ESHRE guidelines for good practice in IVF laboratories

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Dear ESHRE members,

Since the publication of the guidelines for Good-laboratory practice in 2000 in Human Reproduction, a lot has changed in the IVF world. Notably the implementation of the EU Directives will have a major impact in IVF Good laboratory practice. Therefore we thought it was useful to revise importantly the Guidelines. Thanks to the tremendous efforts of Christina Magli, Josiande Van der Elst, Kertsti Lundin, Dominique Royere, the revised version of the guidelines in compliance with the EU directives is now accessible on the Embryology website. Please feel free to comment on this revised form. You can send in your suggestions during a period of three months following the placement of the revised guidelines on the website. After that period and after taking into account pertinent suggestions, the revised guidelines will be submitted to Human Reproduction for publication.

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(Revised) ESHRE GUIDELINES FOR GOOD PRACTICE IN IVF LABORATORIES

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Introductory remarks

In the year 2000, ESHRE introduced guidelines constituting the minimal requirements for any laboratory offering Assisted Reproduction Techniques (ART). This was done with the aim of implementing a quality system for all the embryologists and IVF laboratory workers of ESHRE, in the understanding that the embryologist has a responsibility for the correct and justified application of ART in the laboratory. The strict application and further development of these guidelines benefit all patients attending ART clinics, ART professionals and the embryologists themselves.

The implementation of guidelines for good practice in IVF laboratories requires a quality management program to be in place, integrating quality control, quality assurance and quality improvement. This should be performed by the design of a quality policy and a consequent implementation of a quality system that encompasses and integrates the operative units, the processes and procedures that represent the core of ART clinics.

In March 2004, the European Parliament issued the Directive 2004/23/EC "On setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells" (European Union, 2004). The Directive applies to human tissues and cells, including fresh or frozen reproductive cells for application to the human body (see Directive 2004/23/EC, page L102/48, (7) and and Article 1, Objective, page L 102/51).
The Directive requires specifically that tissue establishments have a comprehensive quality management system. The requirements specified by the Directive and the annexed documents (Commission Directives) in which the corresponding technical directives are listed in detail, have to be applied by the Member States by the dates established in the Documents and in the respect of National regulations. The first Commission Directive "On the donation, procurement and testing of human tissues and cells" has been published on 8 February 2006 (European Union, 2006), and must be implemented by member states by 1 November 2006. The second Commission Directive "On technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells" was adopted on the 24th of October 2006 (European Union, 2006), and must be implemented by member states 1 September 2007.
The directives can be found at:

http://ec.europa.eu/health/ph_threats/human_substance/legal_tissues_cells_en.htm

In view of these considerations, the guidelines for good practice in IVF laboratories issued by ESHRE not only respond to the need of embryologists for support and guidance in their duties, but may represent a point of reference for the national competent authorities inspecting according to the Directive.

The current version of the "Guidelines for good practice in IVF laboratories" is based on the previous document (Gianaroli et al., 2000) and should be seen as a complement to the requirements issued by the Tissue Directive.

1. STAFFING AND DIRECTION

1.1 The laboratory should be directed by an appropriately qualified and experienced responsible person with qualifications of diploma and/or expertise in the field of medical or biological sciences according to national rules.

1.2 The responsibilities of the laboratory director should include:

1.2.1 Ensuring that laboratory facilities are appropriate and safe by systematically controlling their functioning and programming periodical maintenance.
1.2.2 Ensuring that written procedure manuals for all procedures exist and are carried out appropriately by the laboratory staff.
1.2.3 To periodically review and update procedure manuals, and ensure that only the updated version of the manual is available in the laboratory
1.2.4 To verify that all the processes are performed in compliance with a quality system.
1.2.5 To ensure that appropriate numbers of staff are in place, having the required experience to undertake the workload of the laboratory in relation to the techniques offered.
1.2.6 To ensure that all new staff are given a comprehensive orientation and introduction programme. Beginners should follow a training scheme under the assistance and control of experienced embryologists and of the laboratory director.
1.2.7 To organize and control laboratory staff training and ensure that the staff receive continual medical education (CME).
1.2.8 Ensuring that individual responsibility of each member of the staff and line of responsibilities are defined and indicated in written procedures, that are known and accepted by all members of the staff.

