



INDIAN FERTILITY SOCIETY

CME : SETTING UP AN ART LAB / CLINIC



Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse.

Infertility though not life threatening, cripples life of a couple. Before the advent of IVF, nothing much could be done to help these distressed couples, but now with many advanced technology of assisted reproduction many of these couples can be helped. For accomplishing that, state of the art centers are needed to give quality treatment to the needy.

IFS has organized three pan India workshops to help budding infertility specialists and embryologists, teaching them ways to set up and operate a successful assisted reproductive technique (ART) laboratory /clinic. Through these workshop delegates would not only know about where and exactly how to build the center but also about requisite manpower and legislative requirements

I am sure you would enjoy the meetings and reading the manual.

Dr M Gouri Devi
President - IFS



According to World Health Organization, infertility is a disease of the reproductive organs and is defined, as the inability of sexually active couples taking no contraceptives to achieve pregnancy with in one year.

As the second most populous country in the world, we have a huge burden of infertility in our country. In our culture, infertility is a social stigma and infertile couples suffer silently. We have a huge need to set up many infertility treating centers in our country, not only in the metros but also in the rural areas. These meetings have been designed to give impetus to many aspiring gynecologists to set up secondary and tertiary infertility clinics in their cities.

For an ART center to run successfully, it needs to be designed carefully taking care of location, building material, paint etc. along with careful instrument selection and last but not the least, choosing a perfect team of personnel.

I am sure you would enjoy these clinical symposiums and the resultant knowledge sharing will help you in your endeavor of setting up your ART Lab.

I am grateful to Dr Rashmi Sharma and her team and team from Vardhman Medicare in organizing this event.

Prof (Dr) Pankaj Talwar
Secretary General -IFS



Dear Friends

“Barrenness amid plenty” applies correctly to India, as on one side we are dealing with the problem of population explosion, and on the other side we have largest number of infertile couples in the world. India is home to almost 27.5 million infertile couples and infertility is on further rise due to various factors. Almost 20-25% of these infertile couples will need IVF sooner or later, so the number of couples in need for ART is extremely high in India, but there is an acute shortage of qualified infertility specialists, embryologists and IVF/IUI centers in our country to take care of these couples. Also most of the advanced infertility treatment is available only in the top metro cities, while our almost 68% population resides in smaller towns and villages. Though recently there has been a boom in the number of IVF centers, there is still huge unmet need for the same especially in second and third tier cities.

These meetings on “Setting up an ART Lab/Clinic” have been especially designed to assist budding gynecologists and embryologists, who are in the process of setting up their IUI or IVF center.

We have also prepared a comprehensive course book detailing every aspect of setting up an ART Lab/clinic. I am sure it will be a very useful tool for someone planning to set up his or her ART clinic.

My sincere thanks to Dr Sangita Sinha (chapter secretary ,IFS Chattisgarh), Dr Neeru Thakral (chapter secretary ,IFS Haryana) and Dr Sangita sharma (chapter secretary, IFS Rajasthan) for the hard work put by them in organizing these meetings in their states.

I would like to thank Vardhman Medicare Pvt. Ltd.for their unflinching support in bringing these much needed meetings to various parts of country and also for their help in publication of course book.

Dr Rashmi Sharma
National Coordinator

- Director, Origyn Fertility and IVF, Pitampura, New Delhi
- Joint Secretary , Indian Fertility Society
- Co-Convenor, PCOS – SIG , IFS
- MBBS, MD (BHU, Obs. & Gynae.), DNB, MNAMS, FICOG
- Diploma in IVF & Reproductive Medicine at Kiel University, Germany
- Ex consultant , Southend Fertility and IVF centre, Delhi (2006-2009)
- Ex- HOD –IVF dept , Moolchand hospital, New Delhi (2009 – 2013)
- Ex – HOD – IVF dept ,Max Hospital ,Pitampura, New Delhi (2013 – 2017)
- Winner of “C.S.DAWN PRIZE” for best paper presentation at All India Congress of Obstetricians and Gynecologists, AICOG Guwahati, 2010.
- Winner of first prize for Paper presentation at IMS International congress, Delhi, 2008
- Winner of 2nd prize for Poster Presentation on “Abdominal tuberculosis with elevated CA-125 mimicking metastatic ovarian cancer” at 28th Annual Conference of Association of Obstetricians and Gynaecologists of Delhi (AOGD, 2006)
- Author of book chapters in 7 reputed books on infertility
- Many paper presentations in national and international conferences
- Organized many workshops and CME’s on various aspects of infertility

- Director, Gouri Hospitals Ltd.
- Director, Ridge IVF Group.(Runs a chain of IVF centres)
- President, Indian fertility society
- Ex-Secretary General, Indian Fertility Society
- Executive, AOGD governing council
- Member, Executive Board, NARCHI, DGES, FPSI
- Ex Vice President, NARCHI
- Chairperson, Advocacy & Ethics Committee, IFS.
- State Quality Assurance Committee (SQAC) Govt of NCT of Delhi.
- Member: MTP advisory committee, Govt Of NCT of Delhi
- Member Advisory committee on ethical practices in the field of obstetrics, Govt of NCT, Delhi
- Recipient of Kanak Goel Award 1995-1996 from IMA.
- Chairman's Appreciation Award by IMA AMS – 2002
- Dr. APJ Abdul Kalam Excellence Award – 2017
- Economic Times Award one of the Most Inspiring Gynecologists of India



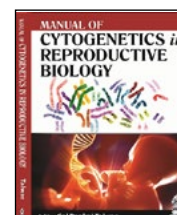
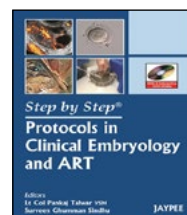
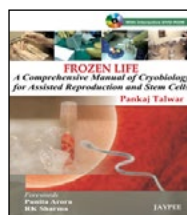
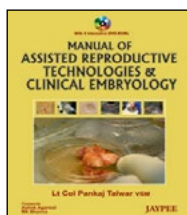
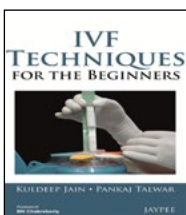
Dr M Gouri Devi
M.D

She is a keen academician, has organized many conferences, has been a speaker in many national and international conferences. Has many publications to her credit

- Sec IFS.
- Secretary Fertility preservation society of India.
- Editorial board of multiple Infertility journals.
- Member Advisory committee ICMR
- Member Infertility committee FOGSI
- Editor Nexus / Artext – E bulletin of IFS
- Awarded Vishisht seva medal by the President of India for working in field of infertility
- Associate Editor FSR
- Set up four centres for Armed forces .
- Experience of 10,000 and ET cycles .
- Member International society of fertility preservation.
- Trained Human Embryonic Stem Cell Derivation – Israel
- Trained in ovarian cortex freezing (fertility preservation) - Paris
- Trained in PGD – Germany, Spain
- Trained in QA/QC-Spain
- Edited 6 books



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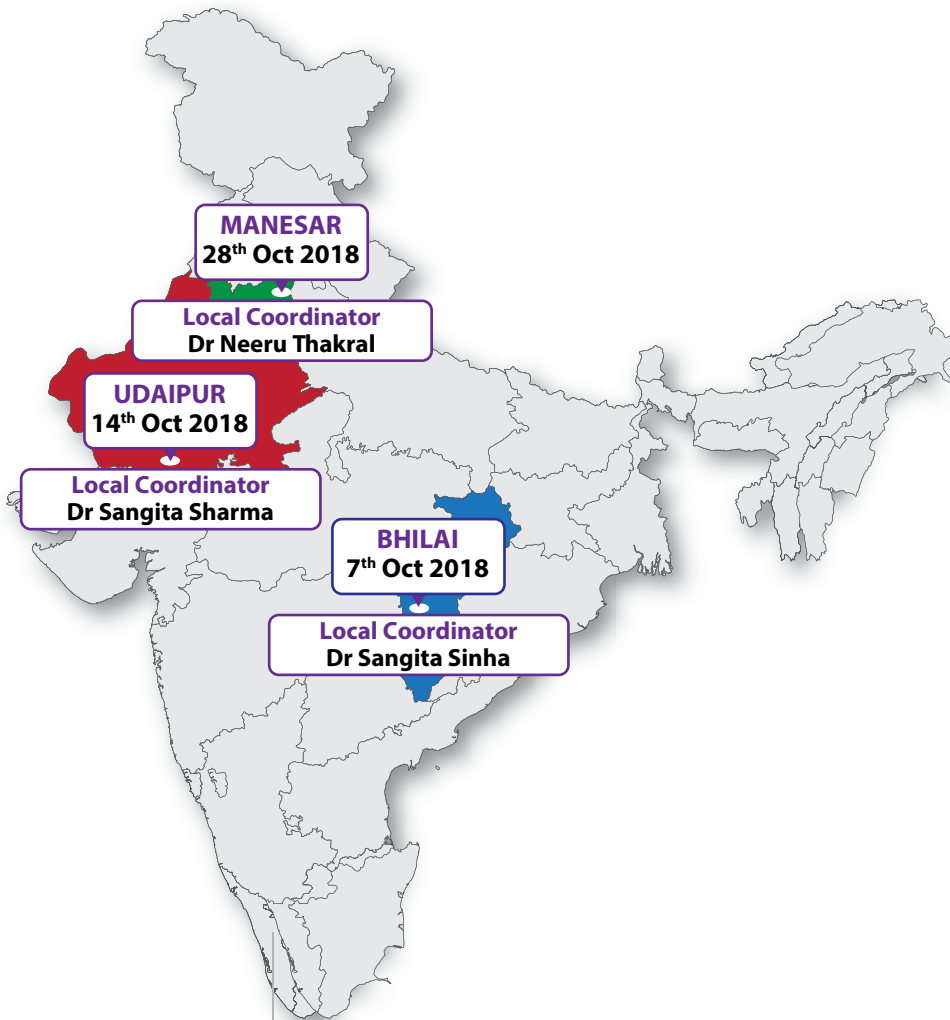
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Venue and Dates



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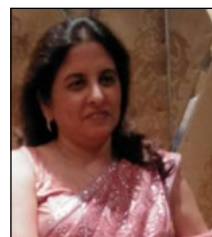
Topic	Contributed by
Set up of an IUI facility	Dr Sangita Sinha
Set up an IVF facility	Dr Sangita Sharma
QA QC at an ART centre	Dr Shalu Gupta
ICMR Guidelines	Dr Neeru Thakral
Desirable and mandatory at an ART lab	Dr Sangeeta Sinha
Panel Discussion : Trouble Shooting at an ART clinic	Dr Sangita Sharma
Infection control in IVF Lab	Dr Subodh Kulkarni



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Programme for the day

Time	Topic
09:30 - 10:00	Registration & Tea
Session 1 : Shift from Gynaecology to ART	
10:00 - 10:20	Set up of an IUI facility
10:30 - 10:50	Set up of an IVF facility
11:00 - 11:20	QA QC at an ART centre
11:30 - 12:00	Inauguration
Session 2	
12:00 - 12:20	ICMR Guidelines
12:30 - 12:50	Desirable and mandatory at an ART lab
13:00 - 13:45	Panel Discussion : Trouble Shooting at an ART clinic
13:55 - 14:10	Infection control in IVF Lab
14:10 Onwards	Lunch

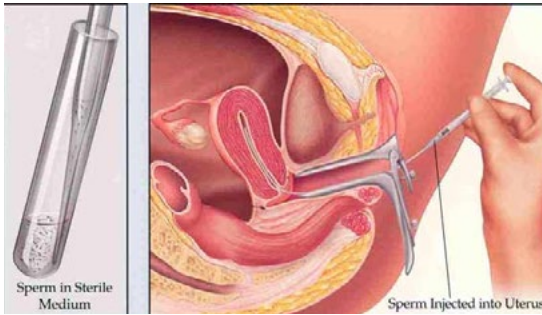
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1. Set up of an IUI facility

IUI

Deposition of processed highly motile, morphologically normal sperms, washed free of seminal plasma and other cells, directly in to the uterus at the anticipated time of ovulation.



Intrauterine Insemination

- **The rationale is to** expose oocyte to more sperms **near the site of fertilization** and make chances of pregnancy better

Is there a need to for registration ?

According to ICMR IUI facility comes under-
Secondary Level (level-2) infertility clinic
and

IT REQUIRES ICMR REGISTRATION

Level II clinic Shall have facilities for —

- 1 Artificial insemination using husband semen
- 2 Artificial insemination using donor semen
- 3 Cryopreservation of there own patient (if needed)

- Non medical members are as important.
- Good IVF nurse can be the backbone of the unit
- Embryologist is the soul of your program
- Don't forget the cleaner!
- Attrition is inevitable
- Keeping updated is important for all the members, not only for the lead clinician

Personnel we need

- GYNAECOLOGIST—minimum qualification is Post graduate degree / Diploma. Additional experience should include—
- understanding the causative factor of male/ female infertility
 - knowledge of practice and use of diagnostic method for determine the cause of infertility
 - knowledge of clinical aspects of reproductive endocrinology
 - Competence in gynecological ultrasonography

Responsibility of gynecologist

- Interviewing of infertile couple
- History taking, physical examination.
- Recommending appropriate test.
- Carrying out endoscopy , USG
- Doing IUI,AIH, AID

Andrologist

- Andrology is a subject related to male reproduction—
- In India there is no formal course , it is the urologist/ surgeon that often takes the task of treating the male infertility.
- They must take additional training in diagnosis of various type of male infertility.

Responsibility of andrologist

- History taking, making appropriate diagnosis and treatment
- Able to carry out surgical procedure like collecting sperm through sperm retrieval technique.
- He/she should be aware of surgical procedure such as epididymovasal re-anastomosis etc.

Clinical embryologist

- Embryologist must also be familiar with ART
- He/she should be either a medical graduate/having a post graduate degree or a doctorate in appropriate area of life science.

Clinical embryologist

In the case of clinic in existence for at least one year before the promulgation of these rules, a person with a B.sc or B.V.Sc degree but with at least three years of first hand, hands –on experience of the techniques mentioned below, and of discharging the responsibilities listed below, would be acceptable –

1. principle and practice of semen analysis and cryopreservation of semen.
2. to ensure that all the necessary equipments are present in laboratory and are functional.

Clinical embryologist

- To maintain records of all the procedures carried out in laboratory.
- In case of shortage of adequately trained clinical embryologist, an individual may act as a clinical embryologist for more than one clinic but each clinic where the persons work must own responsibility of embryologist and ensure that embryologist is able to take care of entire work load of the clinic without compromising on quality of service.
- An embryologist must not be associated with more than two centers.

Counselor

- A person who has a degree (preferably a post graduate degree) in social science, psychology, life science, medicine and a good knowledge of various causes of infertility and its social and gender implication, and the possibilities offered by various treatment modalities, should be considered as qualified to occupy position of counselor.
- A member or staff of an ART clinic who is not engaged in any other full time activity in the clinic can act as counselor.

Program coordinator

- This should be a senior person who has a considerable experience in all aspect of ART. The program coordinator should be able to coordinate the activities of rest of team and ensures that staff and administrative matters, stock keeping , finance, maintain ace of patient, records , statutory requirement and public relation are taken care of adequately.
- The program coordinator / director should have a post graduate degree in appropriate medical / biological science. In addition she/he must have a reasonable experience of ART.

Infrastructure for IUI Facility

- STERILE AREA & NON STERILE AREA

NONSTERILE AREA

Non Sterile Area

1. A Separate examination room with privacy for interviewing and examining male & female partners
2. A general purpose laboratory
3. Store room-for stocking all the essential items like IUI catheters , media , disposables . etc.
4. Record room-it should be computerized –it includes history, diagnosis, treatment. Freezing details.
5. Autoclave room-for sterilizing and autoclaving

Non Sterile Area

Steps for vermin proofing-steps should be taken to make the whole area vermin proof with suitable traps for preventing insects and other form of unwanted creature.

1. Semen collection room-well appointed room with privacy and an appropriate environment, located in secluded area close to laboratory, should be in house.
2. Procedure of semen collection-sterile , wide mouth , non toxic container. The room must have a wash basin with soap and toilet, must also have a toilet not used for any other purpose.

IUI room

- There must be separate clean room for IUI with appropriate table

Sterile area-

- 1 semen processing laboratory(IUI lab)
- There must a separate room with laminar air flow for semen processing preferably close to semen collection room. GLP (good laboratory practice) guidelines as described internationally must be followed.
- Care should be taken for safe disposal of biological waste and other material(syringe, glass slides)

IUI Lab

- Approximately 100 to 200 square feet area
- It is preferable to have the laboratory adjacent to or near the IUI room where the actual insemination would be taking place

IUI Lab

It is important to take the following precautions –

- All electrical wiring should be concealed.
- It is better to avoid false ceiling.
- Split or central air-conditioner is preferable to an window air conditioner . the laboratory should not get contaminated when the AC is being serviced or removed for repairs.
- The floor should be covered with large marble tiles or granite tile. Alternately one can also prepare the floor with vitrified tiles.

