Revised guidelines for good practice in IVF laboratories (2015)

Introduction

In line with the ESHRE scope, this document represents an updated version of the guidelines published in 2008 (Magli, et al., 2008). The aim is to provide a wider coverage of key aspects of the IVF laboratory, to give continuous support to laboratory specialists and consequently contribute to improve IVF patient care.

1 Staffing and direction

Personnel are one of the most important parts of an IVF laboratory. The number of laboratory staff should reflect the number of cycles performed per year and the complexity of the work. Other duties such as administration, training, education, quality management and communication also need consideration.

Appropriate human resources should provide an adequate climate to perform all laboratory tasks in a timely manner to ensure patient safety and quality care. Sufficient qualified personnel should be available to provide back up for the laboratory staff.

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

1.1 Laboratory director

The laboratory should be directed by a person with officially recognised qualifications and expertise in clinical embryology and biological/medical sciences. In accordance with the results of the ESHRE survey on the education and professional status of clinical embryologists (Kovacic*, et al.*, 2015), this would include a higher academic degree, a minimum of 6 years of documented human embryology experience, and preferably attainment of the ESHRE senior clinical embryologist certification.

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

Laboratory director responsibilities include ensuring:

1.1.1 Selection and implementation of the most adequate materials and procedures to reach the highest standards in clinical IVF.

1.1.2 Safe and appropriate laboratory facilities and equipment according to European and/or national regulations.

1.1.3 Implementation of a quality management system (QMS)

1.1.4 Implementation of a laboratory risk management and prevention policy.

1.1.5 Sufficient laboratory staff members with the appropriate skills.

1.1.6 A comprehensive orientation and introduction programme for all new staff members.

1.1.7 Management of laboratory staff training and continual scientific and biomedical education.

1.1.8 Implementation and review of key performance indicators (KPIs) for all laboratory procedures for quality control and quality assurance purposes.

1.1.9 Reporting of clinical data and adverse events according to European and/or national regulations.

1.1.10 Approval of research projects by competent authorities.

1.2 Laboratory supervisors

Some laboratories may require additional managerial positions. These require specific qualifications, e.g. at least a BSc in biomedical sciences, 3 years of documented human embryology experience and preferably the ESHRE clinical embryologist certification.

Laboratory supervisor responsibilities include ensuring:

1.2.1 Efficient organisation of daily work of their areas of responsibility

1.2.2 Effective communication with laboratory staff and clinical colleagues.

1.2.3 Continuous improvement where possible.

1.2.4 Structured training of staff members.

1.3 Clinical embryologists

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

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

Clinical embryologist responsibilities include:

1.3.1 Execution of standard operating procedures (SOPs).

1.3.2 Participation in daily practice, communication and organisation.

1.3.3 Contribution to laboratory clinical decisions.

1.3.4 Training of staff members.

2 Quality management

According to the European directives and recommendations (European Commission, 2006a, c, 2012; Council of Europe, 2013), working in compliance with a QMS is mandatory. The requirements cover the organisation, management, personnel, equipment and materials, facilities/premises, documentation, records and quality review. This includes:

- defining responsibilities and ensuring all personnel are qualified and competent;
- having validated, written instructions for each process (SOP), including management of adverse events;
- ensuring full traceability of cells and tissues, materials, equipment and personnel involved in specific laboratory activities, with records maintained accordingly;
- confirming that all media/reagents/disposables are tested for quality using appropriate assays whenever possible;
- ensuring proper and periodic equipment maintenance, service, and calibration;
- verifying conformance to specifications;
- taking corrective action to keep procedures under conformity;
- reviewing performance to ensure continuous and systematic QMS improvement;
- providing risk assessment analysis for all laboratory activities.

2.1 It is recommend that a clinical embryologist is made responsible for quality management within the laboratory.

2.2 Written, authorised, signed and up-to-date SOPs should exist for all processes in order to optimise outcomes.

2.3 All SOPs must include provision for unique identification of patients and their reproductive cells and

tissues, while retaining patient confidentiality.

