

# **Chapter 1**

## **Introduction, Brief History of ART and Requirements of ART Clinics**

## 1 Introduction

Infertility, though not life threatening, causes intense mental agony and trauma that can only be best described by infertile couples themselves. There are no detailed figures of the extent of infertility prevalent in India but a multinational study carried out by WHO (Diagnosis and treatment of infertility, ed. P. Rowe and E. M. Vikhlyeva, 1988) that included India, places the incidence of infertility between 10 and 15%. Out of a population of 1000 million Indians, an estimated 25% (250 million individuals) may be conservatively estimated to be attempting parenthood at any given time; by extrapolating the WHO estimate, approximately 13 to 19 million couples are likely to be infertile in the country at any given time.

Prevention and appropriate treatment of infertility has been included in the ICPD (International Conference on Population and Development) Programme of Action; it follows that alleviation of infertility should be included as a component of the primary health care system. Most types of infertility such as reproductive tract infections (RTI) and genital tuberculosis, are preventable and amenable to treatment. About 8% of infertile couples, however, need serious medical intervention involving the use of advanced ART (Assisted Reproductive Technologies) procedures such as IVF (*In vitro* Fertilization) or ICSI (Intracytoplasmic Sperm Injection). Such advanced treatment is expensive and not easily affordable to the majority of Indians. Further, the successful practice of ART requires considerable technical expertise and expensive infrastructure. Moreover, the success rate of any ART procedure is below 30% under the best of circumstances. Infertility, specially in our country, also has far-reaching societal implications. Therefore, with the rapidly increasing use of ART in our country, it has become imperative to ensure their safety and have safeguards against their possible misuse.

Scientific societies around the world, such as the ASRM, ESHRE and IFFS, have drawn up guidelines for the safe and ethical practice of ART. The European Union and the Governments of several countries such as Australia, the UK and the USA have taken steps to accredit and supervise the performance of infertility clinics.

At present here are neither guidelines nor a legislation in regard to the practice of ART in India. This document aims to fill this lacuna. It has been prepared after extensive consultations held at both the ICMR and other national institutions, with scientists, medical practitioners, lawyers, social scientists and activists.

The present guidelines are meant to ensure that ART clinics in India are accredited, regulated and supervised to assure the patients as well as the public that our ART clinics offer services that are at par with those available anywhere in the world. Medical malpractice now comes under the purview of the legal redressal machinery of the country; this makes it all the more necessary to have national guidelines for the practice of ART.

## **1.1 Brief History of IVF in India**

The world's first IVF baby, Louise Brown, was born on July 25, 1978, in the UK through the efforts of Dr. Robert G Edwards and Dr. Patrick Steptoe. The world's second and India's first IVF baby, Kanupriya, alias Durga, was born 67 days later on October 3, 1978, through the efforts of Dr. Subhas Mukherjee and his two colleagues in Kolkata.

Dr. Mukherjee and his colleagues published a short note on their above work, in the Indian Journal of Cryogenics (Vol. 3: page 80, 1978). The techniques used by Mukherjee were markedly different from those used by Edwards and Steptoe. Mukherjee was the first person in the world to use

- (a) gonadotropins for ovarian stimulation prior to ovum pick-up in an IVF treatment cycle;
- (b) the transvaginal route by colpotomy for harvesting oocytes; and
- (c) freezing and thawing of human embryos before transferring them into the uterus that led to the successful birth of Durga.

India's first scientifically documented IVF baby, Harsha, was born on August 6, 1986, in Mumbai, through the collaborative efforts of the ICMR's Institute for Research in Reproduction and the King Edward's Memorial Hospital (KEM). This work was executed after being approved by the

Scientific Advisory Committee of the ICMR's Institute for Research in Reproduction and the Ethics Committee for Human Experimentation of the KEM Hospital. Full details of this and other studies in this area were published in the ICMR Bulletin (1986: No. 16) and in peer reviewed national (Natl. Med. J. India 1:10, 1988) and international journals (*J. In vitro Fertilization & ET* 5:376, 1988). Births of IVF babies were reported subsequently during the same year by two other clinics in India. There are an estimated 250 IVF clinics in India today.

### **1.1.1 ART - an alternative to reversal of Sterilization**

Infertility, consequent to use of terminal methods of contraception under the Family Planning Programme, may sometimes need to be reversed for personal reasons such as having lost a child/children born prior to sterilization. IVF is one of the options for women in whom fallopian tubes have been surgically severed and where recanalisation for correction of infertility has failed.

## **1.2 Definitions**

### **1.2.1 Artificial Insemination (AI)**

AI is the procedure of transferring semen into the reproductive system of a woman. This technique comprises artificial insemination with husband's (AIH) or with donor sperm (AID).

### **1.2.2 Aspiration cycle**

Initiated ART cycle in which one or more follicles are punctured and aspirated irrespective of whether or not oocytes are retrieved.

### **1.2.3 Assisted Hatching**

Assisted hatching allows easier release of the embryo from its shell (zona pellucida), helping implantation and increasing the pregnancy rate.

### **1.2.4 Assisted Reproductive Technology (ART)**

For the purpose of these guidelines, ART would be taken to encompass all techniques that attempt to obtain a pregnancy by manipulating the sperm or/and oocyte outside the body, and transferring the gamete or embryo into the uterus.

### **1.2.5 Blastocyst**

An embryo with a fluid-filled blastocele cavity (usually developing by five or six days after fertilization).

### **1.2.6 Controlled ovarian hyperstimulation (COH)**

Medical treatment to induce the development of multiple ovarian follicles to obtain multiple oocytes at follicular aspiration.

### **1.2.7 Cryopreservation**

Freezing and storage of gametes, zygotes or embryos

### **1.2.8 Donation of Gametes**

Donation of gametes is a process by which a person voluntarily offers his or her gametes for the process of procreation.