IUI Lab

- The walls should also be covered with tiles. Alternately one can cover wall with epoxy paint
- If one wants to be more strict and is planning to convert the IUI room into an IVF laboratory in future , one can use pressure modules to create positive pressure in the laboratory . however this is not a must if one is planning just an IUI facility.

For sterility of IUI Lab

- Ultraviolet light is useful in creating a sterile area in the laboratory
- it is important not to run the UV light when work is going on in the lab. This can damage the eyes of the workers.
- One can place the start button of the UV lights outside the lab door.
- This will enable one to switch on and off the lights, without getting exposed to them.
- Normally, one can run the ultraviolet lights on the weekend when there is no work in the lab.

For sterility of IUI Lab

- It is preferable to change into sterile clothes, slippers, cap and masks when one is working in the lab.
- It is preferable to have clothes made up of terry cotton or silk instead of linen. This will prevent the shedding of small threads from the linen clothes, that can contaminate the laboratory environment.
- however this is not mandatory.

Protective measure in lab

- Strict adherence to hygiene.
- Protective laboratory clothing & hair nets
- Non powered gloves and mask
- Food, gum, drinks and tobacco strictly prohibited
- Cosmetic should be minimized and perfumes should be avoided.

IUI Laboratory equipment

For Semen assessment & sperm preparation

- 1 Makler Counting Chamber
- Microscope phase contrast microscope 10x
- Centrifuge machine with swing –out rotor, time and RPM meter
- Dry incubator to maintain sample at 37 degrees
- Test tube warmer
- Laminar flow hood (horizontal/ vertical)
- Wide mouth semen collecting jar.
- cryocan

IUI lab- equipments

Essential

Centrifuge, laminar air flow hood , microscope, Incubator, test tube warmer.

Desirable

Phase contrast microscope, Makler chamber, CO₂ incubator, Experimental-CASA

Sperm counting chambers

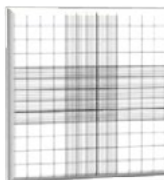
Neubaeur chamber or the Makler chamber are commonly used

Use of makler chamber is highly recommended as it is very accurate, though more expensive

Neubaeur chamber



Makler chamber



Automatic sperm analyzer systems : Systems such as CASA are being routinely used in labs with a large volume of work on semenology is being carried out in the laboratory.

However it is not required to run a routine IUI laboratory

Refrigerator : this is used to store the various chemicals and culture medias. The fridge should not be used for any other activity.

Microscope

Monocular microscope preferably phase contrast to test the semen for count, motility, morphology and other components such as pus cells. The microscope should have 10x, 20x,40x,and 100x objectives, and a 10z eyepiece.



- Centrifuge this should have a timer and a rotor (upto 3000rpm) whose speed can be controlled .
- It is better to go for a swing out rotor head.



Incubator

Plain incubator set at 37 degrees centigrade, which can be primarily be used for placing the semen collection jars for liquefaction, prior to sperm processing. Alternately one can also place the jars on digital heaters whose surface temperature is set at 37 degrees centigrade.



Incubator

Carbon dioxide incubator - optional.
If one is using bicarbonate buffered media, it is important to use an incubator.
However if one is using HEPES buffered media one can use plain simple digital heaters instead of CO₂ incubators.



Digital heater : this can be used as an alternative to CO₂ incubators/dry incubator .
Cheaper alternative
Equivalent results.
Need to use Heppes buffered media rather than bicarbonate based media



laminar air flow

Sterile laminar flow hood : this can be either a vertical or a horizontal flow.

A vertical flow is less harmful to the lab workers the air flow is not directly aimed at their face.

On the other hand a horizontal flow is more effective in reducing contamination.



Laminar flow

For IUI, one can order for a smaller breadth laminar flow.

It is preferable to ask for a stainless steel top, as it is durable, easier to clean and maintain as well as it is more aesthetic.

One can start the laminar flow 1 hour before preparing samples.

Nowadays one can order laminar flows with their tops heated digitally by heaters to maintain 37 degrees table top temperature.

Mandatory at andrology workstation

- 1 Centrifuge machine—separate spermatozoa from semen sample
- 2 Makler counting chamber-to get a reliable reading in million /ml
- 3 Compound microscope with 10X and 40X objectives
- 4 Small size laminar air flow—for ideal work station.
- 5 Test tube warmers-



Andrology Lab – Laminar flow hood , connecting air tight window with Semen collection room , Compound microscope , heating block for testtubes ,Cryostorage for sperm samples

Andrology Lab – Dry incubator, Centrifuge



Culture media

Some laboratory manufacture their own media , in house.

However majority of the IUI and ART laboratory use ready made culture media .

These are easily available, tested for toxicity, quality controlled and in general have consistant performance.



HEPES buffered culture media

These can be
Simple balanced salt solutions (e. g Earles balanced salt solution EBSS or Human tubal FluidHTF)
or
complex solution (eg hams F-10) supplemented with proteins (human serum albumin or synthetic proteinsources), antibiotics (penicillins with streptomycin or gentamycin) and HEPES.

HEPES buffered culture media

Some of the companies supplement their media with phenol red, which is helpful in gauging the ph of the media.

the HEPES molecule in the media maintains the ph between 7.2 to 7.4 when exposed to the atmosphere .

hence these media do not need dedicated 5% carbondioxide incubators to maintain ph.

The ph of 7.2-7.4 and osmolality of 280 milliosmoles, is ideal for gamete and embryo survival.

Bicarbonate based Culture media

- These media are simple balanced salt solutions (EBSS or HTF) or complex solutions(Hams F-10) supplemented with proteins, antibiotics and bicarbonate buffer molecules.
- In these the HEPES molecule is replaced by bicarbonate molecule , which needs 5% carbon dioxide atmosphere to maintain ph at 7.2 to7.4
- In recent times it has been shown that at sea level, a 6% Co2 mixture is better then a 5% mixture to maintain ph between 7.2 and 7.4 hence many laboratory are setting their CO2 incubators to 6% CO₂ concentration

Culture Media

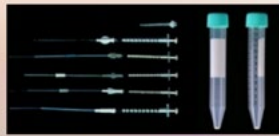
Bicarbonate media are useful for maintaining long term culture conditions, which are needed for IVF

For short term procedures such as semen washing and processing , they are not absolutely essential.

Hence in semen wash procedures done for intrauterine inseminations, HEPES buffered media along with the use of simple digital heaters or incubators is adequate for yielding good post wash semen samples.

DISPOSABLES

- Semen collection containers
- 14ml conical/6ml round bottom tubes
- Pasteur pipette
- IUI Cannulla
- BD Syringe
- Cryovials
- Powder free gloves
- 70% Ethyl Alcohol for cleaning the Lab



Disposables



Consumables and media

Mandatory -
Sterile single use disposable consumables should be used.

Reagents, media and consumables should always be used prior to expiry date.

Size of bottles and other packing must be appropriate to minimize opening and time between first and last case.

Repeated shifts of temperature should be avoided while handling in laboratory.

IUI donor

- Semen for donor IUI should always be procured from accredited ART sperm bank
- A sperm bank /cryo bank is a facility that collects and stores sperms from sperm donor for use by women who need donor sperm for pregnancy

Guideline for IUI donor

- A sperm bank/semens bank or cryo bank is a facility or enterprise that collects and stores human sperm for use by women who need donor- provided sperm to achieve pregnancy.
- Either an ART clinic or a law firm or any other suitable organization may set up a semen bank. If set up by an ART CLINIC it must operate as a separate identity

For donors –only frozen sample?

- Only semen bank to provide the sample.
- Selection criteria must be met
- Screened for STD (VDRL/HBSAG/HIV/HCV)
- One time screen is not sufficient to R/O HIV, HBsAg
- Quarantine for one month , retest the donor and release
- Blood group and physical character be matched

Freezing of semen

- Men that are likely to suffer from psychological stress at the time of IUI, are recommended to have their semen frozen for use at appropriate time.

Quality control

- This is key factor for success
- Results depend upon strict quality control
- Can be achieved by monitoring air quality, Instruments performance, parameter checks
- Instruments should also calibrated regularly to achieve success

2. Set up of an IVF facility

- IVF lab is a Tertiary level Infertility clinic.

Ref: ICMR – The ART Regulations Draft, 2010

Functions of an IVF Lab

These clinics will have three functions to perform;

- diagnostic
- therapeutic
(at the highest level of specialization and with the best of facilities and)
- research (excepting on human embryos).
- If any of the facilities mentioned below does not exist in the clinic, the clinic should have access to such a facility in another appropriately accredited clinic, ART bank, or laboratory.

Diagnostic procedures

For male infertility:

- Diligent history taking and physical examination
- Basic semen analysis with further tests for sperm function and integrity such as acrosome reaction and sperm-oocyte interaction in vitro.
- Assessment of cell contaminants, debris and infection.
- Endocrine assay
- Karyotyping.
- Assessment of seminal plasma for viscosity, thinness, blood contamination and biochemical constituents.

Diagnostic procedures

For female infertility:

- a) Apart from history taking, physical examination , Laparoscopy & Hysteroscopy, also:
- b) Endocrine assays.
- c) Karyotyping.
- d) Transvaginal sonography.

Therapeutic procedures

- a) Induction of ovulation using gonadotropin, a GnRH agonist and antagonist, and other adjuvants.
- b) All varieties of assisted reproductive technologies, including ICSI.
- c) Procedures for IUI using split ejaculate, pooled ejaculate or sperm recovered from post-coital specimen of urine in retrograde ejaculation.
- d) Cryopreservation of gametes (patients' own) and embryos.

Ancillary laboratory facilities

- Can be outsourced to speciality laboratories (located in the neighborhood)
- Hormone and other assays:
 - Ready access to laboratories that are able to carry out immunoassays of hormones (FSH, LH, Prolactin, hCG, TSH, Insulin, Estradiol, Progesterone, Testosterone and DHEA)
 - Clinical biochemistry lab
- Microbiology and histopathology
 - Tests for infections, HPE of specimen
- Genetic lab
 - Karyotyping and other genetic evaluation

Laboratory design

Based on

- Anticipated case-load
- Budget available
- Future plans of expansion
- Any subspecialty

Basic designs:

- Laboratories using only transport IVF (bridge clinic/satellite clinic)
- Laboratories adjacent to clinical outpatient facilities that are only used part of the time.
- Fully integrated laboratories with in-house clinics
- Portable, temporary laboratories.

3 Essential Pillars of an IVF lab

- Infrastructure
- Equipment
- Manpower (Personnel)

Initial Planning

- Location of the building
 - Avoid - near petrol pump, chemical godown, factories emitting fine particles, heavy traffic, parking slots, cement godowns, etc.
- Enquire whether the building or the surrounding site will undergo renovations or demolition in near future.
- Basic air sampling & determination of VOCs inside and outside the proposed building.

Location of the lab

- First floor Ideal- Less disturbance
- Avoid basement
- Check for any damp area or water seepage
- Connectivity with lifts/elevators should be there preferably (transportation of gas cylinders, LN2, etc.)
- If in a hospital, should not be near radiation sources eg CT Scan, radiotherapy units or near waste disposal areas.
- It should be away from the exhaust emission areas and the backup power generators
- Total area depends on the expected work load.

THE ACTION PLAN

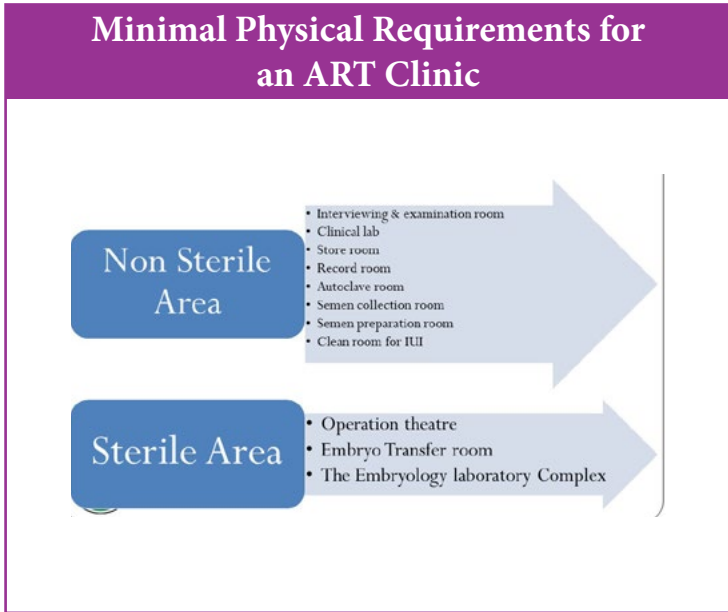
For setting up of your IVF facility info@shivaniscientific.com



Minimal Physical Requirements for an ART Clinic

- Non-sterile and a strictly sterile area
- Same space may be used for more than one purpose (no compromise in quality) of service.
- Sterile and non sterile cannot be combined

ICMR ART Regulation Bill Draft 2010



Non sterile area

1. A reception and waiting room for patients
2. A room with privacy: For interviewing and examining male and female partners independently. It must have:
 - examination table and gynecological instruments
 - USG machine
3. A general-purpose clinical laboratory

4. Store room:
 - for keeping essential stock
 - Facility should be there to store
 - Sterile items (media, needles, catheters, petri dishes etc)
 - Non-sterile material
 - Under refrigerated and non-refrigerated conditions as appropriate.

Location of the lab

5. Record room: should be computerised as far as possible.
 - Essential details of the patient's records
 - History of the cause of infertility
 - Results of new diagnosis
 - Treatment option best suited and carried out
 - Outcome of treatment, and follow-up if any
 - Any adverse reaction to drugs
 - The software must have archival, retrieval and multivariate statistical analysis capabilities.

6. Autoclave room
7. Steps for vermin proofing : should be planned before IVF unit functional as pesticide use cannot be done once the unit is functional.

8. Semen collection room:
 - Privacy and appropriate environment
 - Located in secluded area close to the laboratory
 - Soundproofed
 - Containers - sterile, maintained at body temperature and nontoxic
 - Washbasin, soap and clean towels, Toilet
 - At the end of a hallway
 - Room must not be used for any other purpose
 - Provision for safe delivery of samples
 - Access for disabled persons
 - Requirements for couples
 Facility for audiovisual stimulation

9. Semen processing laboratory: (Functions)

- Semen Samples for diagnostic purposes
- Sperm Processing for ART
- Close to the semen collection room
- Double pass through to collection room

Equipments:

- Laminar air flow
- Centrifuge machine
- Facilities for microscopic examination
- Refrigerator
- Heating block
- Sperm Counting Chambers such as Makler Chamber, Neubaur Chamber.etc

10. Clean room for IUI:

- Separate area/room
- Appropriate table for IUI

Sterile area

- The operation theatre:
 - Endoscopy and transvaginal ovum pick-up.
 - Equipped with emergency resuscitative procedures.
- Room for intrauterine transfer of embryo:
 - Examination table
 - for carrying out the procedure
 - The operation theatre can be used for this purpose
- Adjoining embryology laboratory
 - Entry – strictly controlled
 - anteroom for changing footwear
 - area for changing into sterile garments
 - scrub-station.
- The sterile area must be air conditioned where fresh air filtered through an approved and appropriate filter system is circulated at ambient temperature (22-25 C).

- IVF Procedure Rooms in which procedures such as oocyte retrievals, embryo transfers and percutaneous sperm retrievals are carried out, should be differentiated from invasive surgical facilities, which would usually be subject to separate regulation and licensing.
- Consequently, if the ART procedures room is to be used for invasive surgical procedures, its HVAC system should be separate to the ART suite so as not to require unnecessary and potentially deleterious higher air quality criteria.

(Cairo consensus on the IVF laboratory environment and air quality: report of an expert meeting .D.Mortimer et al Reproductive BioMedicine Online Volume 36, Issue 6, June 2018, Pages 658-674)

21.6.1 IVF Unit (Fertilisation Centres) Functional Relationship Diagram



Laboratory Complex requirements

- Operating room for ovum pick-up
- Embryology lab – including micromanipulation
- Sperm preparation and semen analysis
- Freezing and cryopreservation facilities
- Sterilization and water treatment
- Scrub area
- Preparation and recovery room
- Changing room
- Storage space
- Staff rest room and discussion room

Components (Infrastructure of the Lab complex)

- **Walls & Ceiling**
- **Flooring**
- **Paints**
- **Lights**
- **Electricity fittings and supply**
- **Plumbing & ducting**
- **Furniture**
- **HVAC & Air handling unit**

- 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, adequate work space per person, microscope eye height, efficient use of space and surfaces, sufficient environmental lighting and air-conditioning with controlled humidity and temperature.

