cryoprotectants shall be strictly monitored and standardised, particularly during vitrification procedures, to minimise osmotic and toxic injury. Timers should be used during vitrification and warming procedures to ensure protocol consistency. Any deviation from validated exposure times shall be documented and risk-assessed. 14.X Only personnel who have demonstrated validated competency in cryopreservation techniques shall perform freezing and warming procedures. Competency assessments should include: • Survival rates • Post-warming developmental competence • Adherence to timing protocols • Documentation accuracy Periodic re-evaluation of competency is recommended. 14. X Each cryopreservation protocol shall undergo documented validation prior to clinical implementation. Key performance indicators (KPIs) should include: • Post-warming survival rate (oocytes, embryos) • Blastocyst re-expansion rate • Post-warming fertilisation rate (for oocytes) • Implantation and clinical pregnancy rate (where traceable) • Sperm post-thaw motility recovery Trends in cryosurvival and clinical outcomes shall be reviewed periodically to detect deviations from expected performance. 14.X Liquid nitrogen (LN_2) supply shall be obtained from validated suppliers. Tanks shall be monitored using:	2 items were added to the document. The others are either already included in other sections or considered out of scope for this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
				• Continuous temperature monitoring systems • Alarm systems with 24-hour response capability • Documented LN_2 level checks at defined intervals Backup tanks and emergency response procedures shall be in place and validated. 14.X New storage tanks shall undergo installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) prior to clinical use. Preventive maintenance schedules shall be defined and documented. Tank integrity, vacuum performance, and evaporation rates shall be periodically evaluated. 14.X Risk-based segregation strategies may be implemented, including: • Separate tanks for embryos, oocytes, sperm, and tissues • Dedicated tanks for sero-positive patients • Segregation by storage duration (long-term vs short-term) A formal cryobank risk assessment shall be performed and reviewed regularly. 14.X Electronic witnessing systems (where available) are recommended to reduce risk of sample mix-up during: • Loading of devices • Placement in tanks • Removal for warming • Transfer procedures Where electronic systems are not available, a documented double-witness protocol shall be implemented. 14.X A documented disaster recovery plan shall be in place addressing: • Tank failure • Power outage • LN_2 supply interruption • Fire or flooding • Alarm system malfunction Staff shall be trained in emergency procedures. Simulation drills are recommended at defined intervals.	

NR	Reviewer	Page	Line	Comment	Action / Reply
				14.X Laboratories shall define policies for monitoring storage duration in accordance with national legislation. Systems shall be in place to notify patients prior to expiration of legally permitted storage periods. 14.X Dry shippers shall be validated prior to clinical transport use. Pre-conditioning procedures and hold times shall be documented. Temperature validation studies should demonstrate maintenance below -150˚C for the maximum anticipated transport duration. 14.X Laboratories shall define standardised survival criteria, including: • Oocyte membrane integrity and re-expansion • Embryo blastomere integrity • Blastocyst re-expansion and ICM or TE assessment • Sperm motility and viability assessment Criteria for discarding non-viable material shall be documented.	
15. Contingency and emergency plan					
4	Shridhar S Amanchi	/	/	Brief practical examples on managing unexpected events (equipment fluctuation, delays, sudden workload changes) may help laboratories prepare better.	The most interesting papers are referenced and detailed examples are given in these papers.
12	Durga Rao Gedela	/	/	Periodic Mock Emergency Drill: Guideline may recommend mandatory simulation-based preparedness testing.	The Recommendations paper states that emergency preparedness drills are necessary for the staff. We have added this in the emergency section too.
20	Shivansh Jaiswal	/	/	Incorporation of structured emergency preparedness drills within the Quality Management System and periodic review during management meetings may enhance resilience and alignment with broader biovigilance principles.	The Recommendations paper states that emergency preparedness drills are necessary for the staff. We have added this in the emergency section too.