2. POLICIES AND PROCEDURES

2.1 All laboratory procedures must include provision for unique patient identification, and corresponding gametes and embryos identification, whilst retaining patient confidentiality.
2.2 The updated version of detailed manuals for all procedures used should be available in the laboratory.
2.3 Written, signed and dated protocols should exist for every procedure that is performed in the laboratory.
2.4 There must be written procedures for dealing with and documenting incorrect or incomplete identification of specimens or documentation, as well as with any non-compliance, emergency situation or adverse event.

2.5 Laboratory and clinical results (i.e. fertilization rates, pregnancy rate etc.) should be regularly updated and summarised, and kept available to all staff.

2.6 A log book should be maintained to permit regular evaluation of the results, including the performance of each single operator.

2.7 Every communication with the other operative units (clinicians, geneticists, nurses, secretariat) should be specified by written procedures.

2.8 Taking into account the high degree of attention needed during embryologist's or technician's laboratory work, any communication to the laboratory, such as phone calls, should be kept to a minimum.

3. LABORATORY SAFETY

3.1 Laboratory design.

The embryology laboratory must have adequate space to allow good laboratory practice and should be as close as possible to the operating room in which the clinical procedures are performed.

When commissioning the laboratory, thought should be given to the most recent developments in equipment and facilities. Attention should be given to the ergonomics of the operator: bench height, adjustable chairs, microscope eye height, efficient use of space and surfaces, sufficient air-condition with controlled humidity and temperature, all contributing to a working environment that minimizes distraction and fatigue. Consideration should also be given to local health and safety requirements.

More specifically:

- The construction of the laboratory should ensure aseptic and optimal handling of gametes and embryos during all phases of the treatment.
- HEPA (high-efficiency particulate air) and VOC (volatile organic compounds) filtration of the air supplied to the laboratory and clinical procedure rooms is recommended. In addition, the laboratory should be overpressured to exclude contamination from external areas.
- Changing rooms should be located close to the laboratory, having hand washing facilities.
- Access to the laboratory should be limited to authorized personnel.
- Pass-throughs for all materials and disposables coming into the laboratory are recommended.
- The location of storage areas and equipment such as incubators, centrifuges and cryo equipment should be logically planned for efficiency and safety within each working area.
- Separate office space should be provided for administrative work, such as record keeping and data entry.
- A general wet area in which washing of equipment, sterilisation, etc., is performed, must be separate from the embryo laboratory. Moreover, if fixatives are applied, these analyses must be performed in a separate room, preferably in a fume-hood.

3.2 Laboratory equipment.

- The laboratory equipment must be adequate for laboratory work and easy to clean and disinfect.
- Critical items of equipment, including incubators and frozen embryo storage facilities, should be appropriately alarmed and monitored.
- An automatic emergency generator backup in the event of power failure should be in place.
- A minimum number of two incubators is recommended. Gas cylinders should be placed outside or in a separate room with an automatic backup system.
- Incubators should be frequently cleaned and sterilised. Nitrogen tanks should be cleaned and sanitised at least every year.
- Records of ordinary and extraordinary maintenance on all the equipment must be documented and retained.

- Devices for the maintenance of temperature of media, gametes and embryos during each phase of the procedure, when they are out of the incubators, should be in place (i.e. warm stages, heating blocks).
- Regular checks of functional parameters for devices used to maintain temperature and CO_2 should be performed using calibrated thermometers and extra methods of CO_2 analysis and/or pH measurement. Record of these measurements, as well as those shown by the digital displays of each device, must be retained.
- The instruction manual for every instrument should be available in the laboratory.
- Written instructions should be available for actions to be taken in the case of equipment failure.

3.3 Infectious agents.

All assisted reproductive technologies involve handling biological material, and pose a potential hazard of transmitting diseases to personnel and to other patients' gametes or embryos (cross-contamination). Each unit should establish procedures and policies for the safety of personnel and for preventing cross contamination, taking national and/or local safety regulations into consideration. Therefore:

- Vaccination of the personnel against Hepatitis B or other viral disease, for which the vaccine is available, is recommended
- Screening patients and gamete donors for HIV, Hepatitis B/C and other sexually transmissible diseases before processing or cryopreservation should be routinely adopted according to Commission Directive 2006/17/EC Annex III.
- As patients' admittance to IVF treatment cycles is regulated by physicians, and although the laboratory staff should treat each sample as potentially infectious, the laboratory staff must be informed about the risks of handling infected biological material, whenever the information is available

The treatment of patients positive for HIV or Hepatitis B/C should be only performed in laboratories having dedicated areas, in which the adequate safety measures are followed. Alternatively, patients with positivity for HIV or Hepatitis B/C could be allocated to specific series, which are followed by an accurate cleaning and disinfection of the laboratory.
A Class II laminar flow cabinet that protects both the operator and the specimen should be used when contaminated samples are handled.
Under these safety conditions, the occurrence of disease transmission from semen samples carriers of HIV or Hepatitis B/C to their partners are very rare (Englert et al., 2004; Sauer, 2005), whereas no cases have been reported of infections to the operators. Only one report has been published on nosocomial infection with Hepatitis C after assisted conception (Lesourd et al., 2000).