ESHRE: Revised guidelines for Good Practice in IVF Lab 2015

- Materials used in laboratory construction, painting, flooring and furniture should be appropriate for
 - clean room standards,
 - Smooth with no cracks & crevices, easy to clean, non porous, non odourous,
 - minimising Volatile Organic Compounds (VOC) release and embryo toxicity.

ESHRE: Revised guidelines for Good Practice in IVF Lab 2015

Walls & ceilings

- Walls & partitions – non porous inert material
- Cladding with a non-porous material such as panels made from aluminium tri-hydrate are expensive but provide an inert (low VOC), hypoallergenic, easy to clean wall.
- Ceiling - The ceiling must be composed of a contiguous, solid material, e.g. plasterboard, gypsum panels, Gyprock, Sheetrock®, not tiles, and the need for any access panels must be minimized.
- Essential access panels must have air-tight, silicone gaskets.as sealant.
- False ceilings – should be avoided
 - May be needed to conceal lights and filters
 - Should have solid non porous panels

Paints

- 'Ecological' Water based paints are preferred with NO VOC emission
- Low-volatile paints with acrylic, vinyl acrylic, or acrylic latex polymers.
- Epoxy – Epoxy paints emit VOCs (amine) and take several weeks to cure and thus their use should be avoided.
 - Adv- improved washability and durability.
 - Emission testing on samples is required since amine catalysts can be very persistent
- No paint containing formaldehyde, acetaldehyde, isocyanates, reactive amines, phenols, soluble VOCs

- Some examples – Benjamin Moore paints, Thomsit, Corian, Lindner, etc
 - Disadv- cost
- Practical bargain – White colored, low VOC, low odour epoxy paint with proper burn in period (increased temp and ventilation for a few days initially, with lights on)and assessment for VOCs.

Floors

- Large vitrified tiles/ vinyl sheets or treated marble preferred
- Impervious, sealed, minimum joints
- Edges and corners curved for easy cleaning
- Slip resistant, non staining, non permeable
- Solvent free adhesives/vinyl glues – with low VOC emissions
- Changing vinyl flooring in neighbourhood caused dramatic fall in PR due to adhesive vinyl glue (Cohen et al, 1997)

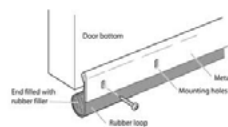
Water supply

- Separate stainless steel tanks for lab – not from common storage tanks of building
- Sinks & drains should be outside lab
- Ducts/pipes should be hidden between wall panels or covered

For Gas supply:

- Inert stainless steel tubing or Teflon coated tubing with medical gasses (CO₂) recommended for incubators.
- Cu to be avoided (prone to oxidation)
- Tubing should be cleaned using inert gases once in 6 months

Doors



- Avoid sliding doors
- Doors must be tight-fitting with bottom 'sweeps' and perimeter seals (top and edges), making them 'Air-tight'.
- Doors – coated or steel
- Sealed doors and pass through windows preferred
- Positive pressure- easier to maintain if the adjoining operating theater also has sealed doors and ceiling.
- Provisions should be made so that doors are wide enough to accommodate large equipment (eg incubators) after the lab becomes permanently sealed and operational.

Furniture

- Wooden furniture to be avoided:
 - binders that release formaldehyde into the space around them for a considerable period
 - Varnished /painted surfaces – could release VOCs
- If completely unavoidable, then should be high density, termite proof and coated with Stainless steel. Cabinets (under and over benches) should be powdercoated metal or stainless steel.
- Stainless steel /Aluminium furniture preferred
 - less likely to release VOCs, although
 - the surfaces may be oiled during the construction process.
 - These furniture items should be cleaned thoroughly with isopropyl alcohol to remove any superficial VOCs before introduction into the laboratory.
 - The issue remains of grease used to lubricate hinges and drawer slides, which should be silicone-based.

(Cairo consensus on the IVF laboratory environment and air quality ,D.Mortimer et ai, June 2018,)

- Furniture should be Ergonomically designed
- Placement of incubators, gamete handling areas, work stations should be such that Embryologist should be able to finish one complete procedure without moving > 3 m in any direction

Light

- The lighting should be within sealed lighting units in order to help reduce airborne particles. Should be dimmable.
- Warm white light (yellow white colour) – less damaging than cool white light
- Direct Sunlight , UV light & Fluorescent light– detrimental to embryos (incandescent light is better)
- At 200 lux lighting –
 - Visible Orange/ Red (wavelength of 620-750 nm) – best development
 - Visible Blue (wavelength of 445-500 nm) - blastocyst formation (most damaging)
- Light damages by:
 - Increasing ROS/free radicals (embryotoxic)
 - Interferes with gene transcription
 - Increases culture medium breakdown products
- Use filters in microscopes

The effect of light on embryos and embryo culture, Pomeroy & Reed, 2013
Effect of light on development of mammalian zygotes, Takenaks et al, 200

Electricity

- Electrical system design
- Categorize equipment and other needs
 - Critical (zero tolerance to interruption)
 - Critical (interruption allowed but needs back up within minutes)
 - Non critical with back up.
 - Non critical without back up
- Calculate the loads and allot for backup with
 - UPS/Inverters/Generators as appropriate
- Proper earthing.
- Alarm systems (personalised to the lab director also)

Electricity

- Power point sockets
 - At Regular distances
 - Number should be more than estimated requirement
- Different electrical phases
- Minimum penetrations with all electrical, gas, AC fittings – concealed but provision for easy maintenance
- NO Window AC

The Embryology Lab (In-Vitro)

- Mimicking in vivo atmosphere is extremely important to optimize fertilization, cleavage, blastulation, implantation and pregnancy rates.
- Embryos are mostly in incubators (controlled temperature, CO2 conc.(pH), humidity)
- Culture Media – controlled temp, pH, osmolality
- But gametes/embryos taken out for- ICSI, assessing fertilization & growth, changing media, other procedures eg LAH or Embryo biopsy for PGD/S
- So ambient atmosphere of lab extremely important to be maintained and controlled in terms of:
 - Temperature
 - Humidity
 - Air quality (Particles, VOC/CAC, microbes, etc)

HVAC

- Heating, ventilation and air conditioning system
- Optimum environment necessary for fertilization and embryo development
- Role of HVAC:
 - Deliver clean air to room
 - Remove contaminants from sources in room
 - Pressurize room
 - Control temperature and humidity

HVAC (Air Quality)

Creating an optimal environment for embryo culture is important for ensuring embryo viability, and thereby maintaining stable pregnancy outcome.

- To optimise environmental conditions, laboratory air should be subjected:
 - Activated Carbon /KMnO4 – VOCs
 - High-efficiency particulate air (HEPA) filters – for particles
 - UV light (optional)– for microbes & photooxidation of VOCs
 - Positive pressure - Ideal target is +38 to +50 Pa in the IVF laboratory (recommended minimum +30 Pa). To minimise air contamination (Embryology lab is positive to the OT, which is positive to the ET room/hallways).
 - Air changes - 15 total air changes per hour, including three fresh air changes per hour, i.e. 20% outside air

ESHRE 2015, Cairo consensus 2018

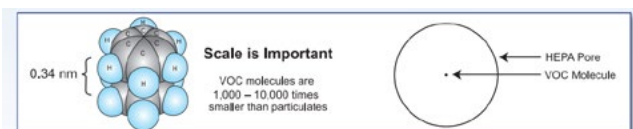
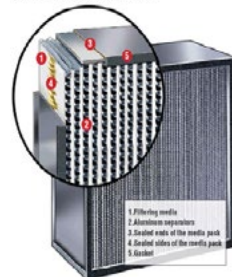
- Temperature – typically within the range of 20– 24°C
- Incubators should not be kept just below the cool air supply
- Humidity - Room relative humidity should be between 40% and 45%.
 - Higher levels will promote growth of moulds, lower values causes high levels of evaporation during dish preparation, which will affect osmolarity of the culture medium and be deleterious to embryos in culture (Swain et al., 2012).

ESHRE Guidelines, 2015
 Cairo consensus on the IVF laboratory environment and air quality:
 Mortimer et al, Reproductive BioMedicine Online , June 2018,

HEPA filters

- Removes 99.97% of airborne particles 0.3µm size
- Do not remove VOC (easily pass through)
- Only activated carbon / KMnO4 can remove VOCs.

A closer look at a HEPA filter



Air quality

- VOC –
 - Isopropanol, benzene, hexane, formaldehyde, vinyl chloride
 - Hydrocarbon based compounds released from solvents, adhesives
 - From instruments – microscopes, TV monitors
 - From building material, flooring
 - Furniture – some amount of particle board (10% formaldehyde resin by weight) – off gases for >20yrs
 - Medical gases – CO2, N2
 - There was a significant drop in the pregnancy rates when a neighbor in the building replaced their vinyl floor, which requires the use of large amount of adhesive (source of VOCs)
- Cohen J, Gilligan A, Esposito W, Schimmel T, Dale B. Ambient air and its potential effects on conception in vitro. Hum Reprod. 1997;12:1742-1749-9.

- VOC - 0.5 ppm : acceptable blastocyst formation and pregnancy rates, but higher miscarriage rates.
- Ideal <0.2 ppm, preferably 0
- VOC > 1ppm – directly toxic to embryos
- VOC meter- can measure till 0 ppb
- But important to know which type of VOC



Better IVF outcomes following improvements in laboratory air quality . Khoudja et al, J Assist Reprod Genet (2013) 30:69–76

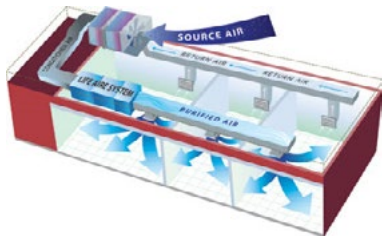
To improve air quality:

- AHU
- Stand alone units (with/without positive pressure), eg Austin Air, United States of America (Lab air purifier)
- Laminar air flow stations
- Inline filters for the gas supply to incubators
- Filters to be kept in incubators

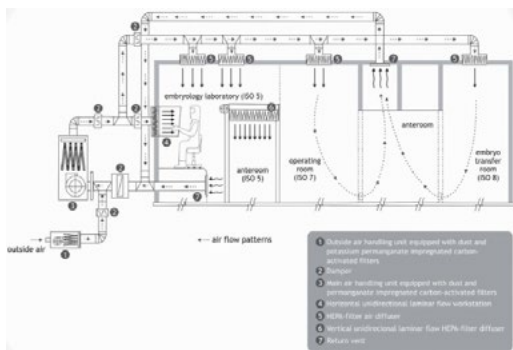


Air delivery (AHU)

- Based on the external air quality, the ratio of external flow and internal recirculation is adjusted.
- If there is an external crisis (fire, smoke, new construction, etc which will increase particles/VOC in ext air), the AHU can be put on 'submarine mode', that is total isolation from outside air.



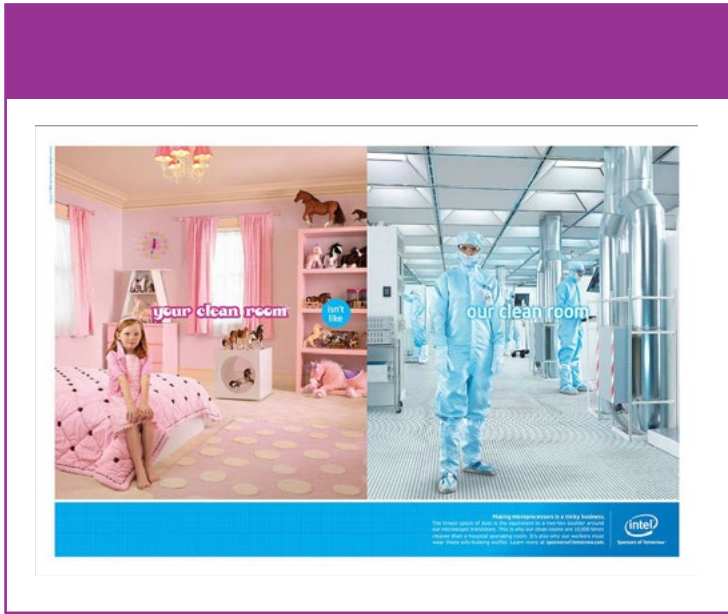
AHU



Clean room

- A cleanroom is defined by ISO 14644-1 as 'a room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity, and pressure, are controlled as necessary'

(International Organization for Standardization, 1999).



Updated 2008, EU GMP Annex 1-Manufacture of Sterile Medical Products

Cleanroom classification

Grade	Max. permitted number of particles per m ³ equal to or greater than the tabulated size			
	At rest		In operation	
	0.5µm	5 µm	0.5µm	5 µm
A	3 520 (5)	20	3 520 (5)	20
B	3 520 (5)	29	352 000 (7)	2 900
C	352 000 (7)	2 900	3 520 000 (8)	29 000
D	3 520 000	29 000	Not defined	

ISO 14644-1 cleanroom standards [\[edit\]](#)

Class	maximum particles/m ³						FED STD 209E equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm	
ISO 1	10	2.37	1.02	0.35	0.083	0.0029	
ISO 2	100	23.7	10.2	3.5	0.83	0.029	
ISO 3	1,000	237	102	35	8.3	0.29	Class 1
ISO 4	10,000	2,370	1,020	352	83	2.9	Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1.0 × 10 ⁶	237,000	102,000	35,200	8,320	293	Class 1,000
ISO 7	1.0 × 10 ⁷	2.37 × 10 ⁶	1,020,000	352,000	83,200	2,930	Class 10,000
ISO 8	1.0 × 10 ⁸	2.37 × 10 ⁷	1.02 × 10 ⁷	3,520,000	832,000	29,300	Class 100,000
ISO 9	1.0 × 10 ⁹	2.37 × 10 ⁸	1.02 × 10 ⁸	35,200,000	8,320,000	293,000	Room air

Minimum criteria for air quality

- The Brazilian National Health Surveillance Agency (ANVISA) established by law in February 2006 the minimum criteria for an IVF laboratory (This regulation asks for at least an ISO class 5 clean room for gametes and embryo manipulations)

Boone WR, Johnson JE, Locke AJ, Crane MM, Price TM. Control of air quality in an assisted reproductive technology laboratory. *Fertil Steril*. 1999;71:150–4.

- The clean room specifications for an IVF lab with appropriate particulate count monitoring are given as follows:
- Should be a Class 100 clean room
- Esteves SC, Bento FC. Implementation of air quality control in reproductive laboratories in full compliance with the Brazilian cells and germinative tissue directive. *Reprod Biomed Online*. 2013;26: 9–21.
- At least an Class 5 ISO clean room
- Boone WR, Johnson JE, Locke AJ, Crane MM, Price TM. Control of air quality in an assisted reproductive technology laboratory. *Fertil Steril*. 1999;71:150–4.
- Should be a Class B clean room
- Knaggs P, Birch D, Drury S, Morgan M, Kumari S, Sriskandakumar R, et al. Full compliance with the EU directive air quality standards does not compromise IVF outcome. *Hum Reprod*. 2007;22:i164–5.

- Directive 2004/23/EC lays down standards of quality and safety for handling human tissues and cells.
- An air quality with particle counts and microbial colony counts equivalent to those of Grade A, as defined in the European Guide to Good Manufacturing Practice, Annex 1 and Commission Directive 2003/94/EC, is recommended.

European Union “Commission Directive 2006/86/EC implementing Directive 2004/23/EC of the European Parliament and of the J Assist Reprod Genet (2013) 30:69–76 75 Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells” *Official Journal of the European Union* L294/32. 2006. Accessed 24 Nov 2006.

Recent consensus on air quality:

- Particulates - Less than 352,000 particles larger than 0.5 um to 10 um per metre³ (equivalent to <10,000 such particles per cubic foot)
- Micro-organisms - Less than 10 cfu/m³ and less than two spores/m³ 'at rest'.
- VOCs - Total VOCs less than 500 g/m³ (~400–800 ppb total VOC, depending on molecular species); less than 5 g/m³ aldehydes.

Cairo consensus on the IVF laboratory environment and air quality:
Mortimer et al, Reproductive BioMedicine Online , June 2018,

Equipments

A. MANDATORY-

- USG machine,
- Suction pumps with foot pedal
- Test tube/petridish warmer, heated blocks
- Laminar flow bench with thermostatically controlled heating plate
- Stereozoom microscope,
- High resolution inverted microscope with phase contrast or Hoffman optics Micromanipulator
- CO₂ incubators
- Routine high powered binocular light microscope
- Laboratory centrifuge, Refrigerator
- Equipment for cryopreservation
- Diposables

Equipments

B. OPTIONAL:
closed chambers, benchtop incubators, LASER

C. EXPERIMENTAL:
IMSI, embryoscope, polscope

Laminar Air Flow



Cryopreservation

- Liquid N₂ containers and medical gas cylinders – placed adjacent to laboratory in a closet or small room with outside access (dirty corridor).
- Pipes and tubes enter the laboratory from this room.
- Medical gases - directed into the laboratory using prewashed vinyl/Teflon-lined tubing or stainless steel tubing.
- Avoid soldered or brazed copper lines – constant contamination
- Extra lines should be hidden behind walls and ceilings for future expansion.
- Adequate ventilation and low oxygen alarms should be installed. Personal low oxygen alarms are recommended.
- Cryostorage units should be continuously monitored and equipped with alarm systems, detecting any out of range temperature and/or levels of liquid nitrogen (LN₂). Extra LN₂ must be available for refilling.