### **1.2.9 Ectopic pregnancy**

A pregnancy in which implantation takes place outside the uterine cavity

### **1.2.10 Embryo**

Embryo is defined as the fertilized ovum that has begun cellular division and continued development up to the blastocyst stage till the end of eight weeks.

### **1.2.11 Embryo donation**

The transfer of an embryo resulting from gametes that did not originate from the recipient and/or her partner.

### **1.2.12 Embryo transfer (ET)**

Procedure in which embryo(s) are placed in the uterus or fallopian tube.

### **1.2.13 Fertilization**

The penetration of the ovum by the spermatozoon and fusion of genetic materials resulting in the development of a zygote.

### **1.2.14 Foetus**

The product of conception starting from completion of embryonic development (at eight completed weeks after fertilization) until birth or abortion.

### **1.2.15 Foetal Reduction**

Foetal reduction is an invasive/interventional process by which a higher order multiple pregnancy is reduced to a single or twin pregnancy in order to improve the perinatal outcome.

### **1.2.16 Gamete**

Oocytes and sperm are called gametes.

### **1.2.17 Hatching**

It is the process that precedes implantation by which an embryo at the blastocyst stage separates from the zona pellucida.

### **1.2.18 ICSI (Intracytoplasmic Sperm Injection)**

In ICSI, a single sperm is injected into the cytoplasm of the ovum to effect fertilization, before the fertilized ovum is transferred to the uterus of the woman.

### **1.2.19 Implantation**

The attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) which starts five to seven days following fertilization.

### **1.2.20 Infertility**

Failure to conceive after at least one year of unprotected *coitus*

### **1.2.21 Intrauterine Insemination (IUI)**

Intrauterine Insemination involves the introduction of sperm into the uterus of the woman. In IUI, specially prepared sperm are injected into the uterine cavity via a fine cannula passed through the cervix. At this site, the sperm are near the uterine entrance of each of the two fallopian tubes and thus have a shorter distance to swim in order to reach the oocyte(s) released at the time of ovulation.

### **1.2.22 IVF-ET (*In vitro* Fertilization-Embryo Transfer)**

*In vitro* Fertilization-Embryo Transfer (IVF-ET) is the fertilization of an ovum outside the body and the transfer of the fertilized ovum to the uterus of a woman.

### **1.2.23 IVMTS & IVMO (*In vitro* Maturation of Testicular Sperm and *In vitro* Maturation of Oocytes)**

*In vitro* Maturation of Testicular Sperm (IVMTS) involves keeping the testicular sperm in a culture medium under optimal conditions where they can attain physiological maturity and acquire motility.

*In vitro* maturation of immature oocytes involves keeping the immature oocytes in an appropriate culture medium under optimal conditions where they can attain physiological maturity.

### **1.2.24 Oocyte donation**

An ART procedure performed with third-party oocytes

### **1.2.25 Ovum/Oocyte**

Ovum/oocyte is the female gamete produced in the ovary.

### **1.2.26 PESA (Percutaneous Epididymal Sperm Aspiration) and TESA/TESE (Testicular Sperm Aspiration/Extraction)**

Percutaneous Epididymal Sperm Aspiration (PESA) and Testicular Sperm Aspiration (TESA) are simplified, minimally invasive outpatient procedures that allow the physician to recover the sperm for fertilization in patients with obstructive azoospermia (lack of sperm in semen).

PESA requires a needle to be introduced into the epididymis and the contents aspirated. The aspirate is observed under the microscope to determine if motile sperm are present.

In TESA, the needle is introduced into the testicle itself.

### **1.2.27 Pre-implantation Genetic Diagnosis (PGD)**

Pre-implantation Genetic Diagnosis is a technique in which an embryo formed through IVF is tested for specific genetic disorders (e.g. cystic fibrosis) or other characteristics prior to implantation.

### **1.2.28 Preterm Birth**

A birth which takes place after at least 20, but less than 37, completed weeks of gestation. This includes both live births and stillbirths. Births are

counted as birth events (e.g. a twin or triplet live birth is counted as one birth event).

### **1.2.29 Semen**

A thick, whitish fluid discharged through the penis during ejaculation containing spermatozoa, secretions from the testes, seminal vesicles, prostate gland, bulbo-urethral and other glands associated with the male reproductive system.

### **1.2.30 Semen Donor**

Semen obtained from third party for purpose of inseminating the wife in cases where husband is unable to produce healthy semen.

### **1.2.31 Sperm**

Sperm are the male gametes produced in the testicles.

### **1.2.32 Spontaneous abortion**

Spontaneous loss of a clinical pregnancy before 20 completed weeks of gestation or, if gestational age is unknown, a weight of 500 g or less.

### **1.2.33 Surrogacy**

Surrogacy is an arrangement in which a woman agrees to carry a pregnancy that is genetically unrelated to her and her husband, with the intention to carry it to term and hand over the child to the genetic parents for whom she is acting as a surrogate.

### **1.2.34 Surrogacy with Oocyte Donation**

Surrogacy with oocyte donation is a process in which a woman allows insemination by the sperm/semen of the male partner of a couple with a view to carry the pregnancy to term and hand over the child to the couple.

### **1.2.35 Zygote**

Fertilized oocyte prior to first cell division is called zygote

## **1.3 Minimal Physical Requirements for an ART Clinic**

A well designed ART clinic of Level 2 or Level 3 (Sections 2.5.3 and 2.5.4) should have a non-sterile and a strictly sterile area as detailed below. Some of the spaces mentioned below could be combined (that is, the same space may be used for more than one purpose) as long as such a step does not compromise the quality of service. However, the space provision for the sterile area cannot be combined with those for the non-sterile area and vice-versa. For level 1B infertility care units (section 2.5.2), a strictly sterile area will not be required. The space requirement, however, will include, a reception area, a waiting room for the patients, a consulting room for the gynaecologist, and requirements mentioned under 1.3.1.8, 1.3.1.9 and 1.3.1.10.