3.4 Protective measures.

All body fluids (follicular fluid, semen etc.) should be treated as potentially contaminated.
The purpose of the protective measures is both to protect laboratory staff and to ensure aseptic conditions for gamete and embryos. The procedures should deal with, but not be limited to, the following:

- Use of laboratory clothing
- Use of non-toxic (non-powdered) gloves and masks where appropriate

- Use of eye and face protection, and of cryogloves if cryogenic materials are handled.
- Use of vertical laminar-flow benches
- Use of mechanical pipetting devices
- Use of fume-hood in case of fixatives
- Use of disposable material; after usage, discard it immediately in the proper waste containers. Potential infectious materials must be disposed of in a manner that protects laboratory workers and maintenance, service, and housekeeping staff from exposure to infectious materials in the course of their work.
- Needles and other sharps should be handled with extreme caution and discarded in special containers. If possible, omit glassware in the laboratory, otherwise discard the Pasteur pipettes and broken glassware in special containers.
- Food, drinks, cigarettes and cigars are strictly forbidden.
- The use of make-up and strong perfumes is strongly counterindicated.

Cross-contamination with infectious material from one patient to another could still occur during the cryopreservation procedure when straws with semen, oocytes or embryos are filled by dipping the straw in semen or in patient medium with gametes or embryos, sealing it and passing it into liquid nitrogen without external disinfection (Tedder et al., 1995). It is advisable to keep the material stored in the cryopreservation tanks in a way that avoids contact of the liquid nitrogen phase with the biological substances.
Safety cryo straws have been conceived in order to fulfil this requirement. Specimen known to be contaminated should be stored in such high security straws and, preferably, in dedicated tanks. Also samples containing body fluids, such as seminal plasma, should be cryopreserved in high security straws.
Another way to avoid contact with liquid nitrogen is to store samples in the gaseous phase of liquid nitrogen. Tanks constructed specially for this have been developed (Bielanski, 2005).

4. IDENTIFICATION OF PATIENTS AND THEIR GAMETES AND EMBRYOS .

4.1 Relevant patient consent should be verified before commencing any procedure.

4.2 The clinical and serological exams performed by the patients before being admitted to IVF treatment should be checked in order to detect any possible positivity to viral infections.

4.3 Written procedures should be present describing in detail the various phases of IVF techniques, including all the laboratory operative procedures, in which the protocols, the equipment and the material list should be specified. In this way, reproducibility and competence in handling gametes and embryos can be assured.

4.4 Rules concerning the correct handling and identification of gametes and embryo samples should be established by a system of checks and, where needed, double-checks by a second person.

- All material obtained from the patients, i.e. tubes with blood, follicular fluid and sperm samples, must bear unique identification of the treated couple
- Incubators should be organised in order to facilitate identification of embryos, oocytes and sperm.

- Verification of patients' identity should be performed at critical steps: before ovum pickup, at semen recovery, and at embryo transfer procedures.
- Double checks are recommended at least at: insemination of oocytes, replacement of embryos, embryo freezing and thawing.
- Documentation of all critical steps in each patient's file is essential.
- The identity of the laboratory person handling the samples at each point of the process, from receipt through final disposition, date and time, should be clearly indicated. This permits tracking of the sample throughout its period in the laboratory, also at later dates.

4.5 Proper training of all the laboratory staff accordingly to these procedures is mandatory.

5. CULTURE MEDIA PREPARATION AND QUALITY CONTROL TESTING

The Commission Directive 2006/86/, page L 294/34 and L 294/38, C.6, indicates the requirement of specifications for critical reagents and materials.