ESHRE 2015

Incubators

- Number of incubator- depends on workload.
- Ideal to start – one CO₂ and one benchtop (Trigas) incubator
- Ratio of cases per incubator – variable
- Minimum number - 4 cases per incubator.
- Separate compartments with double doors may be helpful
- Smaller incubators –not >2-3 cases
- New incubators and equipment – must be “burned-in” or “off gassed”

“burning in”

- Installation of all equipment, electrical lines and UPS needs to be completed before starting cleaning and burning –in.
- Construction should be completed, AHU should be properly configured.
- Fumigation and UV sterilisation
- All surfaces and walls cleaned with 70% ethyl alcohol or Hydrogen peroxide and then with sterile water
- Typical burn-in - increase temperature of new area by 10–20°C and increase ventilation rate
- High temperature + air exchange - aids in removal of volatile organics.
- System must be capable of supplying the space with air at temperature of 30–35°C, at < 40% relative humidity.
- Period - 10 to 28 days

- Laboratory should be closed
- All lights and some auxiliary equipment should be turned on and left running the whole time.
- If these temperatures cannot be reached by the base system – electrical room heaters can be used to reach the desired temperature.
- Before making the lab functional, verification should be done by:
 - Particle & VOC counts
 - Microbial cultures from different walls, LAF, workstations, incubators, etc.
 - Bioassays like sperm survival test

Commissioning of incubators

- As a new incubator has a high VOC output, run it at some other area outside the lab for a month.
- The laminar Air Flow has to be left on for few hours each day during the burning in period and cleaning.
- Off gassing of new disposables is extremely important to reduce VOCs

Cairo consensus on the IVF laboratory environment and air quality: Mortimer et al, Reproductive BioMedicine Online , June 2018,

Consumables & Culture Media

- Consumables should be procured from reliable sources.
- Culture media used for processing gametes or growing embryos - from reliable manufacturers.
- Each batch of
 - consumables & culture media - tested for sterility and endotoxins/ embryotoxicity
 - culture medium - also tested for osmolality and pH.
- Drugs to be taken from reputed & time tested brands, ensuring cold chain maintenance extremely important for the outcome.

Manpower

A successful IVF programme is run by a well co-ordinated team, which includes:

- Programme Director
- Gynaecologists
- Andrologist
- Embryologist
- Counsellor
- Others – Nursing Staff, OT Technician, Housekeeping staff

After setting up an IVF lab

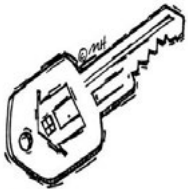
The project doesn't ends, Rather begins !!

- The success (outcome) depends on:
 - Competence of the team
 - Training of staff
 - Quality control & Quality Assurance
 - Proper SOPs
 - Record keeping & Timely audits
 - Patient centred individualised approach.

3. QA QC at an ART centre

Incubators

- Achieve and maintain highest level of patient care
- Highest success (pregnancy) rates



What we want to avoid

- Avoid Mistakes
- Mix up

How can we achieve this ?

By Quality control and Quality assurance

Quality control

- What is it?
- How can it help me?

- Careful quality control can lead to much more stable products with less variability and less likelihood of toxicity.
- Easy to identify problem area and rectify mistakes



What does QC look into

All activities and operational techniques within ART lab

Tangible elements	Intangible elements
Personnel,	Techniques
Instruments	Protocols
Equipment and	Documentation
Supplies	Record-keeping

Goal

- Evaluate : effectiveness of policies & procedures
- Identify & correct: problems
- Assure : Accuracy and precision of procedures
- Monitor : Performance and competency of the laboratory staff

Who all are involved

- All personals working in ART Center
- Laboratory personals
- Clinicians
- Nurses
- OT Staff and Technicians
- Even the Administration department

Difficulties faced during QA

- Final outcome is an ongoing pregnancy
- Dependent on numerous components that are beyond laboratory control .
- Confused by a disparity in way in which each laboratory ascertains outcomes.

Example

- The clinics quality parameters:
 - Implantation rate per embryo above 30%
 - Monitor for every 50 transfer
- Cause for attention: below 25%
- Full overhaul : below 20%
- This happened too often –
 - Likely cause each time was too many low prognosis patients
- Solution: include only good prognosis patients

Guidelines for ART Lab

- International standard for laboratories
 - American Fertility Society (1992)
 - Association of Clinical Embryologists (1996)
 - European Society for Human Reproduction and Embryology (ESHRE) (Gianaroli, 2000)

ART lab - Guidelines

- Very valuable
 - Practical and ART specific guidelines
 - Incorporate basic safety rules.

They form a good basis to establish a quality management system in an ART laboratory.

Disadvantage

- Not approved as official standards, hence not suitable when seeking formal recognition/ accreditation by an authoritative body.
- Whenever formal recognition is pursued, official international standards, such as those developed and released by International Organization for Standardization (ISO), should be implemented

Certification or Accreditation?

- Certification according to ISO 9001:2008 –
You do what... you say..... you should do
- ISO does not specify how good you should be in pregnancy rates or implantation rates
- You need to specify that yourself.
You can be certifiedand have lousy results..
- As long as that is what you aim for....?

Further step

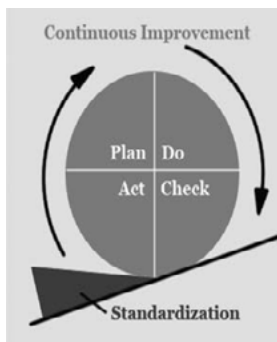
- An ART laboratory should
 - First define its scope of practice and
 - What it ultimately hopes to achieve.
- Is it Certification or accreditation alone?
- or maintaining standards ?

Best documented models or strategies are

- Total Quality Management (TQM) (Packard, 1996)
- Excellence Model of the European Foundation for Quality Management (EFQM, 1992).

Total Quality Management

- Based on PDCA/Deming cycle
- Continuous striving to improve every aspect of a process/service



PDCA / Deming cycle

- **Plan:** establish objectives and identify necessary processes to achieve them
- **Do:** implement the new processes
- **Check:** measure and compare the results against the expected outcome
- **Act:** analyze any differences and the level of performance

EFQM

- **Continuous learning, innovation and improvement**
- **Logic: RADAR**
- **Results**
- **Approach**
- **Deployment**
- **Assessment**
- **Review**

Objectives – minimum targets

- Personnel - appropriately educated and trained
- Well-maintained and efficient ART laboratory and clinic
- Detailed written standard procedures- SOP manuals
- Control of disposables & culture media
- System patient and patient sample identification
- Every action performed with patients' samples is registered and verified by double-checks

Objectives – minimum targets

- Reliable, proper functioning and calibrated equipment and instruments
- Consistent and proper execution of appropriate techniques and methods
- Proper documentation and record-keeping
- System for appraisal of employees, correction of deficiencies and implementation of advances and improvements – keep them motivated and satisfied

- Once implemented - adjusted and improved continuously and reviewed periodically
- Establishing and maintaining a quality system and achieving objectives requires substantial changes at all levels, both within and outside an ART laboratory.

Final result depends on everyone's will to change co-operate and commitment .

Laboratory personnel

Staff requirements

The number of staff has to be adjusted according to the number and nature of the procedures performed in the laboratory

Cycles per year	Positions *
< 250	1.5
250 - 500	2.5
500 - 750	3.0
750 - 1000	4.0
1000 - 1500	5.0
1500 - 2000	6.0
2000 - 2500	7.0
2500 - 3000	8.0

* Including laboratory director

Laid by the German Society for human reproductive Biology

Laboratory personnel

Position	Degree	Experience	ART procedures
Director	PhD, MD	2 years	60
Supervisor	Master's /Bachelor's	6 months	60
Technologist	Bachelor's		30

Ensure

Continuing education program

Training of a new team member

Log Book

Periodic assessment of competency

Periodic assessment of competency

Short term intervals

- Grading oocyte maturity
- Fertilization rate
- Cleavage rate
- Grading embryo quality
- Evaluation of sperm parameters

Long term intervals

- Pregnancy rate/transfer
- Fertilization/degeneration rates following ICSI

Best evidence of good quality control

Pregnancy Rate

Best short-term evidence of good quality control

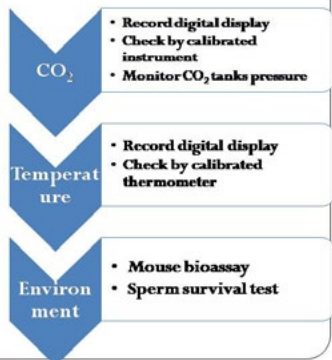
- Unfortunately, real decline in performance may only be identified several weeks later when pregnancy rates fall
- Delaying implementation of corrective action.

Correct operation & calibration of all instruments

Table 1 Internal Quality Control Measures Used to Assess the Performance of New Equipment Installed in the Assisted Conception Unit, Centre for Reproductive and Developmental Medicine, Sheffield, U.K.

Equipment	Manufacturer	Model	Test	Frequency of testing	Method	Range± accuracy
Incubator	Heraeus	BB620	CO ₂ (%)	Weekly	Fyrite gauge	5± 0.5%
			Temperature	Daily	Max/min thermometer	-10°C to +50± 1°C
Water system	Vivendi	Purclub Maxima	Environment	As required	Mouse bioassay	n/a
			Endotoxin	Weekly	Limulus	0.03 EU/mL
					Amoebocyte lysate test	
Refrigerator	LEC	LR500	Temperature	Daily	Max/min thermometer	-10°C to +50± 1°C
Heated stages	MAT*	BioMAT2	Temperature	Monthly	Surface thermometer	-50°C to +150± 0.1°C
			Temperature	Monthly	Surface thermometer	As above
	Hunter*	Hunter N24	Temperature	Monthly	Surface thermometer	As above
	Hunter	200AIN	Temperature	Monthly	Surface thermometer	As above
Hot blocks	Grant	QBT2	Temperature	Monthly	Mercury thermometer	-20°C to +150± 5°C
Hot oven	Gallenkamp	OVB-307	Temperature	Every use	Temperature indicator strips	121°C to 160± 2%
Cryopreservation equipment	Planar	Kryo 360	Function	Every use	Test run with medium only	n/a

Incubator - QC



Incubator - QC



In built



External CO₂ Analyzer

Monitor CO₂ tanks pressure



Heated water/dry baths



Liquid nitrogen tanks



Monitor level using dipstick/ruler

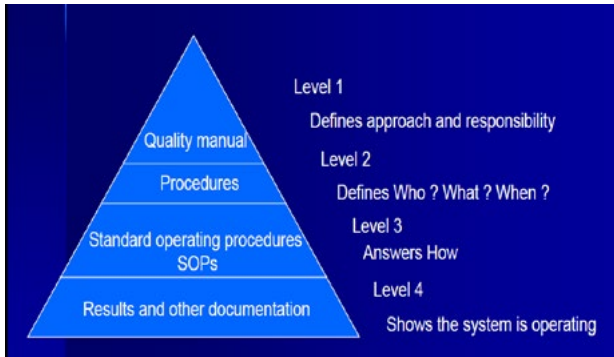
Quality handbook

- Describing main structure and basic elements of the quality system.
- Laboratory protocols and all activities performed within ART laboratory need to be described in detail - SOP

Basic requirement

- Individuals working in ART laboratory should be
 - Aware of the duties and responsibilities
 - Importance and consequences of their work
- Personal goals should not come before

Documental control



Detailed SOPs – why?

- Ensures uniform execution of all laboratory procedures by all personals
- Enables introduction of the protocols to new team members

Control of disposables & culture media

- Culture media prepared in lab should undergo QC using an appropriate bioassay system
- Commercially produced media – integrity of the packages and appropriate delivery conditions should be controlled
- Certificate of analysis which specifies documentation of QC should be supplied by manufacturer
- Reagents and media should be used prior to expiry date

Disposables & culture media



Quality handbook

- Describing main structure and basic elements of the quality system.
- Laboratory protocols and all activities performed within ART laboratory need to be described in detail - SOP

Disposables & culture media



Proper environment

- Low odor specialized paint
- Air quality
- Laboratory lighting
- Microbiology testing - weekly

Air quality

Human Reproduction, Vol.25, No.5 pp. 1317-1324, 2010
Advanced Access publication on March 13, 2010 doi:10.1093/humrep/deq021

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Effect of air quality on assisted human reproduction[†]

Richard S. Legro^{1,†}, Mark V. Sauer², Gilbert L. Motta³,
Kevin S. Richter³, Xian Li⁴, William C. Dodson¹, and Duanping Liao⁴

- NO₂ – consistently associated with lower live birth rates
- Ozone – decreased odds of live birth
- Fine particulate matter – decreased conception rates

Air quality

- Chemical air contaminants (COC) are believed to exert a range of effects, from fertilization failure and delayed embryonic development to a reduction in viability and pregnancy rates
- These effects may or may not be evident morphologically

Air quality requirements in ART lab

- High fresh air changes (15 – 25 air changes/hour)
- Positive pressurization
- HEPA filtration
- Chemical air processing using activated carbon and potassium permanganate
- Active filtration units inside or at the inlets of incubators and in the laboratory area

Air quality



CODA tower



Air Quality Analyzer

Air quality – result of

**Environment
Protection Agency air
quality monitors**

Definite identification of patients & their biological material

- All material obtained from the patients, should bear unique identification
- Incubators should be organized in order to facilitate identification of embryos, oocytes and spermatozoa

Patient Identification

- **Verification** of patients' identity should be performed at critical steps:
 - Before ovum retrieval
 - At semen recovery
 - Embryo transfer
- **Double checks** need to be considered at least at:
 - Insemination of oocytes
 - Replacement of embryos
 - Embryo freezing and thawing

Identification and control of deviations

Laboratory management shall have a policy to be implemented when there is a deviation from a correct execution of its own procedures and regulations

How to adopt a correct procedure for the control of deviations ?

- Team members should understand the importance of transparency and the documentation of nonconformities
- In a case of an incident, immediate corrective actions should be executed
- Possible preventive actions will be suggested
- The effectiveness of these preventive actions will be examined

Deviation from laboratory protocol

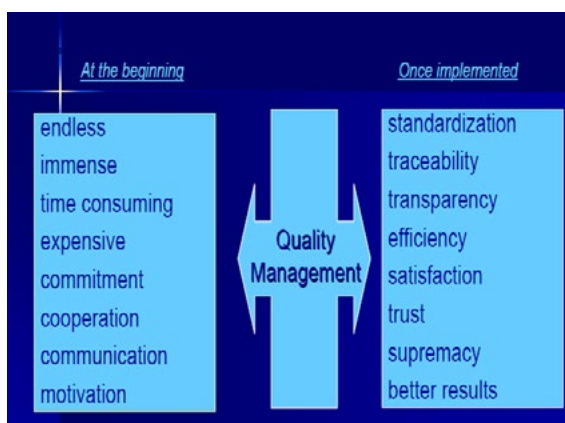
- A Petri dish containing patient's A embryos was found in patient's B compartment in the incubator

Preventive action

- An addition of a second colorful label including patient's name on the inner door of the incubator

Examination of the effectiveness of the preventive action

- No such event reoccurred during a 6 month period



Summarize

Weigh the huge amount of work involved against possibly optimized chance for the patients of delivering a healthy baby

THANK YOU

4. ICMR Guidelines

ICMR Guidelines

- To regulate & Supervise ART Clinics ICMR has come out with national guideline for Accreditation, Supervision & Regulation of ART clinics in India.
- Guideline have been evolved after detailed discussion & debate by experts Practitioners of ART, lawyers & social scientists

- Most of countries have drawn guidelines for practice of ART .
- Some countries like U.K Egypt, and Argentina have both guidelines & legislation .
- These guidelines are useful for infertility clinics as well as patients, who seek services of these clinics.

CATEGORIES of Infertility Care Units

Primary (Level 1A) infertility care units

- These would be clinics where preliminary investigations are carried out and cause of infertility diagnosed.
- Primary infertility care unit or clinic could be a doctor's consulting room, such as a gynaecologist's or a physician's consulting room, or even a general hospital.

