



TRAINING PROGRAMME IN CLINICAL EMBRYOLOGY

Approved by

*The European Society of Human Reproduction and Embryology (ESHRE)
and The European Board and College of Obstetrics & Gynaecology (EBCOG)*

POSTGRADUATE TRAINING AND ASSESSMENT WORKING PARTY

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ANNEX II

PRACTICAL TRAINING PROGRAMME IN CLINICAL EMBRYOLOGY (SYLLABUS)

Main objective:

Trainees should acquire detailed knowledge and skills with which they will become competent to independently perform the activities according to the highest clinical, laboratory, safety and quality standards in the areas described below.

Introduction of trainee in the practical clinical laboratory work:

Trainees should be systematically introduced into the clinical programme in such a way that no damage or injury to equipment, instruments, biological material, patients, colleagues, visitors or to themselves is caused. The quality and safety of treatment should never be endangered. The supervisor has a duty to include the trainee in the clinical programme on a regular basis and in a structured manner from the beginning of the training. The complexity of the training should be increased according to the supervisor's assessment of the trainee's competence. Patient written consent must always be provided prior to use of biological fluids/tissues and gametes/embryos for training purposes.

Recommendations on the biological material to be used during training:

1. Semen and testicular specimen evaluation, preparation for ART and cryopreservation should be performed on parallel samples (one half of the specimen should be processed by an experienced embryologist and the other half by a trainee). The comparison of the obtained counting values (yield number, concentration, % motile, % morphologically optimal sperm) should be evaluated by validated methods.
2. COC handling training should begin with practising the transfer of granulosa residues from a follicular aspirate to the culture medium dish, once a COC harvest has been completed. Training in the identification and isolation of COCs from follicular fluid should first begin with the search for COCs in the last aspirate with constant support by the supervisor. The proportion of examined aspirates should be proportionally increased with trainee experience, but supervisor should make a double checking of all aspirates.
3. Insemination training for conventional IVF should begin with the insemination of one or two COCs, followed by immediate observation of motile sperm in the IVF dish.
4. Insemination training for ICSI should include placement of micromanipulation pipettes on the micromanipulator as well as the identification of optimal sperm in the ICSI petri dish left after an ICSI procedure has been completed. The trainee should learn to immobilize and isolate sperm into a PVP droplet.

5. Movement of oocytes within the same dish should involve abnormally fertilised oocytes ($\geq 3\text{PN}$) and/or at least 48-hour-old oocytes that have failed to fertilize and are donated by patients. This can be followed by transfer between different dishes.
6. At least 48-hour-old oocytes that have failed to fertilize and are donated by patients may first be used to practise the injection procedure. Microbeads or air bubbles (non-biological material and never sperm) should be injected into the cytoplasm. The technique should be evaluated by the supervisor and the lysis rate should be recorded. Once the supervisor considers that the micromanipulation skills are at an appropriate level, the trainee should begin injecting sperm into 'live' oocytes giving them 1 or 2 oocytes from a live ICSI, where there are at least 10 oocytes, and the supervisor ICSIs the first 8. In the next step, the mature oocytes of an individual patient should be injected by a trainee and experienced embryologists in a proportion related to the trainee's skills.
7. Animal models or participation in organized hands-on workshops are ideal for practising cryopreservation and biopsy of oocytes and embryos and cryopreservation of ovarian tissue. Similar to the ICSI procedure, the use of donated oocytes that have failed to fertilize or have matured in vitro from GV and MI oocytes, as well as donated arrested embryos and embryos developing from abnormal fertilisation, is recommended for cryopreservation and biopsy training. Donated ovaries after ovariectomy can be used for ovarian tissue cryopreservation.
8. Vital oocytes or embryos of an individual patient should be cryopreserved / biopsy by a trainee and experienced embryologists in a proportion related to the trainee's skills.