5.1 Disposables and Reagents

- Culture media should be of tissue culture grade, preferably mouse embryo tested and with a purity appropriate for the purpose. Use of commercially produced, quality controlled tested media is recommended. When commercially produced media are used, it is important to check that producers use validated quality control testing, if not this has to be done by the laboratory. In addition, integrity of the packages and appropriate delivery conditions should be controlled. Documentation of quality control testing using an adequate bioassay system must be supplied by the manufacturer for any commercially produced media employed. Correspondence with the delivered batch should be verified.
- Reagents and media should always be used prior to the manufacturer's expiry date.
  · Appropriate refrigeration facilities must be available for media and reagents storage.

If culture media are produced in the laboratory, the following is recommended:

5.2 Water used for the preparation of culture media must be of appropriate purity.
· If the water is purified on site, protocols must be in place for quality control of the water system, including sanitization of the system, regular cartridge changes and replacement of other components, endotoxin and bacterial screening.
· If culture grade water is purchased, there must be strict control over storage and use in relation to temperature and shelf life.

5.3 Reagents must be designated exclusively for use in culture media.
· All batch numbers of reagents, media and disposables used in the preparation of media should be systematically documented, including the date of their introduction to the system.

5.4 Donor serum or follicular fluid are not recommended. In case they are used in culture media they should be screened according to the local rules for blood donors.
· Human serum albumin (HSA) should come from a source screened as above. Commercial suppliers of HSA or media containing a serum derived protein source should supply evidence of such screening.
· Appropriate quarantining arrangements must be in place for use of donor serum or follicular fluid.

5.5 Similarly to commercially produced media, all tissue culture media prepared in the laboratory should undergo quality control including bacterial and endotoxin testing, and an appropriate bioassay system.

5.6 Use of mineral oil.

- Oocytes and embryos may be cultured under equilibrated mineral oil. The oil maintains temperature, osmotic pressure and pH during short-term manipulations of the oocytes.
- Similarly to commercially produced media, documentation of quality control testing including an adequate bioassay system should be supplied by the manufacturer.

5.7 Each lot of culture media and mineral oil should be recorded in each patient's worksheet, being traceable for each step of the procedure.

6. HANDLING OF EMBRYOS, OOCYTES AND SPERMATOZOA

The laboratory procedures regarding the handling of embryos and gametes for assisted reproduction techniques have been standardized. The procedures should be easy, simple and effective and should be performed in a laminar flow hood equipped with stages and pre-warmed heating blocks. Class II hoods should be used for documented contaminated samples, since they give protection also to the operator. Aseptic technique should be used at all times.

6.1 Appropriate measures should be taken to ensure that oocytes and embryos are maintained at 37 degrees centigrade during handling/observation using hot plates or other systems.

6.2 Whenever possible, tissue culture grade disposables should be used for handling body fluids/cells (see Commission Directive 2006/17/ page L 38/50 on medical devices, and Commission Directive 2006/86/ page L 294/34 and L 294/38, C.6 on critical reagents and materials).

6.3 Pipetting devices (pasteurs, drawn pipettes, tips etc.) should be used for one procedure only, should never be used for more than one patient, and should be disposed of immediately after use.

6.4 Simultaneous treatment of more than one patient should never be done in the same working place. Each sample should be handled individually and its treatment should be completed before moving to the following sample.

6.5 Identifying information marked on the culture dish/tube should be cross referenced to the patient and the patient's documentation.

- Procedures must be in place which ensure correct patient identification at all stages.
- Labelling of dishes/tubes containing oocytes, embryos, or sperm must be permanent (Strong odour markers should be avoided).
- Incubators should be organized in order to facilitate identification of embryos, oocytes and sperm for each patient.

6.6 At each stage of the procedure, date, time and identity of the operator should be recorded. This is especially useful in cases of recalls.

6.7 Before receiving samples, the identity of the corresponding patients should be controlled.

6.9 All the above points are also to be applied to thawed gametes and embryos with the exception of temperature requirement during thawing that may vary according to the used protocol.

7. OOCYTE RETRIEVAL

As the meiotic spindle starts to depolymerize at about 35°C, oocytes need to be kept at body temperature (close to 37°C) as much as possible. Following temperature-induced depolymerization, the spindle reassemble spontaneously when the temperature rises again, but errors in this process may cause aneuploidy (Pickering et al., 1990).

7.1 There should be appropriate equipment in use to maintain a 37°C temperature also when laboratory and egg collection areas are on different sites. Scanning petri dishes, collection tubes and heating blocks should be pre-warmed at 37°C.