2.4 All relevant data concerning laboratory work must be recorded in a database that allows KPI extraction and statistical analysis. Corrections, either written or electronic, should be traceable. Data should include:

2.4.1 Morphological characteristics of gametes and embryos.

2.4.2 Detailed information of the procedures, including timing and staff involved.

2.4.3 All information needed to comply with the requirements of national and international data registries.

2.5 Every relevant communication with patients should be recorded in the patient's files.

2.6 Taking into account the high degree of attention needed during laboratory work, distractions should be minimised.

2.7 Proactive risk assessments should be made and preventive actions taken to minimize non-conformities.

2.8 A documentation system should be in place for dealing with non-compliances, emergencies, errors, adverse events and complaints. Corrective and preventive actions, implementation dates and assessments of their effectiveness should be documented. Under certain circumstances, a period of follow-up may be advisable to ensure the adequacy of actions. Non-compliances should be discussed regularly and reviewed at least annually.

2.9 KPIs should be objective and relevant, regularly checked and discussed, and communicated to all staff. KPIs can be based on a reference patient group with good prognosis, as well as on the whole patient population. Appropriate statistics can be used to account for patient variation and the number of previous treatment cycles patients may have already undertaken.

Critical performance levels should be defined for each KPI with reference to national data and European registry data collected by the European IVF-monitoring programme for ESHRE. If necessary, appropriate action should be taken.

2.10 In addition to laboratory and clinical performance, operator performance should be checked regularly to ensure competence, compliance and consistency, via direct observation of procedural skills (DOPS) and/or individual KPIs. If necessary, retraining should be implemented.

2.11 Participation in Internal Quality Control (IQC) and External Quality Assurance (EQA) programmes, either commercial or in collaboration with other laboratories, is recommended. QC records should be maintained and reviewed, including documentation of results and any corrective action.

2.12 The laboratory's QMS should be systematically reviewed annually to ensure continuous improvement of the entire process by identifying current challenges, problems, errors or improvements.

2.13 An audit system, both internal and external, must be in place. An independent, competent auditor should verify compliance of all procedures with SOPs and requirements. Any findings, corrective actions and their effectiveness must be documented.

3 Laboratory safety

3.1 Laboratory design

The IVF laboratory must have adequate functionalities to minimise any damaging effects upon reproductive cells, and ensure good laboratory practice. The laboratory should be adjacent to the operating room where clinical procedures are performed.

When commissioning the IVF laboratory, the most recent developments in facilities, equipment and procedures should be considered. Attention should be given to operator comfort to provide a safe working environment that minimises the risk of distraction, fatigue and thereby making a mistake. Taking into account local, national, and European occupational health and safety requirements, considerations should include bench height, adjustable chairs, microscope eye height, efficient use of space and surfaces, sufficient environmental lighting, air-conditioning with controlled humidity and temperature.

More specifically:

3.1.1 Materials used in laboratory construction, painting, flooring and furniture should be appropriate for cleanroom standards, minimising Volatile Organic Compounds (VOC) release and embryo toxicity.

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

3.1.3 Laboratory access should be restricted to authorised personnel.

3.1.4 A system for clean access of personnel and materials to the laboratory is necessary.

3.1.5 Rooms for changing clothes should be separate to the laboratory.

3.1.6 Hand washing facilities should be placed outside the laboratory.

3.1.7 Separate office space for administrative work should be available outside the laboratory.

3.1.8 A separate laboratory with a safety fume hood should be provided for analyses using fixatives.

3.1.9 The area for cleaning and sterilisation of materials, if present, should be separate to the laboratory.

3.2 Laboratory Air Quality

3.2.1 To optimise environmental conditions, laboratory air should be subjected to high-efficiency particulate air (HEPA) and VOC filtration.

3.2.2 Positive pressure is recommended to minimise air contamination.

3.2.3 Procedures involving gamete or embryo manipulation should be performed in a controlled environment. Background and processing air quality should comply with European and national guidelines, and should be regularly monitored.

3.2.4 According to the European Union Tissues and Cells Directive (EUTCD), tissues and cell processing must be performed in a Good Manufacturing Practice (GMP) Grade A environment with a background of at least GMP Grade D. However, if it is detrimental or not feasible to carry out a specific procedure in a Grade A environment, it can be performed in at least a Grade D environment.

3.3 Laboratory equipment

3.3.1 The laboratory should contain all essential items required for IVF, in a number appropriate to the workload.

3.3.2 The incubator number is critical and should be based on the number of cycles and embryo culture duration. Gametes and embryos should be conveniently distributed across incubators to minimise door openings.