### **1.3.1 The non-sterile area**

The non-sterile area must include what is listed under 1.3.1.1 to 1.3.1.9 below.

#### **1.3.1.1 A reception and waiting room for patients**

**1.3.1.2 A room with privacy:** A room with privacy for interviewing and examining male and female partners independently is essential. Evaluation of infertility necessitates history taking of the most intimate sexual practices between the couples. This is followed by close examination of the reproductive tract and sexual organs. Adequate measures must be taken to ensure that history taking and examination are carried out in strict privacy, maintaining the dignity of the patients. In case a male doctor examines a female patient, there must always be a female attendant present. The room must be

equipped with an examination table and gynecological instruments for examining the female per vaginum, an appropriate ultrasonographic machine with a probe for transvaginal examination of the female and examination of the testes and excurrent male reproductive tract. A colour Doppler would be useful but not essential.

### **1.3.1.3 A general-purpose clinical laboratory**

**1.3.1.4 Store room:** A well-stocked store for keeping essential stock of especially those items that have to be imported, precluding the need to be caught short in the middle of treatment. Facilities must be available for storing sterile (media, needles, catheters, petri dishes and such-like items) and non-sterile material under refrigerated and non-refrigerated conditions as appropriate.

**1.3.1.5 Record room:** Record keeping must be computerized as far as possible so that data is accessible retrospectively for analysis or when called upon by the supervisory agency. There are many software programmes for this purpose, which are commercially available today. A user-friendly one should be chosen that could be used widely. Besides containing essential details of the patient's records, it must contain history of the cause of infertility as diagnosed earlier, results of new diagnosis if relevant, the treatment option best suited for the particular patient, the treatment carried out and the outcome of treatment, and follow-up if any. Any other noteworthy point such as possible adverse reaction to drugs, must be recorded. ICMR should make an effort to devise a form for basic data recording, which would be suitable for India.

**1.3.1.6 Autoclave room:** A separate facility must be available for sterilizing and autoclaving all surgical items as well as some of those to be used in the *in vitro* culture laboratory.

**1.3.1.7 Steps for vermin proofing:** Adequate steps should be taken to make the whole clinic vermin proof, with suitable traps for preventing insects and other forms of unwanted creatures entering the clinic.

This essential detail should be planned at an early stage because no pesticide can be used in a fully functional IVF clinic, as it could be toxic to the gametes and embryos.

- 1.3.1.8 Semen collection room:** This must be a well-appointed room with privacy and an appropriate environment; it should be located in a secluded area close to the laboratory. Such a facility must be available in-house rather than having the patient collect the sample and bring it to the laboratory for analysis as, in the latter case, semen quality and identity is likely to be compromised. Procedures for collection of semen as described in the WHO Semen Analysis Manual must be followed with special reference to the type of container used; these containers must be sterile, maintained at body temperature and non-toxic. This room must have a washbasin with availability of soap and clean towels. The room must also have a toilet and must not be used for any other purpose.
- 1.3.1.9 Semen processing laboratory:** There must be a separate room with a laminar air flow for semen processing, preferably close to the semen collection room. This laboratory must also have facilities for microscopic examination of post-coital test smears. Good Laboratory Practice (GLP) guidelines as defined internationally must be followed. Care must be taken for the safe disposal of biological waste and other materials (syringes, glass slides, etc.). Laboratory workers should be immunized against hepatitis B and tetanus.
- 1.3.1.10 Clean room for IUI:** There must be a separate area/room with an appropriate table for Intra-Uterine Insemination (IUI).

## **1.3.2 The sterile area**

The sterile area shall house the operation theatre, a room for intrauterine transfer of sperm or embryos and an adjoining embryology laboratory. Entry to the sterile area must be strictly controlled by an anteroom for changing footwear, area for changing into sterile garments and a scrub-station. The sterile area must be

air-conditioned where fresh air filtered through an approved and appropriate filter system is circulated at an ambient temperature (22-25° C).

**1.3.2.1 The operation theatre:** This must be well equipped with facilities for carrying out surgical endoscopy and transvaginal ovum pick-up. The operation theatre must be equipped for emergency resuscitative procedures.

**1.3.2.2 Room for intrauterine transfer of embryo:** This room must be a sterile area having an examination table on which the patient can be placed for carrying out the procedure and rest undisturbed for a period of time.

**1.3.2.3 The embryology laboratory complex:** The embryology laboratory must have facilities for the control of temperature and humidity and must have filtered air with an appropriate number of air exchanges per hour. Walls and floors must be composed of materials that can be easily washed and disinfected; use of carpeting must be strictly avoided. The embryology laboratory must have the following:

- a laminar flow bench with a thermostatically controlled heating plate
- a stereo microscope
- a routine high-powered binocular light microscope
- a ‘high resolution’ inverted microscope with phase contrast or Hoffman optics, preferably with facilities for video recording
- a micromanipulator (if ICSI is done)
- a CO<sub>2</sub> incubator, preferably with a back up
- a hot air oven
- a laboratory centrifuge
- equipment for freezing embryos in a programmed manner
- liquid nitrogen cans

- a refrigerator

Appropriate steps need to be taken for the correct identification of gametes and embryos to avoid mix-ups. All material from the operation room, culture dishes and Falcon tubes for sperm collection (including lids), must bear the name of the patient. In the incubator, identified oocytes and sperm should be kept together on the same tray and double-checked. Pipettes used should be disposed off immediately after use. The embryology laboratory must have a daily logbook in which all the day's activities are recorded, including the performance of the equipment.

### 1.3.3 Ancillary laboratory facilities

The infertility clinic need not have in-house facilities to perform all the procedures necessary to diagnose infertility, such as those mentioned below. They can be farmed out to speciality laboratories specializing in delivering such services, as long as they are located in the neighborhood.