7.2 Detailed written procedures for oocyte collection and culture must be available.

7.3 Follicular aspirates are checked for the presence of oocyte-cumulus complexes under a stereo dissecting microscope with transmitted illumination base and heated stage, usually at 8-60x magnification. Exposure of oocytes to light should be minimized whenever possible.

7.4 Morphological criteria for the description of oocyte quality and maturity, as well as modality of observation should be specified. The morphological evaluation of the recovered oocytes should be documented in the patient's worksheet.

7.5 Where donor oocytes are to be used, traceability must be guaranteed according to the rules existing in the laboratory. Whenever freezing donor oocytes, the unique European code must be used, whose implementation is foreseen for the end of 2008.

8. SPERM PREPARATION

Before starting a treatment cycle, semen analysis should be performed according to the protocols described in the WHO manual (WHO 1999).

8.1 The semen sample is collected in a sterile, plastic container without using spermicidal condoms, creams or lubricants. The container should be clearly labelled with the names of the couple. After collection, the sample should be delivered to the laboratory as soon as possible, preferably within one hour from collection, and extreme temperatures should be avoided (WHO, 1999).

8.2 Records should be kept of the type of container used (if this differs from the norm), time and place of collection (with particular reference to samples produced outside clinic facilities), and the time interval between collection and preparation.

8.3 Where donor sperm is used, the necessary identifying information (donor code/clinic code) must be recorded. The definition of a unique European code is foreseen for the end of 2007 with implementation in 2008. From then, the unique European code must be used.

8.4 Written procedures should be available and include:

- medium type
- sperm preparation technique (e.g. swim-up or gradient centrifugation procedure)
- semen to medium ratio
- centrifugation time and force
- incubation time and condition

8.5 The method of sperm preparation should be recorded, including details of any variation on the standard laboratory protocol.

8.6 A record should be kept of pre- and post- preparation sperm parameters and of any dilution carried out prior to insemination.

8.7 In case of surgically retrieved spermatozoa, surplus sperm after insemination should be cryopreserved for further assisted reproductive cycles; this will avoid repeated surgery.

8.8 Sperm preparations must also be protected from extreme temperature (Mortimer, 2005):

- If the sperm suspension cools down below 20°C, a cold-shock occurs due to changes in the membrane phospholipids phase.
- If the temperature rises above the physiological norm, spermatozoa will be irreversibly damaged.

9. INSEMINATION OF OOCYTES

9.1 Preparation of spermatozoa for insemination.

9.1.1 The method of preparation is designed according to individual samples. A trial preparation prior to the treatment cycle may be advisable in order to choose the most adequate technique. Sperm preparation is aimed to:

- concentrate and select the active and motile spermatozoa;
- discard seminal plasma, debris and contaminants;
- select against abnormal forms.

9.1.2 A frozen backup sample may be requested for those patients for which the possibility of sperm collection difficulty is anticipated.

9.1.3 Different methods are used for sperm preparation. Among them, the swim-up technique and the discontinuous density-gradient centrifugation are the most used.
The standard protocols for sperm preparation must be detailed in a written procedure. As a general rule, excessive centrifugation should be avoided especially in oligospermic samples in order to avoid increasing the concentration of reactive oxygen species (Twigg et al., 1998).

9.1.4 The culture media used for the sperm suspension are bicarbonate-buffered and therefore susceptible to pH shifts if exposed to atmospheric air for more than two minutes (Mortimer and Mortimer, 2005). In addition, prolonged

exposure to high velocity air flow will provoke a decrease in the suspension temperature. Hence, the procedure of sperm preparation for insemination must be performed under conditions that maximize the control of temperature and pH.

9.2 Conventional IVF insemination.

9.2.1 A record should be kept of the time of insemination and the sperm concentration used.

9.2.2 The number of spermatozoa must be high enough to yield oocyte fertilization without compromising embryo development.

9.2.3 A double identity check at the time of insemination procedure is recommended.

9.3 Intracytoplasmic Sperm Injection (ICSI) procedure.

9.3.1 Preparation of oocytes for ICSI. Removal of cumulus - corona (CC) cells.
Oocytes are denuded from the surrounding cumulus and corona cells using an enzymatic procedure with hyaluronidase, mostly followed by mechanical denudation using a pipette. Both the enzyme concentration and the duration of exposure to the enzyme should be limited. Care needs to be taken in order to avoid damage to the oocytes, which can result from too vigorous pipetting or from a pipette diameter which is too small.