3.3.3 Equipment must be adequate for optimal laboratory work, easy to disinfect and kept clean to avoid contamination.

3.3.4 All equipment must be validated as fit for its purpose, and performance verified by calibrated instruments. Equipment should preferably be CE-marked.

3.3.5 Gas cylinders should be located outside the laboratory. There should be an automatic change-over system and sufficient cylinders stocked for immediate replacement. High-purity gas and inline HEPA and VOC filters should be used.

3.3.6 Equipment validation, calibration, maintenance and repair must be documented and records retained.

3.3.7 Heating devices should be installed to maintain the temperature of media and reproductive cells during handling.

3.3.8 Accepted ranges of use for all measured parameters should be determined and recorded. If measurements are out of range, corrections should be made and their effectiveness verified.

3.3.9 For every item of equipment, the instruction manual, and simplified instructions where needed, should be available.

3.3.10 Malfunctioning equipment should be labelled as “out-of-use” to avoid its use by mistake.

3.3.11 Critical items of equipment, including incubators and cryostorage units, should be continuously monitored and equipped with alarm systems.

3.3.12 An automatic emergency backup power system must be in place for all critical equipment.

3.4 Cryopreservation facilities and material

3.4.1 Cryopreservation facilities should be rationally and safely located outside but close to the laboratory, and, for safety reasons, with visible access to the interior (e.g. via a window, camera).

3.4.2 Adequate ventilation and low oxygen alarms should be installed. Personal low oxygen alarms are recommended, as additional security measure.

3.4.3 Cryostorage units should be continuously monitored and equipped with alarm systems, detecting any out of range temperature and/or levels of liquid nitrogen (LN2).

3.4.4 Protection devices (e.g. glasses, face shield, cryo-gloves, apron, footwear) should be used during LN2 handling.

3.4.5 All staff dealing with LN2 should be trained in safety aspects of its use.

3.5 Infectious agents

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

3.5.1 Procedures to ensure personnel safety and prevent cross-contamination should be established, taking European and national safety regulations into consideration. Therefore:

- Vaccination of all personnel against Hepatitis B or other viral diseases, for which a vaccine is available, is recommended.
- Patients must be screened for infectious diseases according to national and international regulations.
- Staff must be informed when a viral-positive patient is to be treated and be aware of the risks of handling infected biological material.
- SOPs should be in place to manage eventualities where infection might take place, e.g. needle-stick injuries.

3.5.2 To ensure adequate safety measures the treatment of viral-positive patients should be only performed in IVF laboratories with dedicated areas and equipment. Alternatively, such patient treatments could be allocated to specific timeslots provided processing of their biological materials is followed by a thorough disinfection of the allocated areas and equipment.

3.5.3 Whenever biological material is imported into the IVF laboratory from another clinic, full screening results should be obtained in advance. If any transported material is viral-positive, a dedicated dry shipper may be needed, depending on European and national regulations.

3.6 Protective measures

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

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

- Strict adherence to staff hygiene regulations and aseptic techniques.
- Use of protective laboratory clothing with low particle shedding.
- Use of hairnets and non-toxic, non-powdered gloves and masks where appropriate.
- Use of appropriate vertical laminar flow benches for handling of biological material.
- Use of mechanical pipetting devices.
- Disposal of single use consumables immediately into proper waste containers. Potential infectious materials must be disposed of in a manner that protects laboratory workers and other staff from exposure. Viral-positive waste should be segregated into a separate bin and labelled according to biosafety policies.
- Needles, glassware and other sharps should be handled with extreme caution and discarded into sharps containers.
- Disinfectants with proven compatibility and efficacy for an IVF laboratory should be used.

Alcohol-based disinfectants should be avoided.
- Food, gum, drinks and tobacco are strictly forbidden.
- Use of cosmetics and perfumes should be minimised.
- Staff should be appropriately attired and not wear jewellery, watches and wristbands.

4 Identification of patients and traceability of their reproductive cells

Identification of patients and traceability of their reproductive cells are crucial aspects of ART treatments. Each IVF laboratory must have an effective and accurate system to uniquely identify, trace and locate reproductive cells during each procedural step. A proper identification system should ensure that the main characteristics of patients (or donors) and their tissues and cells, together with relevant data regarding products and materials coming into contact with them, are available at all times.