**1.3.3.1 Hormone and other assays:** The infertility clinic must have ready access to laboratories that are able to carry out immunoassays of hormones (FSH, LH, Prolactin, hCG, TSH, Insulin, Estradiol, Progesterone, Testosterone and DHEA) and tests such as for HIV and Hepatitis B. Endocrine evaluation constitutes an essential diagnostic procedure to determine the cause of infertility. It is also necessary to estimate blood estradiol in samples taken from a woman undergoing controlled ovarian hyperstimulation, and have the result on the same day to determine the dose of drugs to be given for induction of ovulation. Accurate monitoring of endocrine response to controlled ovarian stimulation goes a long way in preventing ovarian hyperstimulation.

**1.3.3.2 Microbiology and histopathology:** Another important facility in an ART clinic (or easily accessible to it) would be that of a microbiology laboratory that can carry out rapid tests for any infection,

and a clinical chemistry laboratory. Facilities for carrying out histopathological studies on specimens obtained from the operation theatre would also be desirable.

- 1.3.3.3 Maintenance of the laboratories:** Each laboratory should maintain in writing, standard-operating manuals for the different procedures carried out in the laboratory. It should be ensured that there is no “mix up” of gametes or embryos. The patient’s name should be clearly labeled on all the tubes, dishes and pipettes containing the gametes and embryos. All pipettes should be immediately discarded after use.

Laminar flowhoods, laboratory tables, incubators and other areas where sterility is required must be periodically checked for microbial contamination using standard techniques, and a record of such checks must be kept.

A logbook should be maintained which records the temperature, carbon dioxide content and humidity of the incubators and the manometer readings of the laminar air flow.

All instruments must be calibrated periodically (at least once every year) and a record of such calibration maintained.

- 1.3.3.4 Quality of consumables used in the laboratory:** All disposable plasticware must be procured from reliable sources after ensuring that they are not toxic to the embryo. Culture media used for processing gametes or growing embryos *in vitro* should be preferably procured from reliable manufacturers. Each batch of culture medium needs to be tested for sterility, endotoxins, osmolality and pH. The embryologist should know the composition of the media that are being used. Most media are supplemented with serum; they should, therefore, be tested for antibodies to HIV 1 and 2, Hepatitis B Surface Antigen and Hepatitis C RNA.

## 1.4 Back-up Power Supply

There should be no interruption in power supply to the incubator and to other essential services in the clinic. Given the power supply situation in India, it is, therefore, imperative that a power back up in the form of UPS systems and/or a captive power generation system is available in infertility clinics offering ART services.

## 1.5 Essential Qualifications of the ART Team

The practice of ART requires a well-orchestrated teamwork between the **gynaecologist, the andrologist and the clinical embryologist** supported by a **counsellor** and a **programme coordinator/director**. The staff requirements given below would be mandatory for Level 2 and Level 3 clinics (see Section 2.5.3 and 2.5.4). In the case of small Level 2 and Level 3 clinics, the services of the andrologist, the clinical embryologist and/or the counsellor could be shared.

### 1.5.1 Gynaecologist

The minimal qualification for a gynaecologist in a Level 1B, Level 2 or Level 3 clinic (see Sections 2.5.2, 2.5.3 and 2.5.4) is a post-graduate diploma or degree in gynaecology. Additional experience should include:

- © Understanding the causative factors of male and female infertility.
- © Acquiring knowledge of the practice and use of diagnostic methods for determining the cause of infertility.
- © Acquiring knowledge of the clinical aspects of reproductive endocrinology and the reproductive defects caused by endocrine factors, and an understanding of the limitations of the currently used hormone assay methods, and of the techniques available for medically or surgically correcting endocrine disorders.
- © Acquiring competence/skills in gynecological ultrasonography to diagnose reproductive tract anomalies, monitoring ovarian and uterine response to ovarian stimulation, picking up oocytes at the most appropriate time, and transferring embryos by any one of the several methods currently available to handle embryo transfer in

‘difficult cases’.

- © The gynaecologist must be well versed, particularly in the pharmacology of hormone action, and know how to avoid situations such as Ovarian Hyperstimulation Syndrome that can pose a great health hazard.

The responsibilities of the gynaecologist would include the following:

- Interviewing of the infertile couple initially.
- History taking.
- Physical examination of the female.
- Recommending appropriate tests to be carried out, interpreting them and treating medical disorders (infections, endocrine anomalies).
- Carrying out laparoscopy or sonohysterosalpingography for determining the status of the uterus and the fallopian tube.
- Advising the couple on planned relationship in simple cases.
- Carrying out AIH, AID, IUI, IVF or ICSI as the case may warrant, based on diagnostic evidence.

In case of male factor infertility, if the gynaecologist is confident and competent, he/she can treat such cases or refer them to the andrologist. The treating doctor must be responsible for maintaining all records of diagnosis, treatment given and consent forms. Before any treatment is given, it is advisable that the couple is referred to the counsellor, with all the details of the case, for proper advise and counselling. It would be the gynaecologist’s responsibility to see that all equipment and instruments in the operation theatre are properly functional and in order, and that a logbook is maintained of their use and operation.

### **1.5.2 Andrologist**

Fifty percent of infertility cases are related to male factors, many of which can be treated by specific ART procedures or other less invasive procedures. Andrology, a subject related to male reproduction, does not constitute a formal course in the medical curriculum in India, although several journals in andrology are published from different parts of the world including China. There is also an International Andrological Society with branches or affiliated societies all over the world. In India it is the urologist with a post-graduate degree in urology that often takes on the task of treating male infertility. Such individuals must receive additional training in diagnosis of various types of male infertility covering psychogenic impotence, anatomical anomalies of the penis which disable normal intercourse, endocrine factors that cause poor semen characteristics and/or impotence, infections, and causes of erectile dysfunction.