9.3.2 The injection procedure.
Record should be kept of the time of insemination (start and end of the procedure).
During ICSI, the following points are important:

- morphological and maturity status of each oocyte should be recorded
- the selection and immobilization of a viable sperm cell
- the correct positioning of the oocyte prior to injection
- the rupture of the oolemma prior to the release of the sperm cell into the oocyte.

Viscous substances such as polyvinylpyrrolidone (PVP) can be used to facilitate the manipulation of spermatozoa and to control the fluid in the injection pipette, limiting the volume injected into the oocyte.
It is important to select vital spermatozoa, as evaluated by their motility. Where only non vital sperm cells are present in the ejaculate, the use of testicular sperm may be tried.

9.3.3 At the end of the procedure, both the holding and injection needles must be discarded.

9.3.4 A double identity check at the time of ICSI dish preparation is recommended.

10. SCORING FOR FERTILIZATION

10.1 All oocytes that have been inseminated or microinjected should be examined for the presence and number of pronuclei and polar bodies at 16 to 20 hours post insemination.

10.2 This examination should be done under high magnification (at least 200x), using an inverted microscope equipped with Hoffman optics or equivalent, in order to verify normal fertilization and pronuclear morphology.

10.3 The morphological status of each oocyte/zygote should be recorded.

10.4 Oocytes with one or more than two pronuclei should be cultured separately from normally fertilized oocytes. Parthenogenetically activated oocytes can develop to blastocyst; if transferred, they will give false expectations of implantation. Oocytes with three pronuclei can also develop and occasionally reach term and delivery, but die early postnatal. In addition, human triploids originated from polyspermic fertilization (that represent by far the major cause of triploidy), often develop to partial hydatidiform moles that can lead to choriocarcinoma (Jauniaux, 1999; Zaragoza et al., 2000).

10.5 Oocytes showing no signs of fertilization at the expected time window should be maintained in culture and observed for late appearance of pronuclei.

10.6 The normally fertilized oocytes are removed from the insemination medium and transferred into new dishes with pre-equilibrated fresh culture medium.

11. EMBRYO CULTURE AND TRANSFER.

Preimplantation embryos are highly sensitive to culture stress resulting in perturbed metabolism with consequent altered cell function, energy production and gene expression (Lane and Gardner, 2005). Therefore, precautions must be taken to maintain adequate conditions of pH and temperature to protect embryo homeostasis.

11.1 The scoring of embryos should be performed at high magnification (at least 200x, preferably 400x) under an inverted microscope with Hoffman optics or equivalent. The evaluation should include, but not necessarily be limited to; number of cells, percentage of fragmentation, size and cytoplasmic appearance of blastomeres, nuclear status (presence of one or several nuclei per blastomere).

11.2 The stage of embryo development at the time of transfer should be documented. Embryos can be grown to day 5 or 6 for a transfer at the blastocyst stage, usually by cultures in sequential media. Current data show no evidence of increased pregnancy or live birth rates from blastocyst culture (Blake et al., 2005), but it might be of advantage for certain groups of patients.

11.3 In some countries the maximum number of embryos to be transferred is established by the local / national legislation. It is advisable not to exceed two embryos for transfer. In cases where two or more embryos are replaced, the couple has to be extensively informed on the risks of multiple gestations. In particular when blastocysts are available, the number of blastocysts transferred should be limited to two. As a general recommendation, the policy of single embryo transfer is highly recommended.
Single embryo transfers are now mandatory in some countries and practiced to a high extent in other countries. A recent Cochrane Collaboration review has concluded that single embryo transfer significantly reduces the risk of multiple pregnancy, but also decreases the chance of live birth in a fresh IVF cycle (Pandian et al., 2005). Subsequent replacement of a single frozen embryo achieves a live birth rate comparable with double embryo transfer (Pandian et al., 2005). Nevertheless, data from randomized control trials have shown

similar cumulative live birth rates (including following frozen-thawed transfers) in young women between single embryo transfer and double embryo transfer (Thurin et al 2004, Lukassen et al., 2005)

Supernumerary embryos may be cryopreserved, donated to research or discarded, according to the couple's wishes and to the national legislation.

11.4 The patient records for embryo transfer must be dated and signed, and should include details of:

- Batch number and type of media used for transfer
- Time from oocyte retrieval to transfer
- Time from oocyte insemination to transfer
- The number and developmental stage of embryos at transfer
- Fate of supernumerary embryos
- Type of catheter used for transfer
- Name of the clinician performing the transfer
- Name of the operator loading the catheter
- Notes about the clinical procedure: whether the transfer was easy, difficult, presence of blood, etc. (optional)

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

11.6 Sterile disposable catheters should be used for transfer. (see Commission Directive 2006/17/ page L 38/50 on medical devices, and Commission Directive 2006/86/ page L 294/34 and L 294/38, C.6 on critical reagents and materials).