CORE TRAINING PROGRAMME (YEARS 1 - 3)

I. Basic principles of working aseptically in a medical laboratory for ART

Objectives: The trainee gets familiar with basic laboratory rules and should be practically involved in all procedures for:

1. Maintaining laboratory hygiene standards by considering:
 - a. Restricted to authorised personnel
 - b. Clean access for personnel and material
 - c. Clothes, shoes, protective accessories: gloves, masks, caps, goggles/face visor
 - d. Hand washing and disinfection
 - e. Disinfectants and cleaning agents, handling
 - f. Cleaning of laboratory floor, walls and surfaces

- g. Sterilisation – types
 - h. Microbiological testing of lab conditions
 - i. Air quality - maintaining and controlling & positive pressure
2. Laboratory safety
 - a. Handling of potentially infectious samples
 - b. Handling and disposal of noxious chemicals
 - c. Handling and disposal of sharp instruments
 - d. Clinical waste disposal
 - e. Fire precautions
 - f. Handling liquid nitrogen
 - g. Lone working
 - h. Incident reporting and alert system
 3. Troubleshooting in basic principles

II. Laboratory equipment and operation

Objectives: The trainee should know the technical characteristics of the indispensable equipment of ART laboratories. Trainee should be able to detect their malfunctions in a timely manner. In addition to the mandatory documentation on the operation of the equipment and instructions for its maintenance, the trainee should also be capable to prepare the mandatory documentation required for traceability in the laboratory (equipment, consumables, gametes/embryos, staff) and instructions for optimal conditions during collection and transport of reproductive cells and tissue to the laboratory. The activities include detailed knowledge and practice in:

4. Indispensable lab equipment function, validation, verification, calibration and traceability, maintenance:
 - a. Incubators
 - b. Workstations
 - c. Microscopes
 - d. Micromanipulators
 - e. Centrifuges
 - f. Heating plates and blocks
 - g. Pipettors
 - h. Pharmaceutical refrigerator/freezer
 - i. Cryopreservation facilities
 - j. Measuring devices: thermometers, CO₂ meters, O₂ meters, pH meters, , VOC meters.
 - k. Control of key physico-chemical variables, alarming, keeping equipment records
5. Extra lab equipment function, validation, calibration and traceability, maintenance (required only if this equipment is in use):
 - a. Laser

- b. Time-lapse imaging
 - c. Genetics lab facilities
 - d. Autoclave, sterilizer
6. Lab disposables:
- a. Using appropriate types of plasticware, needles, pipettes, catheters, checking certificates, storage, entering the lab (off-gassing and sterility) and ensuring traceability
7. Laboratory / patient documentation
- a. Organizational and workflow charts
 - b. Writing and suggesting improvements to SOPs, manuals, laboratory forms, consent forms
 - c. Forms filling and corrections, traceability
 - d. Database setting up and filling, keeping records
 - e. Patients data storage and protection
8. Preparing of laboratory for start-up
- a. Scheduling of measurements / cleanings / testings (regular daily, weekly, monthly, continuously)
 - b. Disinfecting lab and switching on the heating instruments and flow hoods
9. Culture media and buffers, culture systems, oils, supplements:
- a. Knowing of types, formulations, checking QC parameters on certificates, ensuring traceability
 - b. Ensuring safe transportation and storage
 - c. Selecting media and preparation – method of choice
 - d. Handling and preincubation of various culture media
 - e. Measuring pH and temperature of culture media
 - f. Microbiological testing of culture environment
10. Ensuring optimal biological specimen collection and transfer to the lab
- a. Timing synchronisation with the lab
 - b. Documented pre-testing for viral or other contamination
 - c. Ensuring constant collection conditions, preventing of contamination and negative effect of environmental changes (temperature, pH, osmolality)
 - d. Preparing pertaining documentation, patient and biological material identification, marking, coding
 - e. Storage and transport to the lab
11. Troubleshooting in lab set-up, equipment & operation

III. Semen analysis

Objectives: The trainee should be competent to individually perform all standard and advanced tests on semen samples with the highest level of precision and accuracy.

12. Basic semen examination

- a. Initial macroscopic examination
 - i. Volume
 - ii. pH
 - iii. Liquefaction and viscosity
 - iv. Appearance and colour

- b. Initial microscopic examination
 - i. Counting procedure
 - ii. Sperm motility
 - iii. Sperm vitality
 - iv. Sperm concentration
 - v. Examination of samples with low sperm concentration
- c. Staining methods for cytological examination
 - i. Sperm morphology
 - ii. Classification of spermatozoa by morphology (teratozoospermia index - TZI)
 - iii. Leukocyte count (peroxidase staining)

13. Reporting

- a. Reference values and semen nomenclature

14. Quality control managing (related to semen analysis)

- a. Ensuring precision, accuracy, sensitivity and specificity
- b. Identifying the nature of errors
- c. Internal quality assurance (analysing within and among-technician variability)
- d. External quality assessment in andrology

15. Troubleshooting in semen analysis

IV. Sperm processing for ART

Objectives: The Trainee should be able to select and perform the most appropriate sperm isolation procedure based on the characteristics of the sample and enable ART procedures even in the most severe forms of male infertility.