- © The andrologist must have knowledge of the occupational hazards, infections and fever that cause reversible or irreversible forms of infertility, and knowledge of ultrasonographic or vasographic studies of the reproductive excurrent ducts to detect partial occlusion that can be surgically corrected.
- © He/she must understand the principles of semen analysis and their value and limitation in diagnosis of male fertility status. The person should also be able to interpret the fertility status of the male from the result of semen analysis. The andrologist must be able to collect semen by prostatic massage for microbial culture in cases where infection may lie in the upper regions (prostate, seminal vesicles) of the reproductive tract. He/she should also be able to collect spermatozoa from the excurrent ducts or testis for use in ICSI and must also be knowledgeable about the genetic implications of using poor-quality sperm for ICSI as this technique can vertically transfer the genetic defects of the father to the child. He/she should be familiar with the surgical procedures available for correcting an anatomical defect in the reproductive system such as epididymo-vasal re-anastomosis and varicocoelectomy.

- © An individual may act as an andrologist for more than one clinic but each clinic where the andrologist works must own responsibility for the andrologist and ensure that the andrologist is able to take care of the entire work load of the clinic without compromising on the quality of service.

The responsibilities of the andrologist would include the following:

- Recording case histories.
- Prescribing appropriate diagnosis and treatment based on the diagnosis.
- Carrying out such surgical procedures as warranted by the diagnosis.
- Maintaining all the records, from the case history to the treatment given, and the patient consent forms.
- Referring the couple to the gynaecologist for carrying out the appropriate ART procedure if necessary, after the male factor has been duly investigated.
- Referring the couple to the counsellor if necessary.
- In cases of surgical intervention, making sure that the operation theatre is fully functional and all supplies are available before the start of any surgical procedure.
- Entering any deficiency that needs attention in the operation theatre logbook.

### 1.5.3 Clinical Embryologist

The **clinical embryologist** must be knowledgeable in mammalian embryology, reproductive endocrinology, genetics, molecular biology, biochemistry, microbiology and *in vitro* culture techniques. The biologist must also be familiar with ART. He/she must be either a medical graduate or have a post-graduate degree or a doctorate in an appropriate area of life sciences. (In the case of a clinic in existence for at least one year before the promulgation

of these guidelines, a person with a B Sc or BV Sc degree but with at least five years of first-hand, hands-on experience of the techniques mentioned below and of discharging the responsibilities listed below, would be acceptable for functioning as a clinical embryologist in the particular clinic. Such persons would also be eligible to take a test to be designed and conducted by an appropriate designated authority, to qualify for a position of a clinical embryologist in a new clinic.) He/she must be familiar with the following:

- © Principles and practice of semen analysis and cryopreservation of semen.
- © Cytology of mammalian and human oocyte to identify stages of oocyte maturation accurately.
- © All aspects of embryology including developmental biology.
- © Cell biological techniques used in cell and tissue culture.
- © Molecular biology and genetics of human reproduction.
- © Micromanipulation of sperm and oocytes for carrying out ICSI and single-cell biopsies of embryos for preimplantation genetic diagnosis.
- © Principles and functioning of all the equipment used in the laboratory.
- © In vitro fertilization of oocytes after processing the gametes.
- © Principles and practice of embryo freezing.

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 gynaecologist.
- 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 and ensure that the embryologist is able to take care of the entire work load of the clinic without compromising on the quality of service. An embryologist must not be associated with more than two centers at any given time.

#### **1.5.4 Counsellors**

Counsellors are an important adjunct to any infertility clinic. Indeed, in the UK, counsellors are appointed by the clinic but they report to an independent body. This ensures that there is fair play by the clinic and the patients are adequately informed of what and what not to expect from the treatment offered to them. Counselling for ART is not taught as a separate subject anywhere. A person who has at least a degree (preferably a postgraduate degree) in Social Sciences, Psychology, Life Sciences or Medicine, and a good knowledge of the various causes of infertility and its social and gender implications, and the possibilities offered by the various treatment modalities, should be considered as qualified to occupy this position. The person should have a working knowledge of the psychological stress that would be experienced by potential patients, and should be able to counsel them to assuage their fears and anxiety and not to have unreasonable expectations from ART. A member of the staff of an ART clinic who is not engaged in any other full-time activity in the clinic can act as a counsellor.

The counsellor must invariably appraise the couple of the advantages of adoption as against resorting to ART involving a donor. An individual may act as a counsellor for more than one clinic but each clinic where the counsellor works must own responsibility for the counsellor and ensure that the counsellor is able to take care of the entire counselling load of the clinic without compromising on the quality of the counselling service.

#### **1.5.5 Programme co-ordinator/director**

This should be a senior person who has had considerable experience in all aspects of ART. The programme co-ordinator/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. He/she should ensure that the staff are keeping up with the latest developments in their subject, by providing them with information from the literature, making available to them access to the latest journals, and encouraging them to participate in conferences and meetings and present their data. The programme co-ordinator/director should have a post-graduate degree in an appropriate medical or biological science. In addition, he/she must have a reasonable experience of ART.

## **1.6 ART Procedures**

A variety of ART procedures have been described in the literature. Only those procedures that have been widely tested and proven to be satisfactory as of writing this document are listed here. It would be the responsibility of the National Accreditation Committee (Chapter 9) to ensure that the list given in this document is enlarged in real time as progress occurs in the field. It is hoped that the practitioners of ART in the country will bring to the notice of the Committee on a continuing basis, any new procedure for the practice of which there would appear to be a sound scientific case. The National Accreditation Committee or a body appointed by it will approve or disapprove the new procedure within six months of its having been made aware of in writing: if this is not done, the clinic could continue to use the procedure until the above body has taken a decision on it. No new procedure that has not been approved as above should be permitted to be used by an infertility clinic for more than the period mentioned above.

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. The basic tenets of any medical treatment mentioned in the Helsinki Declaration of 1964 and reiterated in October 2000 in Scotland (information available on the Internet) clearly spell out the ethical concerns of treating patients. These basic tenets are also applicable to ART. The clinic must ensure that a particular ART being offered is fully in consonance with the diagnosis made of the cause of infertility. More particularly, the clinic must make sure that patients are well informed about the treatment being offered to them, the reasons

of suggesting a particular form of treatment, and alternative therapies available if any.