7	Liliana Ramos	35	15.1.5	Should not be mentioned that a back up of the system is necessary if the digital system/cloud is unavailable?	This specific situation is an example of malfunctioning of a critical equipment. We are not providing specific examples.
27	Maria Filippa	35	552	Supplementary data on Flowcharts showing event- response-recovery steps would be insightful Flowcharts for Floods, Earthquakes, Fire, Gas leaks other natural disasters or war	It is very difficult to supply these as the content would vary from center to center. We have added some information in the text.

NR	Reviewer	Page	Line	Comment	Action / Reply
7	Liliana Ramos	35	556	Add "water damage" next to fire (sometimes water brings more damage than fire)	Yes, however the reference of Kornfield is specifically on the toxic compound due to fire.
28	Labadi Leila	36	572	In the emergency vitrification protocol it is suggested to difine a minimum viable data set for rapid labelling to ensure sample remain identifiable record keeping is impossible during a hurried evacuation	This is indeed challenging, however you need minimal labelling, how are you going to be able to use the material afterwards otherwise?
Discussion					
25	Irene Cuevas-Saiz	37	617	And biopsy for PGT	Biopsy for PGT was added to the sentence
General comments					
2	G. Sreekanth	/	/	PGT: It should be a common procedure in all IVF clinics.	The working group does not agree with the reviewer.
2	G. Sreekanth	/	/	IVF clinics: Everything should be clearly documented	The working group agrees with the reviewer.
3	AMM Wetzels	/	/	Compliments on this new version of the recommendation!!	Thank you.
4	Shridhar S Amanchi	/	/	The updated recommendations are very helpful and well organized. They cover important areas in IVF laboratory practice. Including more small practical tips from daily lab work would make them even easier to apply in routine clinical settings.	Thank you.
4	Shridhar S Amanchi	/	/	Applicability across labs: IVF laboratories work with different levels of infrastructure and resources. Clearly stating minimum safe standards and ideal standards would help more centers follow the recommendations safely.	Because of the different infrastructure, resources, legislation and methodology, it is not possible to provide standards that are applicable for all laboratories.
12	Durga Rao Gedela	/	/	SOPs in Flowchart Format: The guideline may encourage that critical SOPs be accompanied by **visual process flowcharts** to enhance cognitive clarity and reduce procedural ambiguity.	Templates and flowcharts are often the responsibility of quality managers and are therefore considered outside the scope of the document.
15	Alev Özer	/	/	This updated draft is well structured and aligns with a risk-based quality approach. From a clinical–laboratory governance perspective, four areas would benefit from more explicit operationalisation: (i) quantitative	The sections have been reviewed and adapted where relevant.

NR	Reviewer	Page	Line	Comment	Action / Reply
				VOC/air-quality monitoring with defined action thresholds; (ii) electronic witnessing system (EWS) governance through structured audits and alarm management; (iii) refined, regulation-aware wording and validation steps for open vs closed vitrification; and (iv) embryo transfer (ET) standardisation where high-level evidence is limited, using protocolisation and audit-driven feedback loops. Adding measurable metrics, audit frequency, and clear CAPA triggers would improve harmonisation across centres and strengthen patient-safety credibility without changing the document's overall intent	
15	Alev Özer	/	/	Comment: The draft is comprehensive and risk-oriented. However, several technically critical domains (air quality monitoring, electronic witnessing governance, vitrification risk wording, and embryo transfer standardisation) would benefit from clearer operationalisation through quantitative thresholds, structured audit cycles, and predefined CAPA pathways. Adding measurable performance metrics would strengthen harmonisation and clinical–laboratory integration without altering the document's core recommendations.	The sections have been reviewed and adapted where relevant.
17	Hannah Park	/	/	Much appreciated update! Great work! Minor comments/suggestions	Thank you.
18	Keerti Singh	/	/	The ESHRE recommendations on Good Practice in the IVF Laboratory is well written covering all important areas. The language is lucid and is within the understanding of both experts and public. I have included some references, especially one that is highly relevant to many topics addressed in this guideline document. I have also suggested inclusion of an additional point 14.2.4 which I believe will add significant value and support the evidence base for recommendations.	Thank you.