11.7 A double identity check is recommended at the moment of loading the catheter.

11.8 Before transferring the embryos, the patients' identity must be double checked.

12. CRYOPRESERVATION OF GAMETES AND EMBRYOS

Freezing can be performed at different stages: gamete stage, zygotes, embryos in early cleavage (day-2 or day-3), day 4 or blastocysts. Embryos displaying a high degree of fragmentation, or very slow or blocked cleavage rate should be discarded from storage procedures due to the low survival rate reported after thawing. (Gianaroli et al., 2000).

In some countries, the application of cryopreservation procedures is regulated by law and / or by the patients' consent.

12.1 Techniques and facilities for cryopreservation of embryos should be available in each IVF center with the aim of:

- cryopreserving spare embryos after transfer;
- delaying embryo transfer in a subsequent cycle if the patient is unable to undergo the procedure or is at risk of developing OHSS;
- storing the embryos generated from donated oocytes in order to allow a six-month quarantine so that the potential donors may be controlled for infectious diseases prior to embryo transfer.

If the laboratory performs cryopreservation, a system should be in place for the detection of low levels of liquid nitrogen in the tanks and for high levels of nitrogen in the air. For this reason, it is recommended to keep the nitrogen tanks in dedicated, controlled areas.

12.2 Several protocols for cryopreservation, including slow-freezing protocols and vitrification protocols have been formulated depending on the embryo development stage, type of cryoprotectant, and speed of cooling

12.3 In order to minimize any risk of transmission of infection via liquid nitrogen, gametes and embryos should be stored in specific receptacles (i.e. straws, vials etc.) that can be sealed effectively.

- Transfer of samples to receptacles should be by a method which avoids contamination of the external surface.
- Sealing should be carefully performed before freezing.

12.4 Patients whose gametes or embryos are being processed or are to be cryopreserved must be tested according to the regulations in the Commission Directive 2006/17/EC Annex III, i.e. for HIV 1 and 2, and for Hepatitis B and C.

- When a patient is known to be a source of infection risk, a system of separate storage must be in place.
- Patients having transfer of thawed embryos ideally should be screened for HIV 1 and 2, Hepatitis B and C.

12.5 Documentation on stored embryos should include:
- The method of freezing and thawing
  The type and batch number of cryoprotectant used.
- The stage of embryo development.
- The number of embryos in each straw/vial (should not exceed two).
- The number of straws/vials stored per patient.

12.6 Straws/vials containing samples must be clearly and permanently labelled with reference to patient details and their unique identification code.

12.7 All reproductive cells for application to the human body are subjected to the requirements about traceability and coding that have been established by the European Directive. These rules apply to fresh and frozen cells, but for frozen cells for non partner donation the unique European code has to be used (still to be established by the European Commision; implementation foreseen in 2008).

12.8 Storage records should be kept in both the patient's individual records and the storage records for individual nitrogen banks.

12.9 An annual audit of stored gametes and embryos must be carried out, cross referencing contents with storage records.

12.10 Storage records must include precise details of the location of the vials/straws.

12.11 Documentation of thawing procedures should include morphological changes seen during thawing, number of cells, number of survived cells and the time period of culture prior to transfer.

13. ASSISTED HATCHING

This technique has been designed with the aim of helping embryo hatching and possibly implantation. However there are conflicting reports about its clinical efficacy.

13.1 Three methods are being used; the mechanical technique, that is partial zona dissection with glass microneedles, the chemical assisted hatching using acidic Tyrode's, and the laser assisted hatching.

13.2 Special care should be taken to avoid damage to the embryo during the procedure.

14. PREIMPLANTATION GENETIC DIAGNOSIS

The purpose of the procedure is to identify embryos, generated in vitro, which carry genetic or chromosomal abnormalities and exclude them from transfer.