If a clinic is offering an ART that is not listed in these guidelines now or as modified in the future (vide para 1 of this Section), the procedure must be approved by the clinic's ethics committee (constituted as recommended by the ICMR ethical guidelines, 2000), justifying the need for the procedure and explaining why alternatives are not suitable. [Only clinics of Level 2 or Level 3 (Sections 2.5.3 and 2.5.4) would be required to have an ethics committee.] Informed consent from the patients would be mandatory in such cases as well. As mentioned in para one of this section, the clinic must also bring the new procedure to the notice of the National Accreditation Committee for its approval; if such an approval is not granted, all further use of the procedure must stop.

### **1.6.1 Artificial insemination with husband's semen (AIH)**

The technique consists in placing in the interior of the vagina a sample of the unprocessed semen.

### **1.6.2 Artificial insemination with donor semen (AID)**

The indications for AID are when there is (a) non-obstructive azoospermia; (b) the husband has a hereditary genetic defect; or (c) when the couples have Rh incompatibility.

The main advantage of AID is that it enables a couple to achieve pregnancy even though the husband is not the biological father. However, the possible transmission of diseases from the donor to the future child and the risk of consanguinity, constitute some drawbacks that must be brought to the notice of the patients. It is necessary to get the informed consent of both the partners after they are counselled about the possible psychological conflict they may face later in their life with the knowledge that one of them is not the biological parent of their child.

AID is an ethically acceptable procedure provided there is a medical indication and psychological confirmation for its use. Also, the normal

conditions of anonymity and screening of the donor must be met and only frozen sperm samples that have passed appropriate quarantining for infectious diseases such as HIV, hepatitis B and C, and syphilis should be used (for details see Chapter 3). AID involves the placing of a donor's semen into the interior of the vagina.

**Common indications:**

- ⊙ Husband has non-obstructive azoospermia.
- ⊙ Husband has a hereditary genetic defect.
- ⊙ The couple has Rh incompatibility.
- ⊙ The woman is iso-immunized and has lost previous pregnancies and intrauterine transfusion is not possible.
- ⊙ Husband has severe oligozoospermia and the couple does not wish to undergo any of the sophisticated ART such as ICSI.

**1.6.3 Intrauterine insemination with either husband's or donor semen (IUI-H or IUI-D)**

IUI involves the processing of semen in the laboratory so as to yield pure, activated sperm, devoid of seminal plasma, which are then directly placed into the uterus.

**Common indications:**

- ⊙ Hostile uterine cervix that does not respond to medication. (Cervical hostility can readily be determined by carrying out proper tests such as the sperm-mucous interaction test or post-coital tests. Technical skills constitute an important factor in carrying out these tests correctly and reading the results.)
- ⊙ In cases where husband's sperm cannot be used for reasons as described above for AID.

**1.6.4 *In vitro* fertilization and embryo transfer (IVF-ET)**

The technique of IVF consists of bringing about the fertilization of the oocyte and the spermatozoa in the laboratory instead of in the woman's fallopian tube. IVF involves induction of ovulation in order to obtain multiple oocytes, thus making available more embryos with which higher pregnancy rates can be achieved. Serial determination of plasma estradiol levels and daily monitoring of ovarian follicular growth by ultrasonography would indicate the response to ovarian stimulation. At the appropriate moment of follicular growth, the follicles are aspirated to obtain the oocytes. The oocytes are mixed with appropriately capacitated spermatozoa from the husband (or the donor, if the medical condition indicates the use of donor sperm) and kept in an incubator for fertilization which is observed microscopically after 16 to 18 hours. Embryos are transferred into the uterine cavity between days 2 and 6 after oocyte aspiration. If implantation ensues, pregnancy can be confirmed by 14 to 16 days after embryo transfer by determining the presence of hCG in a blood or urine sample. Such a test is reliable only when progesterone is used for luteal supplementation instead of hCG.

The success rate of IVF is approximately one in every 4-5 women. IVF is the therapeutic option of reproductive medicine with the highest yield per attempt, coming close on many occasions to that achieved by fertile couples conceiving naturally.

### **Common indications:**

- © The original indication for IVF was irreversible pathology of the fallopian tubes, resulting from an inflammatory process or from previous surgery. However, in recent years the indications for IVF include infertility due to a subnormal male factor.

Other indications include:

- © Idiopathic infertility.

- © Endometriosis.
- © Infertility of immunological origin.

### **1.6.5 IVF-associated techniques**

Gamete Intrafallopian Tube Transfer (GIFT) or Tubal Embryo Transfer (TET) has been recommended for patients with undamaged fallopian tubes. Access to the tube is gained by laparoscopy or by retrograde catheterization through the uterine cervix. GIFT is associated with higher levels of pregnancy than IVF but it has the drawback that it is unable to demonstrate the fertilizing capacity of the gametes.

### **1.6.6 Intracytoplasmic sperm injection (ICSI) with ejaculated, epididymal or testicular spermatozoa**

It is well known that the incidence of fertilization with sub-optimal semen is much lower in contrast to normal semen samples. It has been argued that since a sizeable number of couples are not suitable for IVF because their sperm count is far below 10 million/ml with less than 30% sperm being motile and more than 30% having abnormal morphology, alternate methods must be found to facilitate fertilization. Several approaches have been developed to circumvent the barriers (the zona pellucida and the ooplasmic membrane) that prevent the sperm reaching the ooplasm. Notable amongst these are: partial zona dissection (PZD), subzonal insemination (SUZI), and intracytoplasmic sperm injection (ICSI).

Live births have been reported using all these methods. The use of PZD or SUZI must be discouraged, as they do not offer any distinct advantage. ICSI is the most widely accepted choice of treatment for male factor infertility. ICSI can be carried out with fresh or frozen-thawed ejaculated or epididymal/testicular motile or live spermatozoa.