19	Fayezi, Shabnam	/	/	This is a highly valuable and comprehensive update of the 2015 ESHRE IVF laboratory recommendations, with strong emphasis on safety, quality, and modern ART workflows. • Comprehensively covers core IVF lab processes (organisation, QMS, safety, traceability, and procedures). • Strong alignment intent with emerging European regulatory landscape (SoHO) and EDQM guidance.	Thank you.

NR	Reviewer	Page	Line	Comment	Action / Reply
				• Highly relevant to daily laboratory operation and audit preparedness. • Overall recommendation: Accept with minor to moderate revisions.	
19	Fayezi, Shabnam	/	/	The manuscript notes that not all recommendations are supported by evidence, but there is no explicit system to grade evidence level or recommendation strength. • Introduce a transparent grading framework (e.g., GRADE or an ESHRE-specific classification) to label: (i) level of evidence; (ii) strength of recommendation. • Where evidence is limited, clearly flag 'expert consensus' versus evidence-based statements.	This is not an evidence-based guideline. Owing to the format of ESHRE Recommendations papers, recommendations are based on (very low quality) evidence, where available, and on expert opinion.
19	Fayezi, Shabnam	/	/	Several recommendations are conceptually clear but difficult to implement without operational tools, particularly for smaller or resource-limited laboratories. • Add practical examples and minimum workable templates (e.g., staffing calculator example, KPI dashboard examples, CAPA and change-control forms). • Provide simple flowcharts for key workflows (risk management tool selection, witnessing/traceability steps). • Distinguish minimum standards vs. 'ideal'/aspirational best practice.	Templates and flowcharts are often the responsibility of quality managers and are therefore considered outside the scope of the document.
19	Fayezi, Shabnam	/	/	Wording such as 'must', 'should', 'recommended', and 'preferably' appears to be used interchangeably, which can create ambiguity regarding regulatory compliance. • Adopt standardised normative language throughout (e.g., must = regulatory/mandatory; should = strong recommendation; may = optional). • Consider adding a short legend at the start and/or visually tagging requirements derived from SoHO/EDQM.	The working group has used wording appropriate to the strength of the recommendations.
19	Fayezi, Shabnam	/	/	The text refers to alignment with the new SoHO regulation and EDQM guidance; however, readers would benefit from explicit mapping of changes and requirements. • Add a 'what's new since 2015' summary table and highlight the most impactful updates. • Explicitly identify recommendations introduced/changed due to SoHO	The working group has reviewed the entire 2015 document, and updated and added recommendations where necessary or where new evidence was available. It is not possible to deliver the explicit mapping you are asking for.

NR	Reviewer	Page	Line	Comment	Action / Reply
				requirements (QMS, traceability, biovigilance, documentation retention). • Clearly state which regulatory/guidance documents and versions were used (SoHO articles, EDQM edition/year), and where updates may still be pending.	
19	Fayezi, Shabnam	/	/	Some role terms appear interchangeable (e.g., laboratory manager vs supervisor; clinical embryologist vs embryologist). • Provide short definitions for key staff roles and then use terms consistently throughout.	This reflects variability in terminology across Europe.
19	Fayezi, Shabnam	/	/	Quick-reference tables would improve usability and implementation. • Suggested tables: staffing requirements (ranges), essential equipment and redundancy expectations, critical control points (HACCP-style), key traceability requirements and retention times.	The working group has made sure that all information is easily digestible in the document, as much as possible.
19	Fayezi, Shabnam	/	/	Please consider adding a clear evidence grading framework (e.g., GRADE or an ESHRE-specific classification) to indicate (i) level of evidence and (ii) strength of recommendation for key statements.	This is not an evidence-based guideline. Owing to the format of ESHRE Recommendations papers, recommendations are based on (very low quality) evidence, where available, and on expert opinion.