16. Sperm preparation for IUI and ART

- a. Sperm washing, swim-up, gradient centrifugation - choice of method according to the quality and characteristics of the specimen
- b. Preparing semen from
 - i. cryptozoospermia

- ii. viral-positive patients
- iii. retrograde ejaculation samples
- iv. total immotile samples
- v. frozen/thawed semen samples

17. Preparation of testicular specimen

- a. Preparing of aspirate or testicular biopsy material for a:
 - i. cytological examination / histological examination
- b. Identification of sperm cells, spermatid, Sertoli cells in a native sample
- c. Preparing fresh and frozen/thawed testicular/epididymal samples (mechanical, enzymatic method)

18. Troubleshooting in sperm processing

V. Oocytes processing for ART

Objectives: The trainee should be skilled to identify oocytes and safely prepare them for ART procedures by:

19. Follicular fluid examination

- a. Distinguishing between the content of a follicle, cyst, endometriotic cyst, granulosa cells and cumulus oophorus cells,
- b. Identification and isolation of cumulus -oocyte-complexes (COC)

20. COC morphology assessment

- a. Normal vs luteinized vs immature COC

21. COC culture, considering optimal timing

- a. Required lag time after ovulation triggering and ovum pick-up before further processing

22. COC harvesting

- a. Enzymatic and mechanical denudation
- b. Possible damage

23. Oocyte morphology and maturity evaluation

- a. Nuclear maturity
- b. Cytoplasmic and extra-cytoplasmic evaluation (zona pellucida, perivitelline space, polar body, other dysmorphisms)

VI. Oocyte insemination

Objectives: The trainee should be trained to choose and implement such an insemination method that will ensure the highest fertilization rate, and will prioritize

the use of the classic IVF procedure in non-male factor infertility. The training should include:

24. Reasoning with clinical colleagues about which patient are appropriate for conventional IVF to be used as an insemination method
 - a. Male: normo-, astheno-, teratozoospermia, fresh/frozen sperm, viral/bacterial contamination
 - b. Female: age, response to ovulation hyperstimulation, fresh/frozen oocytes
 - c. Couple: fertilization rate in previous ART cycles, PGT cycles
25. Conventional IVF
 - a. Preparing for IVF in a well or in a droplet
 - b. Ensuring the correct sperm concentration, timing and duration of coincubation

VII. ICSI

Objectives: The trainee should be trained to ensure the highest fertilization rate in different types of male infertility. The training should include:

26. Intracytoplasmic sperm injection (ICSI) procedure
 - a. Preparing ICSI dish and loading gametes
 - b. Identification and isolation methods of optimal spermatozoa in ICSI dish
 - c. Performing ICSI with sperm immobilization and injection
 - d. ICSI with epididymal / testicular sperm
 - e. ICSI in patients with cryptozoospermia
 - f. ICSI in patients with totally immotile spermatozoa or Kartagener syndrome
 - g. ICSI in patients with globozoospermia
 - h. Troubleshooting in micromanipulation
27. Troubleshooting in oocyte insemination

VIII. Embryo culture, evaluation of fertilization and embryo development

Objectives: The trainee should be able to maintain safe culture conditions for embryos, to evaluate their morphokinetic characteristics that predict implantation, and to rank embryos by their developmental potential. The training should cover the following area:

28. Evaluation of fertilization
 - a. Harvesting remaining corona cells from oocytes, inseminated by classical IVF
 - b. Distinguishing between normal, abnormal fertilization, (triploidy, micronuclei, parthenogenesis) and fertilization failure
 - c. Identifying possible reasons for fertilization failure