#### **1.6.6.1 Indications of ICSI with ejaculated spermatozoa**

- Severe male-factor infertility.
- Fertilization failure after standard IVF treatment.

- Number of spermatozoa in the ejaculate too low for IVF.

#### **1.6.6.2 Indications of ICSI with epididymal spermatozoa obtained by microsurgical epididymal sperm aspiration (MESA/PESA)**

- Congenital bilateral absence of the vas deferens (CBAVD).
- Failed vasoepididymal anastomosis.
- Failed vasovasal anastomosis.
- Obstruction of both ejaculatory ducts.
- Anejaculation because of spinal cord injury.
- Retrograde ejaculation.

#### **1.6.6.3 Indications of ICSI with testicular spermatozoa (TESA)**

- Extensive scarring, rendering MESA/PESA impossible.
- Germ-cell hypoplasia (hypospermatogenesis).
- Germ-cell aplasia with focal spermatogenesis.
- Sertoli cell-only syndrome with focal spermatogenesis.

#### **1.6.6.4 Indications of ICSI with *in vitro* matured oocytes**

- Polycystic ovary.
- History of ovarian hyperstimulation.

### **1.6.7 Oocyte donation (OD) or embryo donation (ED)**

Oocyte donation would necessitate using the husband's semen for fertilization and transferring the resultant embryo to the infertile female partner. Embryo donation would obviate the necessity of using the husband's semen. The choice of oocytes and embryos for oocyte or embryo donation would depend entirely on the circumstances prevalent at the time the infertile couple comes for treatment, and the access of the infertility clinic to frozen oocytes or embryos.

#### **1.6.7.1 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.

Donors should be healthy (as determined by medical and psychological examination, screening for STDs, and absence of HIV antibodies) women in the age group of 18-35 years. Oocytes may be obtained for donation, mostly by surgical intervention from women participating in an IVF program, or those undergoing elective sterilization or surgery.

The recipient should be a healthy woman (determined by medical and psychological examination) having normal genitalia (as determined by physical examination) and uterine cavity (as determined by hysterosalpingography). In case of OD, the semen characteristics of the husband must be determined to see if they are in conformity with those associated with normal fertility. The blood group of the donor should be noted; the donor should also be tested for antibodies to rubella, HIV, hepatitis, CMV, gonorrhea, syphilis, chlamydia, mycoplasma and trichomonas.

Ovum/embryo donation can be carried out in menopausal women with no surviving child and desiring to have a child. The endometrium of menopausal women has the ability to respond to sex hormones and provide a receptive environment for the implantation of an embryo.

Various protocols are now available to prepare the endometrium of the recipient for OD or ED with estrogens and progestogens until the placenta takes over the function of maintaining the gestation.

### **1.6.8 Cryopreservation**

Facilities for cryopreservation are an essential component of an ART clinic as they are to be used under a variety of conditions such as those described below.

#### **1.6.8.1 Freezing semen**

Men, who are likely to suffer from psychological stress at the time of ovum pick-up or those who cannot be present at the time of ovum pick-up, are recommended to have their semen frozen for use at the appropriate time. One of the important reasons for freezing semen from donors is that any donor semen has to be quarantined for six months. The safety of using frozen sperm has been abundantly proven, both by experimental work and the actual results in humans. Matters of concern are the donor's health and the necessity to avoid donors who are infected with venereal diseases, hepatitis B or C, or HIV. One of the drawbacks of sperm freezing is an approximate 20 % loss in motility after thawing. Donors whose semen is frozen for future use are required to report to the semen bank six months after donation to be checked for HIV, HBV or HCV infection/disease status.

#### **1.6.8.2 Freezing embryos**

Embryos are routinely cryopreserved to enable storage of supernumerary embryos, as upto a maximum of only three embryos is allowed for transfer to avoid the risk of multiple pregnancies. Embryo freezing is a widespread routine procedure to increase cumulative pregnancy rates.

Human embryos can be successfully cryopreserved at any stage from zygote to blastocyst, using 1, 2 propanediol (PROH) or dimethylsulfoxide (DMSO) for zygotes and cleaved embryos and glycerol for blastocysts. The formation of ice crystals is of concern during embryo freezing. Using programmed, slow freezers reduces this problem considerably, and slow cooling is the most widely employed method. Human embryos are known to survive a simple ultra-rapid procedure of fast cooling but there is not much data on the efficacy of these techniques when used routinely. Straws or ampoules used for freezing embryos should be carefully and permanently labeled for identification purpose.

Patients should be fully informed before the treatment cycle on the procedure of cryopreservation, the risks and, particularly, what is to be done with their embryos if they do not use them. They should sign a consent form concerning the agreement for embryo freezing as well as for the future use of the embryos (also see Section 3.11).

When a serum supplementation is used in the preparation of freezing and thawing solutions, one must carefully avoid any risk of viral transmission to the embryo through the serum.

### **1.6.8.3 Oocyte cryopreservation**

This procedure has been successfully used in cases where a large number of immature oocytes have been retrieved during ovum-pick-up. The oocyte can be thawed at a later date, matured *in vitro* and used for oocyte donation or similar procedures either on the person from whom the oocytes were retrieved or on other prospective recipients. However, the success rates in terms of fertilization, pregnancy and live births with the use of cryopreserved oocytes are not very encouraging. Much remains to be learnt on identifying the optimal stage of oocyte development when cryopreservation would be of value.

### **1.6.9 *In vitro* culture media**

There has been a spurt of new media introduced for *in vitro* culture of gametes and embryos. If one takes a close look at these media, they are products that have evolved over the years. However, some manufacturers do not give the exact composition of their media but merely state that for reasons of patent protection or as trade secret they are constrained to give full details of the composition of their media (J D Biggers, Reproductive Biomedicine Online Vol. 1, No 3, 2000; also available on the world-wide web: rbmonline.com).