4.1 Proper training in traceability procedures for all laboratory staff is mandatory.

4.2 Before commencing any procedure, the laboratory must be provided with each patient's unique identification code, which has to clearly and easily refer to the patient's documentation. Each treatment cycle must be assigned a unique code.

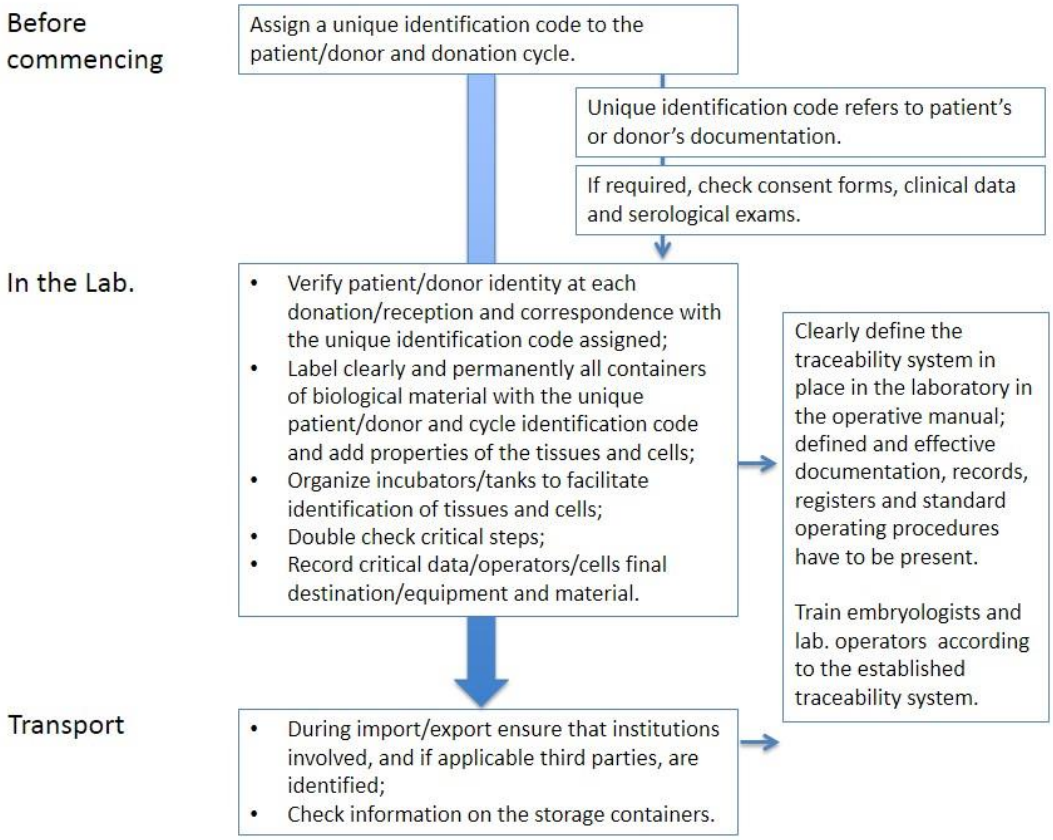
4.3 Corresponding consent forms, clinical data and serological exams undertaken by patients/donors prior to admission to the treatment should be available to the laboratory staff.

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

- Direct verification of patient identity and correspondence with their assigned unique identification code is required at every critical step. Patients should be directly asked to give their own identifying information (at least full name and date of birth) before procurement or artificial insemination / embryo transfer.
- All devices containing biological material must be clearly and permanently labelled with the unique patient and cycle identification code.
- Biological material from different patients must not be processed in the same working area at the same time.
- Incubators and cryostorage systems should be organised to ensure easy access and identification of the biological materials therein.
- During critical steps, double-checks by a second person (witness) and/or an electronic identification system is strongly advised.
- Products and materials used with biological materials must be traceable.
- The date and time of each manipulation and identity of all operators and witnesses must be documented throughout the treatment. These records should be kept for a specified period of time according to European and/or national legislation.
- Gametes and embryos from non-partner donation may require specific coding for those countries that are regulated according to European Commission Directives (European Commission, 2006c) (additional Directive anticipated in 2016).

4.5 Transport of reproductive cells and tissue requires identification of importing and exporting institutions, as well as identification of the biological material and its conformity for clinical use. At both institutions, the accompanying documentation and sample identification on the storage device must be checked to correspond with patient records.