- **The responsibilities of a Level 1A primary infertility care unit would be**
- Completion of the basic investigations.
- Introduction of ovulation with clomiphene citrate, (Gonadotropin should not be used at a primary infertility care unit level).
- Correcting minor endocrine disorders such as thyroid disorders or hyperprolactinemia.
- Treatment of oligozoospermia.
- Ability to carry out AIH & IUI using processed semen of husband or donor, obtained from an accredited laboratory or semen bank.

- The gynaecologist or the physician incharge should have an appropriate post-graduate degree or diploma, and must maintain records of the use of the requisitioned semen and of all AIH & IUI done, appropriately and confidentially;
- these records will be liable to inspection by an appropriate Review Committee.
- **Level 1A infertility care unit will not require an accreditation under these guidelines.**

Primary (Level 1B) infertility care units engaging in IUI

- **Infertility clinics falling into this shall require registration.**
- The IUI in such clinics must be done under the supervision of a gynecologists with a post-graduate degree.

Facilities required are:

- Assessment of follicular growth and ovulation by transvaginal sonography (TVS).
- Hysteroscopy, laparoscopy.
- Facilities for semen preparation and for intrauterine insemination (IUI), including an appropriate sterile area for IUI.

Secondary (Level 2) Infertility Clinics

- They shall have facilities for artificial insemination using husband's semen (AIH), artificial insemination using donor semen (AID).
- Provision for semen collection in men with a vibrator or an electroejaculator in functional erectile and ejaculatory problems.
- Require registration under the Act.
- They may have infrastructure for further in depth investigation and extended treatment of infertility except Where oocytes are handled outside the body.

Tertiary (Level 3) Infertility Clinics

- Provision for extended treatment of infertility including oocyte pick up, in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and similar techniques.
- These clinics will require registration.

Requirements for an ART Clinic

- Minimal Physical Requirements are :

ART clinic of level 2 or 3 should have a non-sterile and a strictly sterile area.

A. The NON-STERILE AREA should include :

1. Reception and waiting area.
2. A room with privacy for interviewing and examining male and female partners.

3. A general purpose clinical lab.
4. Store room/Record room, Record keeping preferably should be computerized.
5. Autoclave room.
6. Vermin Proofing /Whole Clinic should be Vermin proof.
7. Semen Collection room.(Room with privacy and an appropriate environment, in a secluded area close to lab).
8. Semen Processing Lab.(Lab with laminar flow)

(B) STERILE AREA

- Sterile area include operation theater and embryology lab complex.
- **Operation Theater** – Must have Ac and filter system along with emergency resuscitation equipment for ovum pick-up.
- **Embryology Lab** - Should have facilities for the control of temperature and humidity and must have filtered air.

- Walls and floors must be composed of material that can easily washed and disinfected.
- Embryology Lab- must have the following :-
 - (I) Laminar flow.
 - (II) Stereo microscope.
 - (III) High-Power binocular light microscope.
 - (IV) High resolution inverted microscope.

- Micromanipulator.
- CO₂ Incubator.
- Laboratory centrifuge.
- Equipment for Freezing Embryos
- Liquid nitrogen.

Ancillary Lab Facilities

- Either in-house or near by located standard lab preferably NABL for hormone assay and other infertility related test.
- ART Clinic should have microbiology lab accessible to it.

Maintenance of Laboratories

- Each lab should have SOP for different procedure there should be no mixing of gametes or embryos.
- All dishes and tubes containing gametes and embryos should be clearly labeled.
- All pipettes should be immediately discarded after use.
- Laminar flow, table, incubator must be regularly checked for microbial contamination.
- Logbook should be maintained which records the temperature, Co2 content and humidity of incubators.

- Quality of Consumable used in lab should be good reliable source, so that they are non-toxic to embryos. Similarly culture media in lab should be from reliable manufacturer.
- Power Back-up ; Embryo lab should have online UPS and power back-up, no interruption of power supply to incubator and other accentual service.

Essential Qualifications of the ART Team

- The practice of ART requires team work between the gynaecologist, the andrologist and the clinical embryologist supported by a counselor and programme coordinator/director.
- Gynecologist - minimum qualification for level 2 or level 3 clinic is post graduate, diploma or degree.
- Knowledge of reproductive endocrinology.
- Acquiring skill in gynae ultrasound.
- Carrying out procedure like IUI, OPU,IVF or ICSI.
- For male Infertility if gynecologist is confident and competent, she can treat such patients or refer them to andrologist

Andrologist

- (I) As 50% cases are related to male factor, many of which can be treated by specific ART procedure.
- (II) Each ART clinic should have andrologist (Urologist having special interest in male infertility), who should be able to treat such patients.
- (III) Carry out special procedure like TESA/PESA and other surgical procedures.

Clinical Embryologist

- (I) Each IVF lab should have clinical embryologist who should have knowledge of embryology, reproductive endocrinology, genetics, molecular biology and in vitro culture techniques.
- (II) Must be familiar with ART.
- (III) He/she should be either a medical graduate or have post graduate degree in appropriate area of life science.

The responsibilities of the clinical embryologist would be:

- To ensure that all the necessary equipments are present in the laboratory and are functional.
- To perform all the procedures pertaining to processing, handling and culturing of gametes and embryos in the laboratory and hand over the embryo to the gynecologist.

- To maintain records of all the procedures carried out in the laboratory.
- In case of shortage of adequately trained clinical embryologists, an individual may act as a clinical embryologist for more than one clinic but each clinic where the person works must own responsibility for the embryologist .
- An embryologist must not be associated with more than two centers at any given time.

Counselors

- Counselors are very important adjunct to every ART Clinic. A person who has at least a degree(preferably a postgraduate degree) in
- Social Sciences/ Psychology/ Life Sciences / Medicine and good knowledge of infertility should be considered for the post of counsellor.

Programme Coordinator / Director

- Programme Coordinator / Director
- This should be a senior person who has had considerable experience in all aspects of ART. The programme coordinator/director should be able to co-ordinate the activities of the rest of the team and take care of staff administrative matters, stock keeping, finance, maintenance of patient records, statutory requirements, and public relations.
- The programme coordinator/ director should have a post graduate degree in medical or biological science.
- He / She must have a reasonable experience of ART.

ART Procedure

- ICMR has given specific guidelines and indications for various procedures like IUI, IVF ET, ICSI and some associated technique like TESA/PESA.
- One of the primary concerns of all ART treatments is the safety of the patients and of their gametes and embryos which constitute the very beginning of a new individual's life.

Indications for oocyte or embryo donation

- Gonadal dysgenesis.
- Premature ovarian failure.
- Iatrogenic (due to ovarian surgery or radiation, or chemical castration) ovarian failure.
- Women who have resistant ovary syndrome, or who are poor responders to ovulation induction.
- Women who are carriers of recessive autosomal disorders.
- Women who have attained menopause

Code of Practice, Ethical and Legal Issues

This Code of Practice deals with all aspects of the treatment provided and the research done at registered clinics.

Codes are summarized as:

Staff

Staff of the registered unit should be sufficiently qualified,

Guidelines for minimum standards and qualifications of clinical, scientific and counseling staffs are already described.

Confidentiality

Any information about clients and donors must be kept confidential.

No information about the treatment of couples should be disclosed to anyone other than the accreditation authority or persons covered by the registration, except with the consent of the person or in a medical emergency concerning the patient, or a court order.

Information to patient

All relevant information must be given to the patient before a treatment is given.

Information should be given on the:

- limitations and results of the proposed treatment
- possible side-effects & the techniques involved
- comparison with other available treatments
- cost of the treatment.
- Rights of the child born through ART
- Need for the clinic to keep a register of the outcome of a treatment

CONSENT

No treatment should be given without the written consent of the couple at all the possible stages, including possibility of freezing.

A standard consent form recommended by the accreditation authority should be used by all ART clinics. Specific consent must be obtained from couples who have their gametes or embryos frozen, in regard to what should be done with them if he/she dies, or becomes incapable of varying or revoking his or her consent.

Counseling

- People seeking treatment must be given a suitable opportunity to receive proper counseling about the various implications of the treatment.
- Counseling is recognized as being beneficial, and couples should be encouraged to go through it.
- The provision of facilities for counseling in an ART clinic (of Levels 1B, 2 or 3) is mandatory.

Use of gametes and embryos

- No more than three embryos may be placed in a woman in any one cycle, regardless of the procedure/s used, excepting under exceptional circumstances like:-
elderly women/ poor implantation/adenomyosis/ poor embryo quality. It should be recorded.
- No woman should be treated with embryos derived from the gametes of more than one man or woman during any one-treatment cycle.

Storage and handling of gametes and embryos:

The 'highest possible standards' in the storage and handling of gametes and embryos in respect of their security, and in regard to their recording and identification, should be followed.

Complaints

All registered ART clinics are required to have procedures for acknowledging and investigating complaints, and to have a nominated person to deal properly with such complaints.

- The accreditation authority must be informed of the number of complaints made in any year and those that are outstanding.

Responsibilities of the Clinic

- To give adequate information to the patients.
- To explain to the patient the rationale of choosing a particular treatment and indicate the choices the patient has with advantages and disadvantages of each choice.
- To maintain records in an appropriate Performa (to be prescribed by the authority).

- To keep all information about donors, recipients and couples confidential and secure.
- The information about the donor, (excluding information on the name and address) should be released by the ART clinic only to the offspring only if asked by him/her after he/she reaches the age of 18 years, or as and when specified and required for legal purposes, and never to the parents (excepting when directed by a court of law).

- To maintain detailed record of all donor oocytes, sperm or embryos used, the technique in which they are used, couple/surrogate mother on whom they are used).
- These records must be maintained for at least ten years after which the records must be transferred to a central depository to be maintained by the ICMR.
- If the ART clinic/centre is wound up during this period, the records must be transferred to the central repository in the ICMR.

- To have the schedule of all its charges suitably displayed in the clinic and made known to the patient at the beginning of the treatment. There must be no extra charges beyond what was intimated to the patient at the beginning of the treatment.
- To ensure that no technique is used on a patient for which demonstrated expertise does not exist with the staff of the clinic.
- To be totally transparent in all its operations. The ART clinics must, therefore, let the patient know what the success rates of the clinic are in regard to the procedures intended to be used on the patient.
- To have all consent forms available in English and local language(s).

Information and Counseling to be given to Patients

- The success rates with the recommended treatments obtained in the clinic as well as around the world (this data should be available as a document with references, and updated every 6 – 12 months).
- The possible side-effects of the drug used and the risks of treatment to the women & the risks associated with multiple pregnancy.
- The need to reduce the number of viable fetuses, in order to ensure the survival of at least two fetuses.

Complaints

- The possible deterioration of gametes or embryos associated with storage
- The advantages and disadvantages of continuing treatment after a certain number of attempts.
- Pamphlets (one-page on each technique in all local languages and English) which give clear, precise and honest information about the procedure recommended to be used will help the couple make an informed choice.

Desirable Practices/Prohibited Scenarios

- A third party donor of sperm or oocytes must be informed that the offspring will not know his/her identity.
- There would be no bar to the use of ART by a single women who wishes to have a child, and no ART clinic may refuse to offer its services to the above, provided other criteria mentioned in this document are satisfied. The child thus born will have all the legal rights on the woman.

- The ART clinic must not be a party to any commercial element in donor programmes or in gestational surrogacy.
- A surrogate mother carrying a child biologically unrelated to her must register as a patient in her own name.
- While registering she must mention that she is a surrogate mother and provide all the necessary information about the genetic parents such as names, addresses.
- She must not register in the name of the person for whom she is carrying the child, as this would pose legal issues, particularly in the untoward event of maternal death.
- The birth certificate shall be in the name of the genetic parents.

- All the expenses of the surrogate mother during the period of pregnancy and post-natal care relating to pregnancy should be borne by the couple seeking surrogacy.
- The surrogate mother would also be entitled to a monetary compensation from the couple for agreeing to act as a surrogate; the exact value of this compensation should be decided by discussion between the couple and the proposed surrogate mother.
- An oocyte donor can not act as a surrogate mother for the couple to whom the oocyte is being donated.

- A third-party donor and a surrogate mother must relinquish in writing all parental rights concerning the offspring and vice versa.
- No ART procedure shall be done without the spouse's consent.
- Sex selection at any stage after fertilization, should not be permitted, except to avoid the risk of transmission of a genetic abnormality assessed through genetic testing of biological parents or through preimplantation genetic diagnosis (PGD)

- No ART clinic shall offer to provide a couple with a child of the desired sex.
- Collection of gametes from a dying person will only be permitted if the widow wishes to have a child.
- Use of sperm donated by a relative or a known friend of either the wife or the husband shall not be permitted.

- It will be the responsibility of the ART clinic to obtain sperm from appropriate banks; neither the clinic nor the couple shall have the right to know the donor identity and address.
- Both the clinic and the couple, however, shall have the right to have the fullest possible information from the semen bank on the donor such as height/weight/skin color/educational qualification/profession/family background/freedom from any known diseases or carrier status (such as hepatitis B or AIDS), ethnic origin, before accepting the donor semen.

- It will be the responsibility of the semen bank and the clinic to ensure that the couple does not come to know the identity of the donor.
- It would apply for oocyte donation as well .
- When DNA fingerprinting technology becomes commercially available, the ART clinic may offer to the couple, a DNA fingerprint of the donor without revealing his/her identity, against appropriate payment towards the cost of the DNA fingerprint.
- Semen from two individuals must never be mixed before use, under any circumstance.

- The data of every accredited ART clinic must be accessible to an appropriate authority of the ICMR for collation at national level .
- Any publication or report resulting out of analysis of such data by the ICMR will have the concerned members of the staff of the ART clinic as co-authors.
- The consent on the consent form must be a true informed consent witnessed by a person who is in no way associated with the clinic

Requirements for a Sperm Donor

- Donor should not have hypertension, diabetes, sexually transmitted diseases, and identifiable and common genetic disorders such as thalassemia.
- The age of the donor must not be below 21 or above 45 years.
- An analysis must be carried out on the semen of the individual, preferably using a semen analyzer, and the semen must be found to be normal according to WHO method manual for semen analysis.

- The blood group and the Rh status of the individual must be determined and placed on record.
- Other relevant information in respect of the donor, such as height, weight, age, educational qualifications, profession, colour of the skin and the eyes, record of major diseases including any psychiatric disorder, and the family background in respect of history of any familial disorder, must be recorded in an appropriate Performa.

Requirements for an Oocyte Donor

- The individual must be free of HIV and hepatitis B and C infections, hypertension, diabetes, sexually transmitted diseases, and identifiable and common genetic disorders such as thalassemia.
- The blood group and the Rh status of the individual must be determined and placed on record.
- Other relevant information in respect of the donor, such as height, weight, age, educational qualifications, profession, colour of the skin and the eyes, and the family background in respect of history of any familial disorder, must be recorded in an appropriate proforma.
- The age of the donor must not be less than 21 or more than 35 years.

How may Sperm and Oocyte Donors and Surrogate Mothers be Sourced?

Semen banks

- Either an ART clinic or a law firm or any other suitable independent organization may set up a semen bank. If set up by an ART clinic it must operate as a separate identity.
- The bank will ensure that all criteria mentioned (Requirements for a sperm donor) are met and a suitable record of all donors is kept for 10 years after which, or if the bank is wound up during this period, the records shall be transferred to an ICMR repository

- On request for semen by an ART clinic, the bank will provide the clinic with a list of donors (without the name or the address but with a code number.)
- The semen bank shall not supply semen of one donor for more than ten successful pregnancies.
- It will be the responsibility of the ART clinic or the patient, as appropriate, to inform the bank about a successful pregnancy.
- The bank shall keep a record of all semen received, stored and supplied, and details of the use of the semen of each donor.
- This record will be liable to be reviewed by the accreditation authority.

- The bank must be run professionally and must have facilities for cryopreservation of semen, following internationally accepted protocols. Each bank will prepare its own SOP (Standard Operating Procedures) for cryopreservation.
- Semen samples must be cryopreserved for at least six months before first use, at which time the semen donor must be tested for HIV and hepatitis B and C.
- The bank must ensure confidentiality in regard to the identity of the semen donor.
- All semen banks will require accreditation.

Surrogacy: General Considerations

- A child born through surrogacy must be adopted by the genetic (biological) parents unless they can establish through genetic (DNA) fingerprinting (of which the records will be maintained in the clinic) that the child is theirs.
- Surrogacy by assisted conception should normally be considered only for patients for whom it would be physically or medically impossible/ undesirable to carry a baby to term.

- Payments to surrogate mothers should cover all genuine expenses associated with the pregnancy.. The ART centre should not be involved in this monetary aspect.
- Advertisements regarding surrogacy should not be made by the ART clinic. The responsibility of finding a surrogate mother, through advertisement or otherwise, should rest with the couple, or an ART bank.
- A surrogate mother should not be over 45 years of age. Before accepting a woman as a possible surrogate for a particular couple's child, the ART clinic must ensure (and put on record) that the woman satisfies all the testable criteria to go through a successful full-term pregnancy.