This is an undesirable situation. Infertility clinics that deal with human embryos and the future life of the products they create in the laboratory must be privy to the knowledge about the media they use, if need be by signing an

appropriate confidentiality agreement which would prohibit the clinic from using or passing on the proprietary information provided by the manufactures of the media to any other organisation that may commercially exploit this information.

When a serum supplementation has to be used in the preparation of media, one must carefully avoid the risk of viral transmission to the embryo through the serum.

### **1.6.10 The future ART technologies**

Assisted reproductive technologies represent a rapidly progressing area in modern biology. It would be the responsibility of the National Accreditation Committee (Chapter 9) to ensure that this list of techniques is kept updated in real time.

### **1.6.11 Caution, precautions and concerns about ART practice**

#### **1.6.11.1 Ovarian stimulation**

It is important that ART procedures aimed to facilitate the bringing together of oocytes and spermatozoa should occur when the oocyte is ready to fertilize. Under normal conditions it is very difficult to predict when ovulation will occur and whether the oocytes released will be fertilizable. It is, therefore, a common practice to induce follicular development by administering clomiphene citrate (CC) and or human menopausal gonadotropin (hMG) prepared from menopausal urine, followed by human chorionic gonadotropin (hCG) for the induction of ovulation just when the ovarian follicle has ripened and grown to its optimal size as determined by ultrasonography. Insemination can be carried out *in vivo*, or the oocyte aspirated and subjected to *in vitro* fertilization or ICSI. The time of oocyte maturation can be predicted by this method to facilitate carrying out the rest of the ART procedure.

Ovarian stimulation should be carried out with the utmost caution to avoid Ovarian Hyperstimulation Syndrome (OHSS). Basal blood levels of FSH and LH should be estimated on day 1 or 2 of the menstrual cycle. LH

levels twice as high as FSH are indicative of the woman having polycystic ovaries; such women are prone to develop multiple follicles when stimulated and also undergo OHSS. Oocytes aspirated from such ovaries usually fail to fertilize. If such women are subjected to mild ovarian stimulation with CC, it is important to carefully monitor their ovarian response ultrasonographically.

### **1.6.11.2 Indiscriminate use of ICSI**

ICSI, one of the latest entrants to the field of ART, has been claimed to be a panacea for severe male infertility. This technique has never undergone critical evaluation in animal models before introducing it to treat human infertility. There are, therefore, some genuine concerns in regard to the use of ICSI; some of the fears underlying these concerns have come true ( S. Oehninger and R. G. Gasolen: Should ICSI be the treatment of choice for all cases of *in vitro* conception ? No, not in light of the scientific data. Human Reproduction 17: 2337, 2002).

Although, ICSI has revolutionized the treatment for male infertility, its widespread use has raised medical concerns about the transfer of genetic defects to future generations. There is a higher than normal frequency of sex chromosome abnormalities in children born of ICSI procedures compared with the normal population (Science 281:651-652, 1988; Human Reproduction 13: 781-782,1998; Human Reproduction 16:115-120,2001; British Medical Journal 327: 852, 2003; Fertility and Sterility 80: 851, 2003). Besides, infertile men carrying Y chromosome microdeletions pass this defect to ICSI-born sons (Fertility and Sterility 74:909-915, 2000). During ICSI, the process of fertilization is dramatically changed. For example, there is no fertilization occurring *in vivo*, and the physiological maturation of sperm, its selection and penetration through oocyte investments, and its influence on embryonic spatial patterning (Nature 409: 517-521,2001), are bypassed. Because ICSI bypasses a part of the process of natural selection and certain early developmental mechanisms, concerns are expressed on the possible reproductive health risk(s) to the offspring.

In India, it is estimated that about 15% of married couples are sub-

fertile or infertile. Treatment of male-factor infertility in the country has improved dramatically with the introduction of ICSI, which is currently being practiced rather extensively in various major ART clinics in the country. It is, however, extremely important that this approach to treating male-factor infertility is carried out with caution, in view of the possible risk of vertically transmitting defective (spermatogenetic) fertility gene(s) to the male progeny, when the etiology of infertility is genetic in origin (Human Reproduction 13:219-227,1998). Thus, ICSI may fall below the general expectations of the Helsinki Declaration (WMA 1964 and 2000). ART clinics accredited under the present programme must therefore take due note of the above before resorting to ICSI, and counsel the couple for whom ICSI is being recommended, appropriately. In spite of what has been said above, in some cases, ICSI may still be the preferred choice of treatment for infertility.

#### **1.6.11.3 Possible misuse of ART – sale of embryos and stem cells**

There is a growing interest in embryonic stem cells because of their potential use for developing spare organs or replacing defective tissues such as parts of the brain destroyed due to Alzheimer's disease, or pancreatic cells in diabetic patients. The range of their potential use is limited only by one's imagination.

ART clinics are the only source of embryonic stem cells. Spare embryos are either frozen or returned to the infertile couple for replacement during a later cycle, or donated to another infertile couple, or discarded after five years using a suitable protocol (Section 3.11).

Recently, the USA banned all federal support for embryonic stem cell researches unless the laboratories could demonstrate that they had developed embryonic stem lines before August 10, 2001. However, private funding is allowed which encourages scientists in the USA to procure stem cells from abroad. Germany has banned all research on embryos produced in that country but permits the use of embryos brought from abroad.

The stand taken by the foreign governments on embryo research opens up the possibility of embryos from developing countries that do not have

appropriate national guidelines in this area, being commercially exploited and sold to foreign countries. Therefore sale or transfer of human embryos or any part thereof, or of gametes in any form and in any way – that is, directly or indirectly – to any party outside the country must be prohibited. Within the country, such embryos or gametes could be made available to bonafide researchers only as a gift, with both parties (the donor and the donee) having no commercial transaction, interest or intent.