Figure 1. Example of traceability system



5 Consumables

Specifications of critical reagents and materials should be in compliance with European and/or national regulations.

5.1 All consumables and media should be fit for their purpose, of embryo culture grade quality and preferably CE-marked. Use of quality controlled media, oil and disposables is recommended. If appropriate quality control testing for IVF purposes is not provided, this must be performed by the laboratory itself or by a designated company. In addition, packaging integrity and appropriate delivery conditions should be checked. Documentation of quality control testing must be supplied for any commercial media and this must correspond with the delivered batch.

5.2 Sterile single-use disposable consumables should be used.

5.3 Reagents, media and consumables should always be used prior to the manufacturer's expiry date.

5.4 Size of the bottles and other packaging must be appropriate to minimize openings and time between first and last use.

5.5 Appropriate refrigeration facilities must be available for storage of media and reagents. The correct temperature during their shipment to the clinic should be verified. Repeated shifts of temperature should be avoided while handling in the laboratory.

5.6 Patient or donor serum and follicular fluid should not be used as a protein supplement. Commercial suppliers of human serum albumin or media containing a serum derived protein source should provide evidence of screening according to European and/or national regulations.

5.7 An appropriate stock management system for media, oil and consumables, including the batch number, date of entry and expiration date should be available. Bottle sizes should be appropriate to the workload.

5.8 Risk assessments should be performed to ensure all consumables and media are easily identified to avoid any misuse.

6 Handling of biological material

6.1 Handling of biological material should be easy, simple and effective and should be performed in laminar flow hoods equipped with heating stages and pre-warmed heating blocks, using aseptic techniques at all times.

6.2 Measures must be taken to ensure that oocytes and embryos are always maintained at the appropriate temperature, pH and osmolality during culture and handling. Exposure to volatile or toxic substances, or harmful radiation should be minimised.

6.3 Buffered media (HEPES, MOPS or similar) should be kept in atmospheric air, while bicarbonate-buffered media should be kept in 5-7% CO2.

6.4 “In-house” made or sterilised devices for handling human gametes and embryos should be avoided. Pipetting devices (handling or denudation pipettes) should be used for one procedure only.

6.5 Traceability should be confirmed at all times (see Section 4 and Figure 1).

7 Oocyte retrieval

Oocyte retrieval is a particularly sensitive procedure and special attention should be given to temperature, pH as well as efficient and quick handling.

7.1 An identity check before the oocyte retrieval is mandatory.

7.2 The time between oocyte retrieval and culture of washed oocytes should be minimal. Prolonged oocyte exposure to follicular fluid is not recommended.

7.3 Appropriate equipment must be in place to maintain oocytes close to 37°C. Flushing medium, collection tubes and dishes for identifying oocytes should be pre-warmed.

7.4 Follicular aspirates should be checked for the presence of oocytes using a stereomicroscope and heated stage, usually at 8-60x magnification. Exposure of oocytes to light should be minimized.

7.5 Timing of retrieval, number of collected oocytes and the operator should be documented.

8 Sperm preparation

Before starting a treatment cycle, at least one diagnostic semen analysis should be performed according to the protocols described in the World Health Organization (WHO) manual (World Health Organization, 2010). In addition, a test sperm preparation may be advisable in order to propose the most adequate insemination technique (IVF/ICSI). Patients should be given clear instructions regarding the collection of the sperm sample (hygiene, sexual abstinence, timing, etc.). A frozen back-up sample should be requested if sperm collection

difficulty on the day of oocyte retrieval is anticipated.

Sperm preparation aims to:

- eliminate seminal plasma, debris and contaminants;
- concentrate progressively motile sperm;
- select against morphologically abnormal sperm.

8.1 Semen samples should be collected into sterile, plastic containers (tissue grade, sperm-toxicity tested). The use of spermicidal condoms, creams or lubricants must be avoided. The container should be clearly labelled and correct identification should be confirmed by the patient. Collection should be preferably performed in a room near to the laboratory. After collection, the sample should be delivered to the laboratory as soon as possible avoiding extreme temperatures (<20°C and >37°C). Analysis should start within one hour of collection.

8.2 Records should be kept of the type of container used, the time and place of collection, and the time interval between collection and analysis/preparation. The use of medication, fever during the previous months, and completeness of the ejaculate collection should be documented.