- d. Detecting contamination with microorganisms
 - e. Patient communication about fertilization results, including 100% failed fertilisation
29. Evaluation of embryo development
- a. Standard assessment of cleavage-stage embryo morphology
 - b. Standard assessment of morula-stage embryo morphology
 - c. Standard assessment of blastocyst-stage embryo morphology
 - d. Recognizing irregularities in embryo cleavage by using bright microscopy: blastomere asymmetry, multinucleation, fragmentation
 - e. Annotation of embryo morphokinetic parameters by a time-lapse
 - f. Recognizing irregularities in embryo cleavage by using time-lapse microphotography
 - g. Predicting embryo developmental potential and viability and ranking them according to predicting markers
 - h. Distinguishing viable embryos for embryo transfer or cryopreservation from non-viable embryos
30. Quality management
- a. Internal quality control (IQC) in embryology
 - b. External quality assessment (EQA) in embryology

IX. Embryo transfer

Objectives: The trainee should be skilled in selecting and preparing the best embryos for embryo transfer according to their morphology and morphokinetic characteristics. The training should cover the following area:

31. Embryo transfer

- a. Selecting embryos for ET
- b. Recommendations to clinical colleagues about the optimal day of ET and number of embryos for ET
- c. Patient communication
- d. Loading catheter and its verification after the transfer
- e. Troubleshooting

32. Troubleshooting in embryo culture & ET

X. Cryopreservation

Objectives: By knowing basic principles of cryobiology, the Trainee should be competent in performing the following procedures and should enable the highest possible survival of frozen / thawed reproductive cells and embryos:

33. Sperm cryopreservation and thawing

- a. Viral contaminated semen cryopreservation

34. Testicular tissue cryopreservation and thawing

- a. Cryopreservation of TESE and micro-TESE
- b. Evaluating testicular sperm vitality

35. Oocyte vitrification and warming

- a. Evaluating oocyte survival

36. Embryo / blastocyst cryopreservation / thawing, vitrification / warming

- a. Evaluating embryo / blastocyst survival

37. Troubleshooting in cryopreservation

ADVANCED TRAINING PROGRAMME (YEARS 4 – 6)

The Trainee should continue all activities from the core training programme in clinical embryology and manifest training activities by registering procedures in a logbook. Advanced training programme covers the following (additional) areas:

XI. Cells, tissue and embryo cryobanking

Objectives: The Trainee should be competent to manage all types of cryobanks for reproductive cells tissues and embryos. These skills should be obtained through:

38. Testicular tissue cryopreservation and thawing
 - a. Cryopreservation of TESE and micro-TESE
 - b. Evaluating testicular sperm vitality

39. Ovarian tissue cryopreservation and thawing (optional)
 - a. Assessing vitality of follicles

40. Activities in cryobank of reproductive cells, tissues and embryos
 - a. Maintaining safety measures for biological material and personnel
 - b. Organizing stored material in cryo-tanks
 - c. Keeping data and documentation
 - d. Organizing quarantine and storage of contaminated specimens
 - e. Maintaining contact with patients regarding their stored biological material
 - f. Allowing biological material to perish after the expiration date
 - g. Importing / exporting and coding of frozen biological material
 - h. Preparing frozen material for transportation
 - i. Preparing frozen material for distribution
 - j. Donor specimen evaluation and selection

XII. Reproductive cells and tissue maturation *in vitro* (optional)

Objectives: This module is mandatory only if the clinic that the advanced trainee works in actually performs this technique on a regular basis. The trainee should be competent to perform *in vitro* maturation of immature oocytes and testicular sperm and to assess the maturation stage. These skills should be obtained through:

41. *In vitro* maturation of COC
 - a. IVM of immature oocytes post hCG/trigger
 - b. Preparing *in vitro* maturation media with hormone supplements
 - c. Immature COC isolation by using cell grid
 - d. Assessing oocyte maturity

42. *In vitro* maturation of testicular cell suspension
 - a. Assessing sperm maturity

XIII. Micromanipulation on embryos (biopsy) and genetic analysis (optional)

Objectives: This module is mandatory only if the clinic that the advanced trainee works in actually performs this technique on a regular basis. The trainee should be competent to perform the most delicate micromanipulation techniques on embryos to prepare them for vitrification or to enable biological material for genetic testing:

43. Performing biopsy
 - a. Polar body, blastomere and/or trophectoderm cells
44. Preparing biopsied cells for chromosome and gene analysis
 - a. Fixation and/or tubing for PGT-M (monogenic defects) and/or for PGT-SR (chromosomal structural rearrangements) and/or PGT-A (aneuploidies)
45. Understanding genetic analysis reports

XIV. Laboratory set-up

Objectives: The trainee should know how to independently set up a new laboratory or cryobank. This is a part of theoretical knowledge needed, but any practical experience in this topic is a good reference for the trainee. The activities include detailed theoretical knowledge and eventual practice in:

46. ART laboratory setting up
 - a. Lab location, construction, design
 - b. Lab infrastructure: dressing filter, lab space, walls, ceilings and floors, furniture, paintings, electrical connections, UPS, gas supply, O₂ / CO₂ sensors, entry control and security
 - c. Lab air: air exchanges and filtering, temperature control and humidification, control of particles, control of VOCs
47. Andrology laboratory setting up
 - a. Lab location, design
 - b. Lab infrastructure
48. Cryobank setting up
 - a. Location and design of cryo-storage room,
 - b. Infrastructure: space, floors, electrical connections, LN₂ supply, O₂ / CO₂ sensors and air exchanges, entry control and security
 - c. Cryotanks, alarming system & safety procedures

XV. Preparation of laboratory results and counselling

Objectives: The Trainee should be able to design a laboratory report and interpret it in consultations with other specialists or patients.

49. Reports

- a. Writing a report on the results of testing, processing, storage or distribution of reproductive tissues, gametes and embryos including reference values where possible
- b. Interpreting and communicating of lab reports with other specialists.
- c. Understanding reports from hormonal, serological, microbiological laboratories

50. Communication

- a. Suggesting the most appropriate ART laboratory tests or procedures.
- b. Communicating with patients and giving information regarding the quality and functionality of their biological material and suitability for clinical use.
- c. Predicting of success of treatment with ART according to the quality of biological material (gametes, embryos) and patient characteristics.
- d. Communicating with auditors during inspection

XVI. Managing laboratory for ART and cryobank

Objectives: The Trainee should be able to manage laboratory and cryobank according to the highest QM standards including areas listed below:

51. Organization of work, distribution of tasks, monitoring the implementation of tasks

52. Understanding and working according standards, guidelines

- a. International standards: ISO 9001 (also possible ISO 15189, GMP, GLP)
- b. Good practice in IVF laboratories
- c. WHO laboratory manual for the examination and processing of human semen 2010

53. Quality control

- a. Documenting control system
- b. Personnel administration
- c. Method documentation and validation
- d. Equipment quality control, monitoring (temperature, gases, humidity), validation, service
- e. Evaluation of suppliers of consumables, kits and culture media and their quality
- f. Control of laboratory environment (temperature, overpressure, particles, microbiological tests)
- g. Quality control records of audits

- h. Nonconformities and improvements

54. Quality assurance

- a. Laboratory information system (Excel or specific lab software)
- b. Definition of important data (variables): patient characteristics, history, indications for infertility treatment, serology testing, IUI/IVF/FER cycle characteristics (hormonal treatment, ovarian response, retrieval of reproductive cells, characteristics of reproductive cells, laboratory procedures, staff involved, material used, storage, laboratory outcome (sperm preparation outcome, maturation of reproductive cells, fertilization, embryo development and quality, embryo usability, survival after cryopreservation and thawing, stages and number of transferred or cryopreserved embryos), transfer outcome (biochemical pregnancy, implantation, multiple pregnancy, clinical pregnancy, miscarriages, live births, singletons, twins, triplets), delivery outcome
- c. Control over database: completeness of collected data, storage of data in compatible format on safe place
- d. Definition of reference patient groups: young, older patients, poor-responders, hyper-responders, IUI group, IVF group, ICSI group
- e. Definition of key performance indicators (KPI): fertilization rate, maturation rate, oocyte degeneration rate, total fertilization failure rate, embryo cleavage rate, good quality embryo rate, blastocyst development rate, survival rate after thawing, pregnancy, implantation, clinical pregnancy, multiple pregnancy, miscarriage, delivery rate
- f. Definition of benchmarks including suitable reference values and minimum/maximum acceptable levels for each KPI.
- g. Validation and monitoring of procedures, keeping statistical records on performance of individuals and the whole laboratory by comparing KPI values
- h. External quality control