- A relative, a known person, as well as a person unknown to the couple may act as a surrogate mother for the couple. In the case of a relative acting as a surrogate, the relative should belong to the same generation as the women desiring the surrogate.
- A prospective surrogate mother must be tested for HIV and shown to be seronegative for this virus just before embryo transfer.
- No woman may act as a surrogate more than thrice in her lifetime

Preservation, Utilization & Destruction of Embryos

- Couples must give specific consent to storage and use of their embryos. The Human Fertilization & Embryology Act, UK (1990), allows a 5- year storage period which India would also follow.
- Research on embryos shall be restricted to the first fourteen days only and will be conducted only with the permission of the owner of the embryos.
- No commercial transaction will be allowed for the use of embryos for research.

Rights of a Child Born through various ART Technologies

- A child born through ART shall be presumed to be the legitimate child of the couple, having been born in wedlock and with the consent of both the spouses. Therefore, the child shall have a legal right to parental support, inheritance, and all other privileges of a child born to a couple through sexual intercourse.
- Children born through the use of donor gametes, and their “adopted” parents shall have a right to available medical or genetic information about the genetic parents that may be relevant to the child’s health.

- Children born through the use of donor gametes shall not have any right whatsoever to know the identity (such as name, address, parentage, etc.) of their genetic parent(s). A child thus born will, however, be provided all other information.
- about the donor as and when desired by the child, when the child becomes an adult. While the couple will not be obliged to provide the above “other” information to the child on their own, no deliberate attempt will be made by the couple or others concerned to hide this information from the child as and when asked for by the child.

- In the case of a divorce during the gestation period, if the offspring is of a donor programme – be it sperm or ova – the law of the land as pertaining to a normal conception would apply.

General Considerations

Minimum age for ART:

- For a woman between 20 and 30 years, two years of cohabitation/ marriage without the use of a contraceptive, excepting in cases where the man is infertile or the woman cannot physiologically conceive.
- For a woman over 30 years, one year of cohabitation/ marriage without use of contraceptives. Normally, no ART procedure shall be used on a woman below 20 years.

Advertisements of an infertility centre

- False claims via hoardings and paper advertisements are a cheap way of attracting a clientele that is vulnerable and, therefore, easily swayed. Such advertisements shall be banned.
- An honest display at appropriate places or publicity of statistics, fee structure, quality of service and of service provided, will be encouraged, provided the guidelines laid down by the Medical Council of India in this regard, are not violated.

- As already mentioned, sperm banks where a complete assessment of the donor has been done, medical and other vital information stored, quality of preservation ensured, confidentiality assured, and strict control exercised by a regulatory body, must be set up.
- Donor sperm would be made available only through such specialized banks/centers.

- No new ART clinic may start operating unless it has obtained a temporary registration to do so.
- This registration would be confirmed only if the clinic obtains accreditation (permanent registration) from the Center or State's appropriate accreditation authority within two years of obtaining the temporary registration. The registration must be renewed every seven years.
- Existing ART clinics must obtain a temporary registration within six months of the notification of the accreditation authority, and appropriate accreditation (permanent registration) within two years of the notification.

- The Center/State Government would close down any unregistered clinic not satisfying the above criteria.
- If the ART clinic that has applied for a temporary registration to the appropriate accreditation authority, does not receive the registration (or a reply) within two months of the receipt of the application from the concerned office of the authority, the ART clinic would be deemed to have received the registration. The same would apply for the permanent registration after the above-prescribed period.

- Human cloning for delivering replicas must be banned.
- Stem cell cloning and research on embryos (less than 15 days old) needs to be encouraged.
- All the equipments/machines should be calibrated regularly.

General Considerations

Minimum age for ART:

- For a woman between 20 and 30 years, two years of cohabitation/ marriage without the use of a contraceptive, excepting in cases where the man is infertile or the woman cannot physiologically conceive.
- For a woman over 30 years, one year of cohabitation/ marriage without use of contraceptives. Normally, no ART procedure shall be used on a woman below 20 years.

Legal Issues

Legitimacy of the child born through ART

- A child born through ART shall be presumed to be the legitimate child of the couple, born within wedlock, with consent of both the spouses, and with all the attendant rights of parentage, support and inheritance.
- Sperm/oocyte donors shall have no parental right or duties in relation to the child, and their anonymity must be respected .

Adultery in the case of ART

- ART used for married woman with the consent of the husband does not amount to adultery on part of the wife or the donor.
- AID without the husband's consent can, however, be a ground for divorce or judicial separation.

Consummation of marriage in case of AIH

- Conception of the wife through AIH does not necessarily amount to consummation of marriage and a decree of nullity may still be granted in favor of the wife on the ground of impotency of the husband or his willful refusal to consummate the marriage. However, such a decree could be excluded on the grounds of

Rights of an unmarried woman to AID

- There is no legal bar on an unmarried woman going for AID.
- A child born to a single woman through AID would be deemed to be legitimate.
- However, AID should normally be performed only on a married woman and that, too, with the written consent of her husband, as a two-parent family would be always better for the child than a single parent one, and the child's interests must outweigh all other interests.

Paper work for ART clinic level 2

- FORM A - Form of application for registration or renewal of registration of an infertility / ART clinic
- FORM - E - Consent for Artificial Insemination or Intrauterine Insemination with Husband's Semen / Sperm
- FORM - F - Consent for Artificial Insemination or Intrauterine Insemination with Donor Semen
- FORM - Q - Semen Analysis Report
- FORM - T - Contract between the ART bank and the ART Clinic
- FORM - V - Oath of Secrecy

Paper work for ART bank

- FORM - A1 - Form of application for registration or renewal of registration of an ART bank
- FORM - L - Consent Form for the Donor of Sperm
- FORM - M - Information on Semen Donor
- FORM - N- Results of screening of Semen Donors / Oocyte Donors / Surrogate
- FORM - O - Record of use of Donor Gametes and Surrogates gate Mothers
- FORM - R - Contract between the ART bank and the Semen Donor
- FORM - S - Contract between ART bank and pt

FORM - E

Consent for Artificial Insemination or Intrauterine Insemination with Husband's Semen / Sperm (See Rule 15.1)

_____ and _____, being husband and wife and both of legal age, authorize Dr. _____ to inseminate the wife artificially or intrauterine with the semen / sperm of the husband for achieving conception.

We understand that even though the insemination may be repeated as often as recommended by the doctor, there is no guarantee or assurance that pregnancy or a live birth will result.

We have also been told that the outcome of pregnancy may not be the same as those of the general pregnant population, for example in respect of abortion, multiple pregnancies, anomalies or complications of pregnancy or delivery.

FORM - F
Consent for Artificial Insemination or Intrauterine Insemination with Donor Semen
(See Rule 15.1)

We, _____ and _____, being husband and wife and both of legal age, authorize Dr. _____ to inseminate the wife artificially or intrauterine with semen / sperm of a donor (ART bank's no. _____; obtained from _____ ART bank with valid registration no.) for achieving conception.

We understand that even though the insemination may be repeated as often as recommended by the doctor, there is no guarantee or assurance that pregnancy or a live birth will result.

FORM - L
Consent Form for the Donor of Sperm
(See Rule 15.1)

I, Mr. _____ consent to donate my sperm to couples / individuals who are unable to have a child by other means.

I have had a full discussion with Dr. _____ (name and address of the clinician) on _____.

I have been counselled by _____ (name and address of independent counsellor) on _____.

I understand that there will be no direct or indirect contact between the recipient, and me, and my personal identity will not be disclosed to the recipient or to the child born through the use of my gamete.

I understand that I shall have no rights whatsoever on the resulting offspring and vice versa.

(If applicable) My wife has agreed to the donation of my sperm. (Strike off if not applicable.)

Endorsement by the ART bank

FORM - M
Information on Semen Donor
(See Rule 15.1)

Date of filling the form:

BASIC INFORMATION:

1. Identification number (Donor ID)
2. Age / Date of birth
3. Marital status
4. Education :
a. Donor
b. Spouse
5. Occupation :
a. Donor
b. Spouse
6. Monthly income
7. Religion

HISTORY:

FORM - R

Contract between the ART bank and the Semen Donor
(See Rule 15.1)

The ART bank and the Donor agree to come into this contract today on the _____ day of _____ month, _____ (year), in _____ as per the following conditions.

First Part being _____ ART bank, having its office at _____, and the registered office at _____, herein referred to as the Bank (which expression shall, unless repugnant to the context or meaning thereof, be deemed to mean and include legal representatives, administrators, etc., of the said Bank);

And

Second Part being Mr. _____ aged _____ residing at _____, herein referred to as the Donor (which expression shall, unless repugnant to the context or meaning thereof, be deemed to mean and include legal representatives, administrators, etc., of the said Clinic)

Whereas

FORM - S

Contract between the ART bank and the Patient
(See Rule 15.1)

The ART bank and the Patient agree to come into this contract today on the _____ day of _____ month, 2008, in _____ as per the following conditions.

First Part being _____ ART bank, having its office at _____, and the registered office at _____, hereinafter referred to as the Bank (which expression shall, unless repugnant to the context or meaning thereof, be deemed to mean and include legal representatives, administrators, etc. of the said Bank);

And

Second Part being Shri/Kum./Smt. _____ aged _____, residing at _____, and Shri / Kum. / Smt. _____ aged _____, residing at _____

*, herein referred to as the Patient (which expression shall, unless repugnant to the context or meaning thereof, be deemed to mean and include legal representatives, administrators, etc., of the said Patient.

FORM - T

Contract between the ART bank and the ART Clinic
(See Rule 15.1)

The ART bank and the ART Clinic agree to come into this contract today on the _____ day of _____ month, 2008, in _____ as per the following conditions.

First Part being _____ ART bank, having its office at _____, and the registered office at _____, herein referred to as the Bank (which expression shall unless repugnant to the context or meaning thereof, be deemed to mean and include legal representatives, administrators, etc., of the said Bank);

And

Second Part being _____ ART clinic having its clinic at _____, and the registered office at _____, herein referred to as the Clinic (which expression shall, unless repugnant to the context or meaning thereof, be deemed to mean and include legal representatives, administrators, etc., of the said Clinic)

Form - V
Oath of Secrecy
(See rule 15.1)

I, _____, Chairman/Member of _____, or a member of the staff of ART clinic/ART bank, with registration no. _____, hereby declare on oath that I shall not disclose any information pertaining to a patient (seeker of gamete donation, or of a surrogate mother) or to a gamete donor or surrogate mother, to which I may have access, to anyone at any time, without the permission of the patient, donor or the surrogate mother, as appropriate, except when asked by a Court of Law to do so. I understand that I will be liable to prosecution if I violate the above declaration.

Date: _____ Signature
Place: _____ (Name)

Address:

Signature of witness 1 _____ Signature of witness 2 _____
(Names, addresses, and telephone numbers of the witnesses)

Sample consent forms for IVF – Available on ICMR website

4.8 Consent Form for the Donor of Eggs

I Ms. _____ consent to donate my eggs to couples who are unable to have a child by other means.

I have had a full discussion with Dr. _____ (name and address of the clinician) on _____.

I have been counselled by _____ (name and address of independent counsellor) on _____.

I understand that there will be no direct or indirect contact between me and the recipient, and my personal identity will not be disclosed to the recipient or to the child born through the use of my gamete.

I understand that I shall have no rights whatsoever on the resulting offspring and vice versa.

I understand that the method of treatment may include:

- Stimulating my ovaries for multifollicular development.
- The recovery of one or more of my eggs under ultrasound-guidance or by laparoscopy under sedation or general anesthesia.

- National Board shall consist of a Chair person and three full time & 21 part time member to be appointment by central government, Qualification and experience of each member has been specified in the act.
- This board shall advice central government on policy matters relating to ART, Lay down code of conduct to be observed by person working at ART clinics, set minimum standards of infrastructure equipment and expert man power in ART clinics.
- The board will supervise functional of National Registry & state board.

The National Registry

- Shall register all ART clinics and banks in India and issue unique registration number to clinics & bank.
- National registry shall have a power to inspect any ART clinics & bank without prior intimation.

Establishment of State Boards

- There is provision of establishing state boards in every state and union territory.
- Every board will have one chairperson, two full time member and eleven part time members(at least three women) to be appointment by state government
- State board will follow the policy of national board for ART clinics and banks in the state.
- It will receive application for registration with national registry from ART clinics & banks, verify same and forward to national registry

Registration of ART clinics

- Under this act any ART clinics & bank can not practice any aspect of ART without valid registration with national registry.
- No application for registration can be rejected without giving opportunity to the applicant to be heard.
- There is provision of renewal of registration every five year & provision for cancelation registration.

Duties of ART clinics & Banks

- Some changes have been recommended in this bill as per ICMR guidelines 2010.
- (a) ART services can be given to women between age 18 to 45 years
- (b) Similarly Art services given to men from 21 to 50 years.
- (c) ART registered bank only can provide semen and oocyte donor.
- (d) The assisted reproductive technology banks shall obtain semen from males between twenty one years of age and forty five years of age,

- (e) To obtain oocytes from females between twenty three years of age and thirty five years of age.
- (f) An assisted reproductive technology bank shall not supply the sperm of a single donor not more than in one commissioning couple and oocyte of a single donor to more than one commissioning couple.
- (g) An oocyte donor shall be an ever married woman having at least one live child of her own with a minimum age of three years and to donate oocytes only once in her life.

- (h) All unused oocytes shall be preserved by the assisted reproductive technology banks for use on the same recipient, or given for research to an organisations registered under this Act after seeking written consent from both the commissioning couple.
- (i) The gamete of a donor or embryo shall be stored for a period of not more than ten years and at the end of such period such embryo shall be allowed to perish or donated to an research organization registered under this Act for research.

- Other important recommendations:-
- A women shall not be treated with gametes or embryos derived from more than one men or women during any one treatment cycle.
- An assisted reproductive technology clinic shall never mix semen from two individuals for the procedures specified under this act.
- No woman shall act as surrogate for more than one successful live birth and with not less than two years interval between two deliveries.

- The sale, transfer or use of gametes, zygotes and embryos directly or indirectly to any party within and outside India is prohibited.
- The assisted reproductive technology clinic, or assisted reproductive technology bank or agent shall not issue, publish, distribute, communicate or caused to be issued, published, or distributed or communicated any advertisement in any manner including internet, regarding facilities of sex selective assisted reproductive technology

5. Desirable and Mandatory at an ART lab

World wide over 4 million children have been born as a result of an ART treatment and it is estimated that over 800,000 treatment cycle are taken annually.

So lab design is important

- IVF lab should be designed in such a way that the outcome of any procedure is optimal and not affected by environment parameter.

What is the standard

- Standard for IVF laboratories published by professional societies and accrediting agencies are mostly lacking, and those that exist are superficial at best
- ESHRE provide minimum guidelines for laboratory space and design

(Revised guidelines for good practice in IVF laboratories (2015). Santos et.al ,human reproduction jan 2016)

What we are going to discuss

Desirable and mandatory

A Building in laboratory

B Equipments in laboratory

C Media and disposable in laboratory

A GOOD LABORATORY IS EQUALLY IM-
PORTANT AS TO HAVE GOOD EQUIPMENTS

A – Building a lab

- Setting up a new LAB
is an ART

As the practice of **ART itself**

Never be build by architects –
without considerable input from
EMBRYOLOGIST

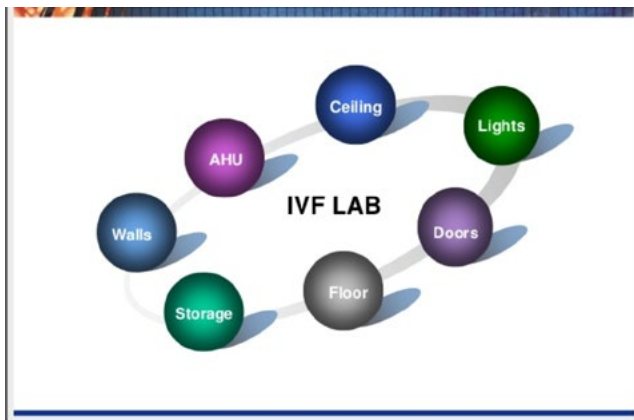
Elements of Good Lab Design

The five elements :

- Prithvi = Building
- Agni = Incubators
- Jal = Water
- Vayu = Lab air
- Aakash= Culture system

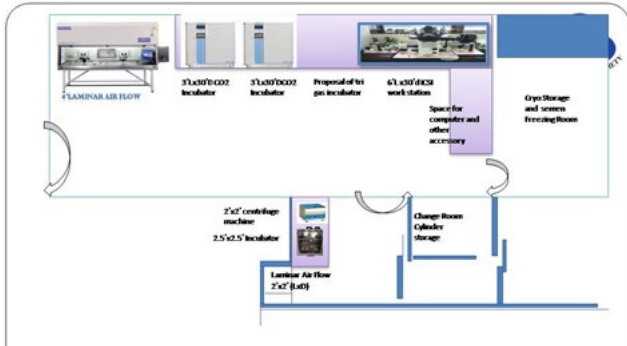
Building a lab-

- **Site**
- **Clean room**
- Air purification
- Air exchange
- **Room pressure**
- **Material**
- **Contamination**



- Laboratory Design
- Consideration of room layout and workflow
- Proximity of lab to other clinical areas (OR, transfer rooms)
- Dedicated space for andrology
- Location of equipment in the lab
- Collection rooms (2+)
- Cryostorage room
- Gas storage room
- Supply storage area
- Office space
- Practical considerations based on budget, space and workflow

Placements of equipments in IVF Lab



Actual photo as per the plan on paper



SITE OF LAB

Things to be taken into consideration—

- A Some building / building sites are intrinsically harmful to cell tissue culture system.(Hum-Reprod1997; 121998)
- It should always taken into consideration is the adjoining building is undergoing demolition/renovation /major changes.
- Working in an area adjacent to laundry , sterilizing /histology department may be avoided.