8.3 The following data on sperm preparation should be documented:

- sample origin (ejaculate/epididymal/testicular, donor/partner, fresh/frozen);
- preparation method;
- pre- and post- preparation sperm parameters and any dilution carried out;

8.4 An appropriate sperm preparation method should be chosen according to the characteristics and origin of individual samples. The swim-up technique and discontinuous density-gradient centrifugation are most frequently used and widely accepted.

8.5 In case of azoospermia on the day of oocyte retrieval, a second semen sample should be requested before considering alternative sperm retrieval procedures or oocyte cryopreservation.

8.6 *Specific treatments*.

- Phosphodiesterase inhibitors (pentoxifylline, theophylline) or the hypo-osmotic swelling (HOS) test may be used in absence of motile sperm.
- Enzymatic digestion of testicular tissue by collagenase may be applied if no sperm are observed.

8.7 For patients diagnosed with blood-borne viruses, extensive semen preparation by density-gradient centrifugation followed by swim-up is recommended. Depending on the serological status, it is recommended to freeze the prepared sperm suspension and to test its viral load before release. Only viral-free suspensions should be used for ART.

9 Insemination of oocytes

Oocytes can be inseminated by conventional IVF or by ICSI. The insemination/injection time should be decided based on the number of hours elapsed from ovulation trigger and/or oocyte retrieval, also keeping in mind that fertilization will need to be checked 16 to 18 hours later.

9.1 Conventional IVF

9.1.1 The number of progressively motile sperm used for insemination must be sufficient to optimise the chance of normal fertilization. Typically, a progressively motile sperm concentration ranging between 0.1 and $0.5x10^6$/ml is used.

9.1.2 The final sperm suspension should be in a medium compatible with oocyte culture. The fertilization medium should contain glucose to allow for appropriate sperm function.

9.1.3 A double check of identity of gametes at the time of insemination procedure is mandatory.

9.1.4 Records should be kept of the time of insemination, the operator and the concentration of progressively motile sperm used.

9.1.5 Co-incubation of cumulus oocyte complexes and sperm is usually performed overnight, although a shorter period may be sufficient.

9.2 ICSI procedure.

9.2.1 *Preparation of oocytes for ICSI.*

When removing cumulus cells from oocytes, hyaluronidase concentration and exposure should be kept to a minimum. In order to prevent oocyte damage, pipettes with appropriate lumen size should be used and vigorous pipetting avoided. After denudation, oocytes should be thoroughly washed to remove traces of hyaluronidase. The maturation stage of the oocytes should be recorded. It is recommended to perform denudation immediately prior to injection.

9.2.2 *The injection procedure.*

Records should be kept of the injection time (start and end of the procedure) and the performing operator. The duration of sperm identification and immobilization followed by injection should be minimised. The number of oocytes transferred to the injection dish should relate to operator's skills and sperm quality. During ICSI, the following points are important:

- Only mature oocytes should be injected.
- Oocyte morphology should be recorded. Giant oocytes should not be injected.
- Morphologically normal, motile sperm should be selected.
- Tail membrane breakage should be below the midpiece, and immediately perfomed prior to the injection of each individual oocyte.
- Polar body should be away from the injection site.
- Oolemma rupture should be assured prior to sperm injection.
- Appropriate temperature and pH should be maintained during injection.

Viscous substances such as polyvinylpyrrolidone (PVP) can be used to facilitate sperm manipulation.

In case of only immotile sperm cells, a non-invasive vitality test can be used to select viable sperm for injection. After injection, oocytes should be washed prior to culture.

9.2.3 A double check of identity of gametes before starting injection is mandatory.

10 Scoring for fertilization

10.1 All inseminated or injected oocytes should be examined for the presence of pronuclei (PN) and polar bodies at 16 to 18 hours post insemination. A normally fertilized oocyte contains 2PN and 2 polar bodies. For conventional IVF, cumulus cells must be removed and 2PN oocytes transferred into new dishes containing pre-equilibrated culture medium.

10.2 Fertilization assessment should be performed under high magnification (at least 200x), using an inverted microscope equipped with Hoffman or equivalent optics, in order to verify PN number and morphology.

10.3 At least the following characteristics of the 2PN oocytes should be recorded:

- PN juxtaposition;
- position of PN within the cytoplasm;
- relative size of PN.