19	Fayezi, Shabnam	/	/	Several recommendations would be easier to operationalise with brief practical examples (templates/flowcharts) and an explicit distinction between minimum standards and ideal/best-practice options, particularly for smaller laboratories.	Templates and flowcharts are often the responsibility of quality managers and are therefore considered outside the scope of the document.
19	Fayezi, Shabnam	/	/	Please standardise normative language (e.g., must = mandatory/regulatory; should = strong recommendation; may = optional) and consider adding a short legend at the start to avoid ambiguity.	The working group has used wording appropriate to the strength of the recommendations.
19	Fayezi, Shabnam	/	/	Please define key staff roles (e.g., laboratory manager vs supervisor; embryologist vs clinical embryologist) and use terminology consistently throughout; a brief glossary could help.	This reflects variability in terminology across Europe.
19	Fayezi, Shabnam	/	/	Where paragraphs are long (e.g., Quality Management and Traceability), consider converting into bullet points and adding subheadings or 'key takeaways' to improve scan-ability.	The section was divided up into smaller lists.

NR	Reviewer	Page	Line	Comment	Action / Reply
19	Fayezi, Shabnam	/	/	Quick-reference tables would improve usability (e.g., staffing requirements, equipment requirements/redundancy, critical control points, traceability documentation requirements).	Working group decided not to comply, such lists would require regular updating.
19	Fayezi, Shabnam	/	/	Minor language and formatting corrections (grammar, consistent abbreviations, uniform wording) would improve readability without changing content.	The document has been reviewed for spelling, punctuation and grammar mistakes.
20	Shivansh Jaiswal	/	/	The updated recommendations represent a comprehensive and well-structured advancement of prior IVF laboratory guidance. The document reflects strong integration of risk-based thinking, traceability principles, and structured governance consistent with contemporary European regulatory frameworks. To further strengthen cross-jurisdictional applicability, the document may benefit from clearer articulation of how the Quality Management System framework aligns with established international quality models, particularly in relation to process-based governance architecture, structured competency documentation, long-term traceability and record retention principles, and integration of continuous improvement methodologies. Such clarification would enhance harmonization across both EU and non-EU centres while preserving ESHRE's evidence-based approach.	Thank you.
20	Shivansh Jaiswal	/	/	The emphasis on structured risk assessment tools such as FMEA is commendable and reflects contemporary laboratory governance standards. The Quality Management System section may be strengthened by more explicit integration of continuous improvement cycles, linking risk identification, corrective and preventive action processes, audit findings, and structured management review. Consideration may also be given to expanding quality indicators to include validated digital embryo assessment tools where evidence supports their use, as well as stronger supportive language for reduced oxygen culture systems where infrastructure permits, given consistent trends in the literature. Further clarification of the relationship between process certification and laboratory competence accreditation models may enhance implementation clarity across diverse regulatory environments.	The working group considers this outside the scope for this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
21	Laboratory for Reproductive Medicine, Erasmus MC, University Medical Center Rotterdam	/	/	I think results of serological screening should also be included, and if the material is intended for donation or within a couple	Serological screening is mentioned in section 3 on laboratory safety.
22	Zuzana Holubcová	/	/	The document would benefit from clearer stratification of recommendation strength and origin. In several sections it is not clear whether statements reflect regulatory requirements, evidence-based recommendations, or expert consensus. Introducing consistent terminology such as “must”, “should”, or “preferably”, or a grading system distinguishing evidence-supported recommendations from expert opinion, would improve clarity and facilitate implementation across laboratories operating under different regulatory frameworks	This is not an evidence-based guideline. Owing to the format of ESHRE Recommendations papers, recommendations are based on (very low quality) evidence, where available, and on expert opinion.