14.1 Genetic counselling should be available to all “at risk” couples.

14.2 The biopsy procedure may be carried out by:

- polar body removal;
- single or double blastomere biopsy at the day 3 stage;
- trophectoderm biopsy at the blastocyst stage

14.3 The cells destined to genetic investigation are removed in the IVF laboratory using glass microtools on a micromanipulation set. The embryology laboratory has the responsibility of providing unique identification between biopsied polar bodies, blastomeres or trophectoderm cells and the corresponding oocyte, embryo or blastocyst respectively, implying the implementation of single oocyte / embryo / blastocyst culture after biopsy. All cells and embryos for genetic investigation must be individually handled, carefully identified and labelled, and tracked during the whole procedure. During these steps, double identity checks are strongly recommended.

14.4 Special care must be taken to avoid damage to the embryo during the procedure. In addition, when blastomere biopsy is performed, integrity of the removed cell is extremely important for the correctness of the genetic analysis.

14.5 The biopsy sample should be subjected to diagnostic procedures in a genetic laboratory.

14.6 Pre-implantation aneuploidy screening is performed in the same way as PGD, and is sometimes used as a complement to ordinary morphological selection of embryos for transfer. It is a method used to find the chromosomally normal embryos when there is no hereditary genetic indication. Clinical efficiency of PGD-AS in randomized studies has not been proven.

Although applied in a few centers world-wide and not allowed by law in some countries, the results obtained indicate that the procedure does not negatively compromise embryo development and implantation despite its invasiveness. The main advantage derived from its application is being an alternative to therapeutic abortion due to a minimised risk of transferring affected embryos.

In the year 2004, the PGD International Society (PGDIS) published guidelines for good practice in PGD (Preimplantation Genetic Diagnosis International Society, 2004). The following year, the ESHRE PGD Consortium published best practice guidelines for clinical PGD and preimplantation genetic screening (Thornhill et al., 2005).

15. QUALITY CONTROL AND QUALITY ASSURANCE

15.1 Working in compliance with a Quality system program is recommended. This implies:

- having validated and written procedures for each aspect of the process, including the occurrence of incidents or hazards;
- whenever possible, assuring that all media / reagents / disposables etc. are tested for quality using an appropriate assay;
- verifying conformance to the specifications;
- taking any corrective action to keep procedures under conformity;
- maintaining and calibrating equipment on a periodical basis (daily / weekly / monthly / yearly)

15.2 A systematic monitoring of the testing process can be performed under Quality Assurance, aimed at improving the entire process by identifying problems, errors or progresses that may have occurred. For this internal quality assurance, results should be evaluated on a regular basis, indicators should be objective and relevant, and adequate thresholds set up. In order to prevent bias due to patient variation, a representative number of procedures in relation of the total number of procedures performed should be selected to establish the corresponding thresholds. Critical levels of laboratory performance for each indicator should be defined.

The following indicators should be regularly reviewed and analyzed:

- Numbers/rates of errors and adverse events
- Rates of normally fertilized oocytes
- Cleavage rates
- Rates of embryos of good quality
- Proportion of patients with failed fertilization
- Pregnancy rates
- Multiple pregnancy rates
- Implantation rates
- Rate of survival of embryos after thawing

For a complete evaluation of the results, this analysis should be performed in collaboration with the clinical staff. In addition, the results should be related to those reported in the specialized literature, including data from the European registry collected by the European IVF-monitoring programme (EIM) for ESHRE (Nyboe Andersen et al., 2007).
To complement the internal quality assessments, participation in external quality assurances programmes, either commercial, or in collaboration with other laboratories, is recommended.

CONCLUSIONS

The expansion of indications for ART has not only increased the number of patients attending IVF clinics, but also the number of techniques that are currently in use. This implies that, after 30 years of IVF, there is a diversification of techniques with a consequent customization of treatment for single categories of patients.
In IVF laboratories, the diversification of the applied procedures necessitates a major attention and concentration, a systematic check of the results obtained, and a more complex training program. Extensive research is necessary before admitting new procedures to standard technique protocols which are routinely performed in IVF laboratories.

As future perspectives, the IVF laboratory is deeply involved in defining novel techniques to be admitted 1) as standard clinical protocols which are routinely performed to expand ART indications, such as freezing of oocytes and ovarian tissue and in vitro maturation of oocytes, and improving embryo implantation through a better selection of gametes and embryos; 2) in basic research such as the generation of embryonic stem cell lines.

The present European situation and the policy adopted by the European parliament comprise a comprehensive management system to be a standard requirement for ART clinics. In this context, the ESHRE guidelines for good practice in IVF laboratories, together with the European Tissue Directives, do not represent anymore an option, but a prerequisite to operate and provide the best clinical outcome in a safe working system.

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