10.4 Embryos derived from ≥ 3PN oocytes should never be transferred or cryopreserved. Under exceptional

circumstances, if no transferable embryos derived from 2PN oocytes are available, embryos derived from 1PN oocytes or oocytes showing no PN but going through normal cleavage may be used for transfer.

11 Embryo culture and transfer

In order to optimise embryo development, fluctuations of culture conditions should be minimised. Precautions must be taken to maintain adequate conditions of pH and temperature to protect embryo homeostasis during culture and handling.

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

11.1.1 A culture medium designed for embryo development should be used, e.g. sequential or single-step media.

11.1.2 The type and number of incubators should be appropriate to the workload.

11.1.3 Oil overlay minimizes changes to temperature, pH and osmolality.

11.1.4 For traceability purposes, single embryo culture is advisable.

11.1.5 For blastocyst culture, a low oxygen concentration should be used.

11.2 Embryo scoring should be performed at high magnification (at least 200x, preferably 400x) using an inverted microscope with Hoffman or equivalent optics. Evaluation of cleavage stage embryos should include cell number, size and symmetry, percentage of fragmentation, granulation, vacuoles and nuclear status (e.g. multinucleation). Blastocyst scoring should include expansion grade, blastocoel cavity size, and morphology of the inner cell mass (ICM) and trophectoderm (TE). Assessment should be performed at standardised times post-insemination. Embryo development can also be assessed using time-lapse imaging, allowing a more precise evaluation of the timing of consecutive events while not interfering with the embryo culture environment.

11.3 Embryo quality assessment records should include the operator(s), date and time of assessment, and embryo morphological characteristics.

11.4 Embryo selection for transfer is primarily based on developmental stage and morphological aspects. Other selection parameters, such as time-lapse kinetics, may be considered.

11.5 Single embryo transfer is recommended to avoid multiple pregnancies. The decision on the number of embryos to transfer should be based on embryo quality and stage of development, female age, ovarian response and rank of treatment. It is advisable not to transfer more than two embryos.

11.6 Supernumerary embryos may be cryopreserved, donated to research or discarded, according to their quality, patient wishes and national legislation.

11.7 For the transfer procedure, the patient records should include:

- date and time of embryo transfer;
- name of the operator;
- name of the practitioner performing the transfer;
- number, developmental stage and quality of embryo(s) at the time of transfer;
- type of catheter used;
- fate of supernumerary embryos;
- details about the procedure e.g. presence of blood, retained embryo(s).

11.8 If the laboratory is some distance from the embryo transfer room, arrangements should be made to maintain temperature and pH whilst transporting embryos.

11.9 A double identity check of the patient, the patient file and the culture dish(es) is mandatory immediately prior to the transfer.

12 Cryopreservation

Cryopreservation can be performed for gametes, embryos and tissues.

12.1 Facilities should be available to cryopreserve and store biological material.

12.2 Different cryopreservation approaches, including slow freezing and vitrification, can be used according to the type of biological material.

12.2.1 For sperm, slow freezing is still the method of choice, but rapid cooling is a possible alternative.

12.2.2 For oocytes, vitrification has been reported to be highly successful and is recommended.

12.2.3 For cleavage stage embryos and blastocysts, high success rates have been reported when using vitrification. However, for cleavage stage embryos good results can also be obtained using slow-freezing methods.

12.2.4 For tissues, the method of choice is slow freezing, but vitrification of ovarian tissue is an option.

12.3 In order to minimise any risk of transmission of infection via LN2:

12.3.1 Contamination of the external surface of cryo-devices should be avoided when loading them with samples.

12.3.2 Sealing should be carefully performed before cryopreservation.

12.3.3 Direct contact of the biological material with the LN2 should be avoided during storage. High-security closed devices have been developed in order to fulfil this requirement. If using open devices, storage in vapour phase LN2 is recommended.

12.3.4 Specimens from sero-positive patients should be stored in high-security closed devices. Dedicated vapour phase tanks are recommended.

12.4 At cryopreservation, documentation on biological material should include:

- Labelling of devices
- Cryopreservation method
- Date and time of cryopreservation
- Operator
- Embryo quality and stage of development
- Number of oocytes or embryos per device
- Number of devices stored per patient
- Location of stored samples (tank, canister)

12.5 Cryo-devices must be clearly and permanently labelled with reference to patient details, treatment number and/or a unique identification code.