55. Risk management: what can go wrong?

- a. Identifying high risks: adverse events / reactions, severe adverse events / reactions, errors, near incidents, signals, deviations / nonconformities, sentinel events, complaints
- b. Adverse events: procedures, how to prevent, how to proceed, reporting
E.g.: mix up of gametes, loss or damage to gametes/embryos during laboratory treatment, transfer of a wrong embryo, malfunctions of equipment/materials
- c. Adverse reactions: procedures, how to prevent, how to proceed, reporting
E.g.: detected malformations of children after using partner donation
- d. Evaluation of risks and production of FMEA Risk Assessments. Effectiveness of preventive actions, corrective actions

56. Continuous quality improvement

57. Management of registers (about patients, cycles, donors, cells and tissues, cryobanking) and regular reporting data to national/international registers

XVII. Research, Statistics and Audit

Objective: The Trainee should be familiar with

58. Experimental design

- a. Understanding of different types of research (observational, experimental; descriptive, analytical; controlled, uncontrolled; cohort studies, case-control studies; prospective, retrospective; randomised, non-randomised; cross-sectional, longitudinal; pilot study...
- b. Understanding randomized controlled trials and techniques of meta-analysis
- c. Distinguishing between evidence-based and non-evidence-based laboratory methods of ART

59. Data acquisition, storage, interpretation and statistical analysis

- a. Understanding population parameters and sampling techniques;
- b. Computing and interpreting measures of comparisons of means and variations
- c. Analysing a presented experiment and construct a hypothetical experiment with respect to the following:
 - a. the question examined
 - b. the null hypothesis
 - c. the sampling technique (including sampling bias and sample calculations
 - d. the expression and correlation of raw data and simple (e.g. log) transformations
 - e. the selection and application of appropriate statistical tests;
 - f. significance of the results
 - g. the conclusions
 - h. the appropriate inferences which can be obtained
- d. Applying the following statistical tests
 - a. parametric tests;
 - b. non-parametric tests;
 - c. correlation and regression;
 - d. multi-variate analysis.
- e. Defining the terms "significance", "confidence interval", "Type I error" and "Type II error"
- f. Performing statistical analysis of assay data and evaluation of quality control
- g. Understanding the value of discussion and collaboration with statistical advisers
- h. Understanding the role of registries
- i. Understanding cumulative rates calculation and assessment of bias

60. Scientific writing and presentational skills including the formulation of a grant application
61. Conducting clinical laboratory audit and feedback and be able to utilise data collection systems
62. Designing, scoping, construction and implementation of laboratory guidelines

XVIII. Teaching

Objective: The trainee should gain experience in teaching which will include:

63. Some responsibility for teaching junior staff in their specialty area;
 - a. full participation in the unit's postgraduate programme with some administrative responsibility for the organisation of teaching in their specialty
 - b. participation in the undergraduate teaching programme
64. Gain experience of appraisal and assessment techniques.

XIX. Ethical and Legal Aspects

Objective: The trainee should be able to discuss the legal and ethical aspects of the clinical laboratory practice. The Trainee should be competent to prepare an application for the Ethics Committee to assess the ethics of research on reproductive cells and embryos. This includes a consent form for patients:

65. Preparing annual report about reproductive cells, tissues and embryos for the EUTCD authority
66. Preparing annual report about adverse events or adverse reactions for the EUTCD authority
67. Participating in any laboratory inspection process as auditee or auditor
68. Preparing a consent form for patients to be included in research:
 - a. Nature of consent (knowledge, capacity, voluntary)
69. Preparing an application for appraisal of research by the Ethical Committee

XX. Continuing professional development

Objective: The Trainees should have the opportunity to attend appropriate national and where possible international meetings relevant to their specialty annually and have the opportunity to fulfil all criteria for acquiring annual ESHRE CPD certificate.

70. Reading professional papers, following the novelties in the professional field and implementing of new laboratory methods in routine practice
71. Continuing professional development for themselves and all laboratory staff by organizing research activities, writing scientific and professional papers, participating in e-learning, organizing and attending of workshops, symposia, congresses
72. Self-reflection activities as part of CPD.