- B- prevalent wind direction, industrial hazards, and general pollution report such as ozone measurement should always be determined otherwise it may lead to phenomena called sick building syndrome
- C basic air sampling & determination of VOC concentration is necessary inside and outside of proposed building.
- This will determine which design requirements are needed to remove VOC

Lay out

- Floor to ceiling height should be 10 feet below the beam.
- AHU should be installed one floor above the lab (either in terrace or in utility room)
- The minimum OT dimension should be 14 into 14 feet
- The minimum culture room dimension should be 14 into 10 feet without any pillars

Wall & ceiling

- Minimum of penetration
- Solid ceiling- clean room typically use tiled ceiling with strip sealing to make them air tight. Clean room tiles are non-shedding and can be sealed.
- Avoid false ceiling
- Air tight utility connection
- Door will require seals and sweeps and should be lockable.

Light

- Light leads to increase reactive oxidative species that leads to apoptosis in blato cyst.
- **Best practice is ---Dim light in lab and close to all day light.**
- UV lamps had been used in American hospitals for reducing the number of air born bacteria .
- Special attention is given to UV-C(100-280nm) which is highly efficient as germicide and for improving indoor air quality. (scheir and Fencil 1996)

Detrimental Effect of Visible Light on Meiosis of Mammalian Eggs in Vitro

Y. HIRAO AND R. YANAGIMACHI
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ABSTRACT Short wavelength visible light (<470-480 nm) emitted from ordinary light sources is detrimental to unfertilized hamster eggs in that prolonged exposure to the light disturbs the completion of normal meiosis after the eggs are penetrated by spermatozoa. The fluorescent light commonly used in modern laboratories is more harmful than the light from incandescent lamps. In experiments involving the handling of eggs in vitro, minimal exposure to the light or the use of appropriate filters (e.g., red cellophane sheets) is recommended.

TABLE 2
Effects of light intensity^a during embryo manipulation on preimplantation development of hamster embryos cultured in vitro.

Light strength (lux)	No. of replications (treated hamsters)	Total no. of 2-cell embryos retrieved	No. (%) of embryos developed to		
			4-cell	Morula	Blastocyst
200	6	110	109 (99)	106 (96) ^b	68 (62) ^c
500	6	101	100 (99)	95 (94) ^b	51 (50) ^{b,c}
900	6	114	111 (97)	96 (84) ^c	44 (39) ^c

Note: Model treatment effect in the development to the 4-cell, morula and blastocyst stages, which was indicated as P value, was .5022, .0026, and .0022, respectively.
^aLight intensity derived from electric bulb of stereo microscope during embryo manipulation in dark room was measured by illuminometer.
^{b,c} Different superscripts within the same parameter were significantly different; P< .05.

- Precaution should be taken that overall illumination intensity should never be more than 50 lux during normal time , while operating being 30 lux

Floor

- Floor covering using seamless sheet vinyl base flushed at least 4 inch up the wall were selected for there low environment impact.
- Flooring should also be flushed to the base of cabinet in lab.
- Polyurethane/epoxy-nitrous oxide/vinyl can be used.
- Furniture-avoid wood, stainless steel furniture and work bench

Paint

- All interior painting throughout the facility should be done on prepared surface with water based paint.(least voc should be emission tested).
- Water based
- Low -volatile
- Acrylic/vinyl acrylic/alkyd/acrylic latex polymers
- NO TO—
- Formaldehyde. Acetaldehyde, isocynate , reactive amines, phenols, and water soluble volatile organics.

- Adhesive glues, sealants, caulking material should never be used
- Silicon material are preferred particularly for ceiling and caulking work.²¹
- Epoxy enamel has low reflection level (5-10%) making it suitable for use in IVF lab

Protective measure in lab

- Strict adherence to staff hygiene.
- Protective laboratory clothing & hair nets
- Non powered gloves and mask
- Food, gum, drinks and tobacco strictly prohibited
- Cosmetic, nail polish, should be minimized and perfumes should be avoided

Two common design

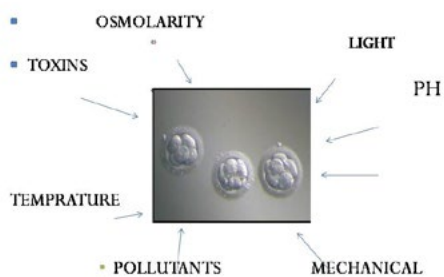
- **1 Program me with enough space**
- There may be a full size door which connects the two room and allow a circulating technician to carry follicular aspirates directly into lab & embryologist to carry transfer Cather.
- **2 Programmed with limited space** have a pass through window to transport tubes from OR to lab same for embryo Cather.

- Air pressure should move from embryology laboratory – andrology laboratory—hallway.
- Air pressure should move from embryology laboratory – OPU room to hallway.
- Controlling air quality in ART laboratory has shown beneficial effect regarding fertilization and embryo development (Boone et al.1999)

Clean Room

- **ONE OF THE MOST IMPORTANT AND INVISIBLE FACTOR INFLUNCEING THE SUCCESS RATE IS QUALITY OF AIR.**

Minimize In-vitro stress



Do we have data to support

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Control of air quality in an assisted reproductive technology laboratory

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Greenville Hospital System, Greenville, South Carolina

Main Outcome Measure(s): Particle counts (sizes 0.3, 0.5, 1.0, and 5.0 µm); IVF rates; and embryo quality (stage and grade).

Result(s): Clinical pregnancy rates decreased from 35% in 1993 to 16% in 1994 (numerous construction odors were detected during 1994) and increased steadily after the cleanroom was built (rates for 1995–1997 were 20%, 32%, and 59%, respectively). Fertilization rates decreased between 1993 (74%) and 1994 (60%) and then steadily increased after cleanroom installation (62% in 1995, 71% in 1996, and 69% in 1997). The proportion of embryos past the four-cell stage decreased from 66% in 1993 to 61% in 1994 but then increased steadily in the years after the cleanroom was built (78%, 77%, and 83% in 1995, 1996, and 1997, respectively). During the same 5-year period, there were no differences in embryo quality or number of embryos transferred.

Conclusion(s): Construction of a Class 100 cleanroom improved air quality and IVF rate and increased the number of embryos past the four-cell stage available for transfer. (Fertil Steril® 1999;71:150–4. ©1998 by American Society for Reproductive Medicine.)

J Assist Reprod Genet (2013) 30:69–76
DOI 10.1007/s10815-012-9906-1

ASSISTED REPRODUCTION TECHNOLOGIES

Better IVF outcomes following improvements in laboratory air quality

Rabea Youcef Khoudja · Yanwen Xu · Tao Li ·
Canquan Zhou



What is ideal

- **Over pressured** (0.10 – 0.20 inches of water) + **7-15 air changes per hour.**
- 100% outside air
- Lab temperature 22 to 24 degrees
- Humidity <40%
- Layout of equipment
- Space air outlets to **avoid drafts**

- 100% outside air sourcing will maximize the life of chemical filter and will provide lower concentration of VOC **BUT**
- IN climates where temperature routinely exceeds 32degree and 85% relative humidity
- 100% outside air will lead to unacceptable level of humidity > 60% which could allow mould growth.
- So limited number of return air from lab is acceptable then
- 50% of outside air and 15-30 total air changes per hour.

Air handling unit

- Must not use an open plenum design
- IDEAL CASE—
- 100% outside air with chemical and physical filtration should be used with sealed supply and return duct.
- Duct and equipment should be laid in such a way that routine and emergency maintenance and repair work can be performed outside the laboratory with minimal disruption to the laboratory.

Clean room classification

Grade	AT REST (B)		IN OPERATION	
	0.5 Um	5 Um	0.5 Um	5 Um
A	3500	0	3500	0
B (a)	3500	0	350 000	2000
C (a)	350 000	2000	3 500 000	20000
D (a)	3 500 000	20 000	not defined (c)	not defined (c)

Temperature

- All lab equipment is designed to operate at room temperature usually between 20-22 degree Celsius.
- Embryologist are happy with high room temperature, it prevents the embryo cooling during transport. But high temperature will provide perfect environment for growth of microbes and contaminants.
- A laboratory without temperature control cant be accredited.

- Transient cooling to room temperature can cause irreversible of meiotic spindle in human oocyte. Pickering et al.(Fertil Steril 54,102-108)
- Limited recovery of meiotic spindles in live human oocytes after cooling rearming observed using polarized microscope. Wang et al (Hum-Rep 16,2374-2378)

Humidity

- Completely controlled according to climate and seasonal variation.
- AHU must be capable of supplying the space with air temperature 30-35 degree and humidity <40%.

BURING IN

- In new facility the--
- Temperature is increased by 10-20% and ventilation rate is increased
- THIS AIDS IN REMOVAL OF VOLATILE ORGANICS.

- SO BURN-IN THE NEW CONSTRUCTED AREA.
- Period can be from 10-28 days lab should be closed during this period.

Maintenance planning and sterilization

- Heating , ventilation and air conditioning(H-VAC)—require filter changes, coil cleaning, replacement of drive belt and chemical purification media
- These require inspection and periodic changes.
- USE OF COLD STERLIZING AGENT IS NOT REQUIRED.

- Sabouraud dextrose agar (SDA-for detection of fungus)
- Trypticase soya agar(tsa -for detection of bacteria)
- Acceptable limits are zero colonies inside the flow hood /handling chambers and < 10 colonies outside the hood in laboratory.

EQUIPEMENT-- DESIRABLE AND MANDATORY

LAB REQUIRES

- Two Workstation—
- 1 EMBRYOLOGY Workstation
- 2 ANDROLOGY Workstation
- &
- CRYOPRESERVATION AND STORAGE AREA—should be located outside but close to the lab for safety reason and visible access to interior

- Laminar flow hoods and micromanipulations workstation should not be located too close to air supply fixtures to avoid disruption of sterile field and minimize cooling on microscope stage.

Placement of equipment

- Appropriate placement of equipment ensures safety and comfort in the over pressurized IVF laboratory.
- **IDEALLY** – An embryologist should be able to finish one complete procedure without moving more than three meters in any direction ; not only is this efficient , but also it minimizes accidents in busy laboratory.





Mandatory equipments -1

- **1 CO2 incubators** — Provides humidity, temperature and PH for early human life to start and grow up to blast cyst.
- **Bench top incubator (triple gas)** – It is mandatory if blast cyst culture being done.
- **2 integrated laminar air flow workstation** with thermostatically controlled heating plate.— clean and sterile environment for handling sperm , oocytes and embryo.

Mandatory equipments -2

- **5 Suction pump-** to provide a smooth, low volume vacuum at a predetermined negative pressure for ovum pickup.
- **6 Heating blocks**
- **7 Air cleaner**—it prevent all the harmful gases, odor and toxic material, germs present in room.
- **8 Pressurizing module**—fixed outside the culture room with sterile air and replaces the room air .A positive pressure is build in the room and does not allow outside air into culture room

Integrated laminar flow work station



Incubator - numbers

- In a small size IVF lab there should be at least two incubator
- >500 cases per year may have half a dozen of incubators
- >2000 cases per year may have two dozen of incubator.
- Large laboratory needs two micromanipulators'



Large Box

Slow temperature and CO₂ recovery

Large volume

Temperature stratifications



Benchtop

Quick recovery of CO2 and Temperature

Smaller footprint

Control and consistency of temperature

Mandatory equipments -1

- The result of systemic review and met analysis suggest that culturing embryo at low oxygen concentration improves the success rate (Hum Repro vol 19 2013)

Low Oxygen & Embryo Culture

- O2 levels in the female reproductive tract are $\leq 8\%$
- Atmospheric O2~21%
- High O2 may result in increased oxidative stress apoptosis, membrane damage, DNA damage, altered gene expression & epigenetic
- Low O2 improves embryo metabolism, low o2 may improve air quality
- Filtered N2 instead of air



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- Dumoulin et al. 1999 Hum Reprod 14:464-469
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- Guoet al. 2014 IntJ ClinExpPath.7(9):6191-8
- KastersteinE. 2013 J AsstReprodGenet 30(8):1073-9

Desirable – Inline filters for air quality control in incubators





Gas cylinder manifold should be outside the laboratory
 Automatic switch over with back up cylinder should be present
 Colour coded tubings for different gases
 Preferably stainless steel tubing

Microscope and visualization of cell

- **Mandatory—**
- Dissecting microscope as well as inverted microscope.
- Still camera and/video camera & monitor
- Interference optics such Hoffmans and Normarski are preferable because they permit best measure of detail and depth.

Trinocular microscope integrated with heated stage



Inverted microscope



Microscope and visualization of cell

- **Desirable**
- Visualisation of internal elements such as spindles using polarised microscopy
- Time lapse

Desirable equipments in

- Embryology lab—
- Bench top incubator- if not doing blastocyst culture
- Laser for PGD and Embryo biopsy
- Co2 analyses
- IMSI ,Poloscope, Metabolomics

Mandatory at andrology workstation

1. Centrifuge machine—separate spermatozoa from semen sample
2. Makler counting chamber-to get a reliable reading in million /ml
3. Compound microscope with 10X and 40X objectives
4. Small size laminar air flow—for ideal work station.
5. Test tube warmers-

Andrology Lab – Lamina



Andrology Lab – Lamina flow hood , connecting air tight window with Semen collection room, Compound microscope, heating block for test-tubes, Cryostorage for sperm samples

Andrology Lab – Dry incubator, Centrifuge



Desirable in andrology lab

- **DNA fragmentation test**

Mandatory at storage area

- **cryopreservation container , liquid nitrogen's, storage dowers, and refrigerators.**



Cryopreservation

1. Nitrogen gas can be provided in at least three ways;
2. 1 compressed N₂ in cylinders, from a nitrogen generator that fills a compressed tank
3. 2 from the vapour phase of a liquid nitrogen tank
4. A nitrogen generator is an effective alternative but the units are loud are relatively large and require maintenance.



Liquid nitrogen alarm system







Protective gear while working with liquid nitrogen to avoid burns

Liquid Nitrogen Burns



1. Mandatory-
2. Appropriate stock management system of media ,oil and consumables, including the batch number date of entry and expiry should be available.

C-Consumables and media

1. Mandatory—
2. Sterile single use disposable consumables should be used.
3. Reagents, media and consumables should always be used prior to expiry date.
4. Size of bottles and other packing must be appropriate to minimize opening and time between first and last case.
5. repeated shifts of temperature should be avoided while handling in laboratory.

IVF culture Media

Past
 Individual clinics manufactured
 Non defined ingredients used
 Variable across labs
 Use of cell culture media not developed for embryos

Present
 Comercially manufactured
 Defined ingredients e.g. HAS, Dipeptide Glutamine
 Quality controlled
 Sterility
 MEA tested
 Endotoxin tested
 Additional supplements
 Vitamins , growth factors
 Sequential and single step

Emergency plan

1. There may be exceptional failure of infrastructure and facilities , either of human or natural origin—
2. Aims-
3. 1 protection of all fresh and cry preserved human material.
4. 2limitation of damage to equipment and medical records.

Emergency measure

1. Communication measure should be clear for all personal
2. In case of loss of electricity there should be UPS
3. In case of failure of automatic supply lines of liquid nitrogen-facility for manual of filling of tank should be there.
4. Medical records in a secure Web –server.
5. **THIRD PARTY ARRANGEMENT SHOULD BE IN PLACE WITH ANOTHER IVF LABARATORY FOR EMERGENCY TRANSFER OF GAMETB AND EMBRYO.**

Liquid Nitrogen Burns

- No place for paper
- Not the place for card board boxes
- Not the storage room for disposables
- Not a storage room for old lab equipments

General cleanliness

- 70% alcohol with sterile water or
- Disinfectant Cleaning Solution from Bioguard IVF Certified, No VOC and long lasting impressions
- Steam cleaners for cleaning floors.