12.6 A periodic inventory of the contents of the cryobank is recommended, including cross-referencing contents with storage records.

12.7 At thawing, documentation on biological material should include:

- Thawing method;
- Date and time of thawing;
- Operator;
- Post thawing sample quality.

12.8 A double check of patient identity is recommended in the following steps: transfer of samples into labelled cryo-dish, loading of the labelled device, deposition in the cryobank, removal from the cryobank.

12.9 During storage and handling of cryopreserved material, care should be taken to maintain adequate and safe conditions. Temperatures should never rise above -130°C.

13 Emergency plan

As a part of the clinic's general emergency plan, all IVF laboratories should, develop an emergency plan with specific procedures in case of an exceptional failure of infrastructure and facilities, either of natural or human origin.

Emergency planning aims to describe the actions to be taken for (in order of importance):

- safety of personnel and patients;
- protection of all fresh and cryopreserved human material;
- limitation of damage to equipment and medical records.

13.1 The following factors should be considered:

13.1.1 Communication measures in emergency situation: contacts (responsible persons, technical services, contact numbers) should be clear for all personnel.

13.1.2 Facilities:

- Electricity: loss of electrical power should be compensated by generators or uninterrupted power supply (UPS) systems.
- LN2: in case of failure of automatic supply lines, tanks should be filled manually. A fully filled reserve LN2 tank should be available.

13.1.3 Equipment:

- In case of power failure, critical equipment should be prioritised.
- A second item of critical equipment should be available if the first item fails. All reserve equipment should be fully validated and ready for use.
- Freezer (-20 °C) and refrigerator: Back up cooled freezers and refrigerators should be available.
- Cryopreservation vessels: It may be necessary to move tanks to another location.

13.1.4 Medical records: Records to identify the ownership of human tissue should be kept on a secure web server.

13.2 Regular revision of the emergency plan is necessary.

13.3 Third party arrangements should be in place with another IVF laboratory for emergency transfer of gametes and embryos (fresh and cryopreserved).

ANNEX 1

Methodology

The steering committee of the ESHRE SIG Embryology decided there was a need for an update of the "Revised guidelines for good practice in IVF laboratories" (Magli*, et al.*, 2008). After approval of the ESHRE Executive Committee, a guideline development group (GDG) consisting of 10 embryologists was composed.

During a first meeting, a six-step procedure was set-up for the development of the current document.

1. Formulations of comments to the Revised guidelines for good practice in IVF laboratories (Magli*, et al.*, 2008)
   The GDG members were asked to formulate comments to the 2008 document, including which recommendations needed to be rewritten, deleted or added.
2. Rewriting of the different subsections
   Based on the comments formulated by all the GDG members, every section was rewritten by an assigned GDG member.
3. Formulations of comments to the rewritten subsections
   All GDG members were asked to formulate comments to each of the rewritten sections. These comments were taken into account during the discussion of the recommendations.
4. Discussion and reformulation of the recommendations until consensus
   Two 2-day meetings were organised in which the GDG members discussed and reformulated each individual recommendation until consensus within the group was reached. After the meetings, the whole document was revised by the GDG members focussing on clarity, consistency and completeness of the sections.
5. Review by the ESHRE membership and relevant stakeholders
   The draft version of the guideline was published on the ESHRE website between .. and … ESHRE members and relevant stakeholders were invited to formulate comments to the document. Each comment was revised by the GDG members and resulted in either modification to the guideline text, or an answer formulated on the comment explaining why no modification was made. A report listing the reviewers and summarizing all comments will be published on the ESHRE website.
6. Publication of the guidelines
   The guidelines will be published on the ESHRE pages of Human Reproduction. The guidelines and accompanying documents will also be published on the ESHRE website.

As evidence on most of the issues is scarce, a formal assessment of any scientific evidence was not performed.

The GDG have taken into account the recommendations published in the EUTCD (European Commission, 2006a, c, 2012) and recent documents, manuals and consensus papers (Magli*, et al.*, 2008; Zegers-Hochschild*, et al.*, 2009; World Health Organization, 2010; The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011; Alpha Scientists in Reproductive Medicine, 2012; Asociación Española de Normalización y Certificación, 2013; Council of Europe, 2013; Kovacic*, et al.*, 2015)

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