22	Zuzana Holubcová	/	/	Several sections would benefit from reducing ambiguous wording. For example, phrases such as “where relevant”, “if possible”, and “when deemed appropriate” should either be defined or linked to a documented risk assessment approach. This would enhance reproducibility and reduce interpretative variability during audits.	The working group needs to take into account that resources and legislation differs across Europe.
22	Zuzana Holubcová	/	/	The citation style throughout the document appears inconsistent, with references sometimes integrated within the narrative text and sometimes presented in brackets after recommendations or bullet points. It is not always clear whether cited literature directly supports a specific recommendation or provides general background context. A more uniform referencing approach would improve readability and help readers distinguish evidence-supported recommendations from expert consensus statements.	Indeed, where narrative paragraphs were used, references were integrated in the text. In tables, the references were included in a separate column to take into account the layout for publication in the ESHRE journal.
24	Stefan Matik	/	/	General impression of the entire guideline: Great update of the guideline, and highly need one – precise, exact and up-to-date	Thank you.
25	Irene Cuevas-Saiz	/	/	Congratulations to all the working group for this updated version, mainly with all the issues related to the QMS of the IVF lab.	Thank you.

NR	Reviewer	Page	Line	Comment	Action / Reply
26	Sheila Mae Poulain	/	/	The document structure is logical and clearly organised, progressing appropriately from methodology to documentation and traceability. The scientific tone is appropriately cautious and avoids overstatement, which strengthens its credibility. Quality management language could be more uniformly embedded across all sections to enhance audit readiness. The level of operational detail varies between sections and could be balanced for uniform depth. Stronger and more consistent linkage to KPIs and outcome monitoring would reinforce continuous quality improvement. Overall, the document reflects strong laboratory leadership, governance awareness, and advanced clinical embryology expertise.	Thank you.
27	Maria Filippa	/	/	Dear colleagues you have done an excellent work but i strongly believe that supplemented material would be of great help for reference	Thank you.
27	Maria Filippa	/	/	IVF lab: Supplementary Data on air quality values, Grade, particles & VOC levels to optimize environment, Supplementary Data on temperature values on heated surfaces to handle gametes & embryos Supplementary Data on pH ranges and osmolarity to prepare and work on culture media dishes, as well as optimal oxygen levels and humidity effect of culture in the incubators	The working group considers this out of scope for this document.
27	Maria Filippa	/	/	Surgery room for Oocyte Retrieval & Embryo Transfer: Supplementary Data on optimal range of temperature of OR & ET equipment, On the range & optimal pressure values of aspiration pumps to perform OR On the range & optimal grading of ET by the Embryologist & IVF specialist Supplementary Data on the adverse effects and findings of the OR and the ET by degree of severity	The working group considers this out of scope for this document.
27	Maria Filippa	/	/	Andrology lab: Supplementary Data concerning the way to handle TESE optimally for best survival and higher fertilization results	The working group considers this out of scope for this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
27	Maria Filippa	/	/	Cryo lab: Supplementary Data concerning ways to prevent contamination by assigning specific tanks to specific viral infected specimen ie Tank for Hep C only, Tank for Hep B only	The working group considers this out of scope for this document. The reviewer is referred to the ESHRE guideline on MAR in patients with a viral infection/disease for more information on this topic.
27	Maria Filippa	/	/	Infection control: Supplementary Data on key points to prevent & handle major bacterial and viral infections, especially HIV I & II, Hep B & C, Syphilis and Covid-19	The working group considers this out of scope for this document. The reviewer is referred to the ESHRE guideline on MAR in patients with a viral infection/disease for more information on this topic.
27	Maria Filippa	/	/	Fate of surplus embryos: Supplementary Data on legal & ethical issues concerning the fate of gametes spermatozoa, oocytes and preimplantation embryos for donation, research-therapy and/or destruction timing & policies in Europe	Fate of supernumerary embryos is included in section 14 on cryopreservation. However, the fate of supernumerary embryos is often covered under national legislation.
27	Maria Filippa	/	/	IQC: Supplementary Data on checking performance to practitioner optimal values -results & appropriate timing- indoor KPIs	The working group considers this out of scope for this document.