VARDHMAN MEDICARE PVT. LTD

Plan in advance

- Supply and evacuation routes should be planned in advance.
- Route of delivery of liquid nitrogen and other gas cylinder should be relatively easy and without staircase between the lab and delivery track.
- Storage area should be different—these sterile disposable item, release multiple compounds for prolonged period, gassing has been determined to be a major cause of VOC where they are stored inside lab.

Electrical system (back up plan)

- All the critical equipments should have their own uninterrupted power supply (UPS).
- And there should be a long term power back up in case of an emergency.

Keep in mind

- That resisting the urge to cut corners in wrong places avoid future headaches and position you and your patients on path to success.

Alarm system

- System provides prompt notification when a device fails it critical for IVF lab.
- 1 sense phone call out device
- 2 computerized system
- 3 wireless technology

IVF lab of future

- Systems to mimic reproductive tract-
- 1 Micro fluids – technology of handling small volume of liquid LAB ON A CHIP
- 2 Application in co-culture(Mizuno 2007)
- Sperm sorting, fertilization (Suh 2006)

Invasive genetic analysis has evolved with IVF techniques

- Euploid selection (PGS) not fulfilled exceptions.
- Global view-array CGH, SNP, New generation sequencing
- RCT underway– promising (Yang 2011)
- Search for non invasive molecular techniques—
- DNA array-cumulus cells (Assou 2008), Follicular fluid Hamel2010,

Future development in IVF lab

- 1 Automation of ICSI– auto injection, use of micro robotics
- RICS-720 per second with an accuracy of -24, immobilization of sperm in 6-7 sec.
- Call for NEW GENERATION MEDIA with an bio active factors.
- Robot assisted oocyte retrieval--

Take home message

- Automation and standardization will allow each step of IVF process to be optimized resulting in good standard of care and an over all increase baby rate.

6. Trouble Shooting at an ART clinic

An ART laboratory

- An ART laboratory consists of multiple procedures which are closely correlated to each other.
- The success of an ART laboratory is highly dependent on the control of these procedures.
- In order to have accurate control on these complex procedures, it is important to have a total quality management system in the ART laboratory to improve the final outcomes.

Troubleshooting

- Troubleshooting is a systematic approach to solve a problem quickly and efficiently.
- Operator skill and culture conditions are epicenter to the IVF laboratories' performance.

Factors Affecting the IVF Lab Process

Recognize ALL sources of influence:

- Patient-derived : biology (age, ovarian rometrial thickness, hydrosalpinges, end etc.)
- Clinical: stimulation / OPU / ET / luteal support
- Environmental (macro): lab construction, air, etc
- Environmental (micro): temperature, pCO₂ / pH, etc
- Equipment: calibration, malfunction, etc
- Materials: suitability, lack of toxicity, manufacturing QC, etc
- Methodology: suitability, choice, correct SOP, etc
- Staff: training, skill, competence
- Errors

Proper Documentation & Record Keeping

- Proper documentation is the key to troubleshooting.
- In the absence of proper documentation, identification of the exact cause becomes difficult.
- Corrective measures can only be implemented once the causative factor is identified.
- Unfortunately it may not be a single but multiple factors contributing viz. clinical, procedural, patient factors, operator and culture conditions

- A well-written standard operating procedure (SOP) is mandatory for all procedures.
- A protocol for management of adverse events should also be in place.
- Every lab should determine its own Key Performance Indicators (KPIs), and their critical levels.
- Internal quality control (IQC) and external quality assurance (EQA) programs, either commercial or in collaboration with other laboratories, are highly recommended.

KPIs

The following need to be continuously monitored and evaluated:

- Oocyte retrieval rate
- Fertilization rate
- Cleavage rate
- Blastulation rate
- Cryosurvival in frozen embryo transfer (FET) cycles
- Embryo Utilization rates
- Implantation rates
- Pregnancy rates
- Clinical pregnancy rates
- On-going Pregnancy rates/ Live birth Rates
- Oocytes/Zygote/Embryo grades

Lab Factors (Affecting Embryogenesis)

These parameters are important to be controlled strictly in all the places, where embryos are exposed, even for a few seconds:

- Ambient Air
- Incubators
- Culture Media

Culture Medium

There can be issues with:

- Maintenance of cold chain
- Package integrity
- Storage temperature (ammonia levels)
- pH/ Osmolality changes
- Use of expired media/ bottles opened for long
- Non compliance with the manufacturer's instructions for use (for proper equilibration)

Challenge - 1

- **Failure to aspirate a follicle**
OR
- **Failure to create negative pressure**

Approach

- Ensure the functioning of the suction pump & power supply to it (should have an UPS backup)
- check the digital pressures displayed,
- confirm the needle is inside the follicle
- any break/interruption in the circuit should be looked for, e.g. cracked tubes, loose caps of the tubes or kinks and bends in the tubing.
- Slight rotation of the needle might be helpful
- a blocked needle can be a possibility (blood clot/tissue)
- The emergency button, present in some aspiration pumps, can be pressed, which generates sudden high negative pressures to dislodge any clot
- withdrawn and flush the needle.

Challenge - 2

- NO oocyte retrieved
OR
- Less than expected number of oocytes retrieved

Empty Follicle Syndrome (EFS)

- Reported incidence – 0.45-7 %
- **False EFS**
 - Faulty administration of the final oocyte maturation trigger in terms of dose, time, date or administration of the injection
 - Check hCG in –serum/urine/follicular fluid
 - Give trigger and plan OR after 36 hours
- New batch of urinary hCG with low biological activity
- incomplete aspiration of the follicles, too low suction pressures.
- With GnRH agonist trigger- check LH levels (? >15mIU/ml)

True/Genuine EFS

- failure to retrieve oocytes in spite of a correctly and timely administered trigger
- More common in conditions with dysfunctional folliculogenesis eg old age, poor ovarian response, poor ovarian reserve
- ? Specific genetic factors, mutations at LH/hCG receptor/ precocious atresia of the OCCs

Different strategies proposed to prevent EFS in the subsequent IVF cycles are:

- changing the batch of hCG
- using recombinant hCG,
- changing the protocol to antagonist with a GnRH agonist trigger
- administering a dual trigger with hCG and GnRH agonist
- increasing the interval between the trigger and oocyte retrieval

Challenge - 3

- Only immature oocytes retrieved !

Causes:

- Too early aspiration after the oocyte maturation trigger (<34 hours).
- Wrong timing of the trigger by patient.
- Overestimation of the size of the follicles on ultrasound before the trigger
- Genetic predisposition to partial and slow response to hCG (same etiology as of genuine EFS)

What can be done now?

- The immature oocytes to be followed for any IVM
- Prognosis ?

What can be done in next cycle?

- Avoiding all above mentioned iatrogenic causes
- Delayed aspiration (>36 hours) after the oocyte maturation trigger should be planned in the subsequent cycle.

Poor Oocyte Quality

The reasons can be - Intrinsic or Extrinsic

- Intrinsic Factors :
 - higher age, poor ovarian reserve, long-standing unexplained infertility, PCOS and endometriosis.
 - Environmental changes/ psychological stress
- Extrinsic Factors:
 - A particular batch of urinary human menopausal gonadotropin (hMG) or hCG might have higher protein impurities or lower bioavailability, thus resulting in poor quality oocytes.
 - Repeated problem in a few patients, especially during a batch IVF, should alert us toward a drug-related issue.
- Poor oocyte quality may indicate chromosomally abnormal oocytes
- May be associated with low fertilization rates, poor embryo grades, lower IR and PR, and higher miscarriage rates.

Challenge - 4

Husband unable to produce semen on the day of pickup

- This can be due to unfriendly/new surroundings with the stress of the treatment cycle.
- Ideally all couples should have a frozen semen sample for backup

Approach

Approach:

- Reassurance, try again after 30-40 min.
- Sildenafil
- ? Home collection (should be recorded and signed by both partners)
- Penile vibratory stimulation (PVS), Prostate massage
- Electroejaculation - usually reserved for spinal cord injury cases where even PVS fails, as it requires general anesthesia, special equipment and expertise.
- Surgical sperm retrieval (PESA / TESA/ TESE)
- Oocyte vitrification. (best results when vitrified within 1-2 hours of retrieval, so timely decision must)

Challenge - 5

- Non availability of the embryologist at the time of oocyte retrieval !!
- Till when can Insemination/ ICSI be done after oocyte retrieval?

Timing of ICSI

Outcome	< 38 h post-hCG	≥ 38 h post-hCG	P
Spindle imaged	61.6%	78.1 – 81.5%	<0.001
Fertilized	62.6%	70.4%	0.035

Cohen et al. (2004) Hum. Reprod., 19: 649.

PROTOCOL	A	B	C
Stripping	Immediate	Delayed 4h	Immediate
Injection	Immediate	Promptly	Delayed 4h
Fertilization	43.4%	72.5%	<0.05
Blastocysts	16.7%	42.9%	18.8%
Pregnant	8.7%	26.2%	11.8%

Masnan (2001) J. Assist. Reprod. Genet., 18: 539.

- ICSI performed before 38 hours and after 41 hours of the hCG trigger results in poorer fertilization, blastulation and pregnancy rates.
- OCCs should be incubated atleast for 3 hours after aspiration for completion of the cytoplasmic maturation of the oocyte.

Challenge - 6

- Total Fertilization Failure (TFF)
 - Occurs in 5-10% of IVF cycles (usually sperm defects)
 - And in 2-4% of ICSI cycles (usually problem in oocyte activation)
- Role of rescue ICSI- ?
 - Which sperm to use (day 0 or ask for fresh)?
 - FR poorer than fresh oocytes (28-60%) with few live births also reported.
 - The poor outcome is due to aged oocyte, poor embryo quality, chances of cytogenetic abnormality, ? Asynchrony with endometrium, but still it can be performed as the last ray of hope in case of failed fertilization.
- Early check after 4-5hours of ICSI, for calcium ionophore oocyte activation on same day, rather than next day

TFF

- In poor or TFF with IVF, the factors contributing can be:
- Semen parameters (count, motility and morphology) or contaminated sample
 - Sperm processing method and final insemination count.
 - Sperm holding post preparation – time, media, temp.
 - Thick zona pellucida and immature oocyte , poor responders (probably having incomplete cytoplasmic maturation of the oocytes)
 - Too early insemination after oocyte retrieval (short incubation, incomplete cytoplasmic maturation)

TFF

- The inseminating technique—too low sperm count and more oocytes,
- Even faulty ICSI technique- too gentle preventing oocyte activation, too harsh damaging the oocyte, or poor sperm selection, too long exposure
- Suboptimal culture conditions - frequent openings of the incubator doors and contamination of the culture media or oil.
- Temperature of the heated stage
- Fertilization media/PVP/Hyase/dishes/pipettes/tubes
- Observation window correct ?

TFF

What to do in next cycle ; if semen parameters normal;
eg. IVF for tubal factor with TFF?

Next cycle - ?

Ans – IVF (80% will have normal FR in next cycle)

Or practically – IVF in half oocytes and ICSI in the other half

Indications for ICSI after IVF:

- No/poor FR in the first IVF cycle when TMC < 10 million
- No/poor FR in two IVF cycles when TMC > 10 million

TFF

- Persistent TFF after ICSI; what next ?
- Role of Artificial oocyte activation ?
- Role of sperm DNA fragmentation ?
- Gamete donation ?
- ICSI with Half partner's + half donor sperms
- If still poor FR, then – oocyte activation/ donor oocyte (cytoskeletal abnormalities)

Challenge - 7

- Poor quality/ fragmented Embryos
- Low cleavage rates/ Arrest/Slow cleavage
- Embryo quality is a strong determining factor for the success rates of IVF programs.

Fragmentation

- Fragmentations of variable degrees are observed in about 75% of human embryos produced in vitro (also seen in in-vivo embryos).
- Fragmentation is associated with higher chances of chromosomal abnormalities.
- With increasing Fragmentation:
 - Embryo viability is severely compromised
 - The early miscarriage rate increases
 - The live birth rate decreases

In vitro culture conditions affect embryo quality.

- In vitro culture conditions that can affect embryo quality are
- inadequate media and oil equilibration and media pH,
- frequent incubator openings, inadequate water level in the incubator
- the gas in the incubator should be filtered by attaching an inline filter.
- poor laboratory air quality with increased VOC.
- Improper /No off gassing of the consumables
- To prevent drastic change in pH and temperature:
 - Media buffered with HCO₃ / HEPES/ MOPS
 - Oil overlay preferred over open cultures.
 - Overexposure of the embryos should be prevented.

So here comes the role of QC & QA.

- Maintaining a log book with daily entries of the temperature, CO₂, functioning of the apparatus eg LAF, heating blocks, stages, etc is must.
- Timely calibration of the equipments is crucial.

Preparation of dishes , if improper, implications

- Poor quality embryos if dishes not prepared properly:
- poor FR/ fragmentation/ arrest, etc.
- Should be prepared aseptically
- Run the laminar flow before dish preparation, turn off while preparing the dishes.
- Do not prepare the dishes with drops at 37 degree (to avoid evaporation)
- Culture media should be checked for expiry dates/ any precipitation/contamination

How to prevent mixups (Challenge – 8)

- Embryology Lab – zero tolerance for errors.
- Proper labelling of the semen containers, culture dishes.
- Immediately discarding the pipettes used.
- Strict segregation of work areas, not more than one specimen at a time at any one work area.
- Confirmation of patient identity at every step, before any procedure.
- Double witnessing at every step.
- Documentation of any spillage/ doubt/mixing
- ? Informing/counselling the couple in that case ??

Challenge - 9

On the next day of Insemination (Day 1)- Dish found to be contaminated , what next?

- Sources of contamination? – semen/vagina;follicular fluid/media/oil/consumables
- How to proceed?
- ET to be cancelled to prevent any intrauterine infection, and other unknown consequences.
- Microbiological e/m and cultures of the contaminated culture media along with the inseminate/ high vaginal or cervical swabs.

- **Precautions in next cycle:**

- Treat any infection found in the semen or vagina (usually the contamination is by commensals)
- Semen preparation – density gradient f/b swim up
- Stringent culture conditions in IVF lab
- Extensive washing of OCCs before culture
- Appropriate antibiotics in culture media
- Proceed with ICSI

Troubleshooting in cryopreservation (Challenge – 10)

- Two most important issues are:
 - Poor post thaw embryo survival rate
 - Poor pregnancy rates in FET cycles

Factors affecting the FET cycles

- Prefreezing Embryo selection critical
- Experience of the embryologist
- Timing of exposure to the cryoprotectants
- Equilibration of the freeze and warming media and temperature at which they are used become crucial.
- Following the manufacturer's instructions.
- Loading volume

Challenge - 11

- Not able to negotiate the internal os for Embryo Transfer. How to proceed?
- Embryo transfer should always be USG guided, with partially full bladder.
- A prior information of any D&C/IUI/ET/Hysteroscopy done in past.
- Better to do mock ET in the previous cycle to have an idea of any difficulty, acute ante/retroversion, any false passage, cervical stenosis.

Difficult ET

If stuck during the actual ET:

- Tilt the Cusco speculum to change alignment
- Use of ET catheters with echogenic tip is better
- Try ET catheters with stylet
- Holding the anterior lip of cervix and straightening the uterocervical axis (caution - can induce uterine peristalsis)
- Uterine sounding (just beyond the internal os)
- Freezing all embryos followed by a hysteroscopy if not done earlier.
- ? Transmyometrial ET for cervical stenosis

Challenge - 12

- Poor pregnancy rates inspite of good embryos (Repeated Implantation Failure)

Repeated implantation failure can be due to multiple factors

- Embryonic – Blastocyst culture, LAH, PGS, change of gamete
- Uterine cavity issues – Hysteroscopy/3D USG
 - Septum, polyps, fibroids, synaechiae
- Endometrial – ERA (Displaced WOI), Endometrial scratch
 -
- Fibrosis in Endometrium – many drugs available with conflicting evidence; aspirin, Vit E, Pentoxifylline, Sildenafil, G-CSF, PRP, stem cell therapy

Repeated implantation failure can be due to multiple factors

- Hydrosalpinges – salpingectomy
- Thrombophilia, Immunological factors
- Seminal issues – DFI (can be normal even with normal semen parameters), Testicular sperm retrieval
- Embryo transfer technique – well trained clinician
- Of special relevance in anatomical defects like false passage, acute ante/retroversion, cervical stenosis

Conclusions

- Troubleshooting in an IVF lab is a systematic approach to the apparent problem.
- It is not always possible to find a cause for the problem faced, moreover sometimes the cause is multifactorial.
- Proper documentation, quality control and quality assurance can prevent most of these challenges.
- Troubleshooting is a team effort where not only the clinician, but also the embryologist, the lab technician, the nursing staff and the counsellor have an equally important role to play.



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