27	Maria Filippa	/	/	EQA: Supplementary Data on timing of KPIs to be checked & optimal thresholds	The working group considers this out of scope for this document. The reviewer is referred to the ESHRE and Alpha Vienna Consensus for more information on laboratory KPIs.
27	Maria Filippa	/	/	QMS: Supplementary Data on troubleshooting issues especially for no-to-low fertilization results, for low pregnancy results, for low blastulation rate results, for high pregnancy losses results, for high immature oocyte at retrieval results etc	The working group considers this out of scope for this document.
27	Maria Filippa	/	/	Endocrinology & ultrasound values which diagnose cause of infertility: Supplementary Data given from WHO, European Society of Endocrinology, ESHRE by showing tables on hormonal optimal reference values and types of infertility causes ie AMH, FSH, Estogen and Progesterone values according to female age & timing in their menstrual cycle	These are clinical parameters, which are out of scope for this document.

NR	Reviewer	Page	Line	Comment	Action / Reply
27	Maria Filippa	/	/	Sperm number, motility , morphology & DFI values that show the potential of sperm performance: Supplementary Data on the initial sperm parameters and their recovery performance after handling to decide whether an IUI or IVF or ICSI will be performed. A range of optimal values per IUI, IVF and ICSI would be valuable. Supplementary Data on the expected rate of DFI values measured and their expected outcome at fertilization	The working group considers this out of scope for this document.
27	Maria Filippa	/	/	Age & BMI of both parents: Supplementary Data on the effect of advanced maternal age & paternal age on the ART outcome Supplementary Data on the maternal & paternal BMI on their fertility outcome	These are clinical parameters, which are out of scope for this document.
27	Maria Filippa	/	/	Genetic analysis of both partners & genetic matching: Supplementary Data on the role of karyotype test of both partners Supplementary Data on the examination of major carrier diseases ie β-thalassaemia , cystic fibrosis etc Supplementary Data on carrier screening matching of partners	These are clinical parameters, which are out of scope for this document.
27	Maria Filippa	/	/	Reporting of clinical data & adverse events: Supplementary Data on the grading and severity of adverse events by type of procedure and how to report and overcome its unwelcomed effects Ie At oocyte retrieval, At sperm preparation, At fertilization, At Embryo culture, At Embryo Transfer, At vitrification, At thawing, At Embryo biopsy, At receiving and interpreting PGT results, At consulting patients, before, during and after ART, At giving bad results, At bleeding, infection, mismatching, Hypestimulation	The working group considers this out of scope for this document.
28	Labadi Leila	/	/	Separation of culture and transfer: Splitting the embryon culture and transfer ; section into two separate chapters is a major structural improvement that allows for more elaborate and detailed guidance on each critical stage	Thank you.
28	Labadi Leila	/	/	Fertilization scoring: The introduction of term (PN not observed) to replace (0PN) is a significant innovation that prevents the premature discarding of zygotes with normal development potential	The working group agrees with the reviewer.

NR	Reviewer	Page	Line	Comment	Action / Reply
31	Koen Wouters	/	/	Some general comments: IVM is not mentioned anywhere. Nothing is said about automation in the IVF/andro laboratory. Time-lapse and embryo culture are not explained very well in comparison with PGT, which is covered in great detail.	IVM is not a standard procedure of the IVF laboratory. Similarly to automation and AI solutions. Time-lapse is sufficiently described and reference is made to the relevant ESHRE recommendation paper for further information.
32	AGRBM	/	/	Thanks a lot to the working group for this revised version of the Good Practice in the IVF labs guideline. It is very much appreciated!	Thank you.
36	David Morroll	/	/	There are a number of spelling, punctuation and grammar mistakes but it is assumed these should be rectified on submission and before publication. Note also a mix of UK and US English.	The document has been reviewed for spelling, punctuation and grammar mistakes.