Chapter III

DEFINITIONS

3.1. Basic Definitions

a. Sperm: The male reproductive fluid containing spermatozoa or highly differentiated cells wonderfully adapted to their function as carriers of genetic material.

b. Gamete: Mature germ cell able to unite with other. Eggs and sperm are called gametes.

c. Ovum / Oocyte: Mature reproductive cell of female animals, produced by the ovary. Ovum / Oocyte is the female gamete produced in the ovary.

d. Zygote: Cell formed by the union of two gametes. Fertilized egg prior to first cell division is called zygote.¹

e. 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.

3.2 Definitions of Assisted Reproductive Techniques

a. Fertilization: Fertilization is the union of two gametes namely, (the egg and the sperm). It takes place in the fallopian tube or oviduct in the fallopian tube. The fertilized egg divides to form a ball of cells, known as pre implantation embryo, which passes into sixth day after fertilization.

b. Assisted Reproductive Technologies (ART): Any medical technique that attempts to obtain a pregnancy by means other than by coitus is defined as Assisted Reproductive Technique. In other words, these techniques manipulate the sperm and ovum outside the body, and the gametes or embryos are transferred into the uterus. ART includes artificial insemination (AI), In Vitro Fertilization-Embryo transfer (IVF-ET) and Gamete Intra-Fallopian Transfer (GIFT).

3.3 Treatment of Male Infertility

a. Microsurgical Epididymal Sperm Aspiration (MESA): Where the male partner is
azoospermic and no sperm can be obtained by either micro surgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA) for use in IVF, with Intra cytoplasmic sperm injection (ICSI) then artificial insemination using donor sperm may be offered.

b. Intra Cytoplasmic 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 then 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), sub zonal insemination

Indications of ICSI with ejaculated spermatozoa
1. Severe male-factor infertility
2. Fertilization failure after standard IVF treatment
3. Number of spermatozoa in the ejaculate too low for IVF

Indications of ICSI with epididymal spermatozoa obtained by microsurgical epididymal sperm aspiration (MESA/PESA)
1. Congenital bilateral absence of the vas deferens (CBAVD)
2. Failed vasoepididymostomy
3. Failed vasectomy
4. Obstruction of both ejaculatory ducts
5. An ejaculation because of spinal cord injury
6. Retrograde ejaculation.

Indications of ICSI with testicular spermatozoa (TESA)
1. Extensive scarring, rendering MESA/PESA impossible
2. Germ-cell hyper plasma (hypo spermatogenesis)
3. Germ-cell aphasia with focal spermatogenesis
4. Sterol cell-only syndrome with focal spermatogenesis
c. Percutaneous Epididymal Sperm Aspiration (PESA) and Testicular Sperm Aspiration / Extraction (TESA): Percutaneous Epididymal Sperm Aspiration and Testicular Sperm Aspiration 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.

d. In Vitro Maturation of Testicular Sperm (IVMTS): The recovered testicular sperm are sometimes so immature that they have minimal motility or none at all. According to Human Fertilization and Embryology Authority (HFEA) of UK Guidelines, non-motile sperm should into be used to inject the eggs. There is a risk that non-motile sperm might not be alive. Such sperm may also carry a higher risk of chromosomal abnormality. 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.

e. 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 it 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.

f. Gamete Intra-Fallopian Transfer (GIFT); Gamete intra fallopian Transfer is the placement of ova and sperm in the fallopian tubes to effect fertilization. It is recommended for patients with undamaged fallopian tube[s]. 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 its own drawback. It
cannot demonstrate the fertilizing capacity of gametes.

g. 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 egg(s) released at the time of ovulation.

h. Intrauterine Insemination with either Husband’s or Donor semen (IUI-H or IUI-D):
Intra Uterine Insemination 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.

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, the processed donor semen is used.

i. In Vitro Fertilization-Embryo Transfer (IVF-ET):
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. The technique of IVF consists in bringing about the fertilization of the egg and the spermatozoa in the laboratory instead of in the women’s fallopian tubes. 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, 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 a period of time when fertilization is monitored microscopically. Embryos are transferred into the uterine cavity between days 2 and 6 after oocyte aspiration. If implantation
ensues, pregnancy can be confirmed by about 18 to 24 days after embryo transfer by determining the presence of HCG in an early morning 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.

**Indications:** The original indication for IVF was irreversible pathology of the fallopian tubes, resulting from an inflammatory process of previous surgery. However, in recent years the indications for IVF include infertility due to an abnormal male factor. Other indications include idiopathic infertility, residual endometriosis and infertility of immunological origin. An added advantage of IVF is that it also serves as a diagnostic tool to determine if the couple suffers from a block to fertilization. IVF is ethically acceptable as long as it is used to optimize the possibilities of pregnancy for an infertile couple.

**j. Zygote Intra Fallopian Transfer (ZIFT) or Pro-nucleate State Ovum Transfer (PROST):** ZIFT is the placement of the zygote into the fallopian tube(s).

**k. Tubal Embryo Transfer (TET):** Tubal Embryo Transfer involves the transferring of a 2-8 cell embryo into the fallopian tube within two days of insemination.

**l. 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.

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

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

**o. 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.
p. **Surrogacy with Egg Donation**: Surrogacy with egg 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.

q. **Artificial Insemination with Husband's Semen (AIH)**: The technique consists in placing in the interior of the vagina a sample of the total semen (Artificial Insemination of Husband’s Semen, AIH), or of semen processed in the laboratory into the uterine cervix or the uterine cavity (Intra-Uterine Insemination).

**Indications:**
1. The impossibility of intra-vaginal ejaculation due to psychogenic or organic impotence
2. Severe hypospadias
3. Retrograde ejaculation
4. Vaginal dysfunction
5. When cryopreservation of sperm has to be used as in cases of cancer treatment or before vasectomy.
6. Unexplained infertility

r. **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; (c) when the couples have Rh incompatibility. The main advantage of AID is that it enables a couple to achieve pregnancy even through the husband is not the biological father.

**Indications:**
1. Husband has non-obstructive azoospermia
2. Husband has a hereditary genetic defect
3. The couple has Rh incompatibility
4. The woman is immunized and has lost previous pregnancies and intra-uterine transfusion is not possible.
5. Husband has severe oligozoospermia and the couple does not wish to undergo any of the sophisticated ART techniques such as ICSI.

s. **Freezing embryos or Cryo preservation of embryos**: Embryos are routinely cryopreserved to enable storage of supernumerary embryos, as up to 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. 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. 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 semen.

**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 unfrozen at a later date, matured *in vitro* and used for oocyte donation and similar procedures either in the person from whom the oocytes were retrieved or for 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.

**Egg Donation:** Many women have fertility problems that can only be overcome by the use of donated eggs as part of IVF programme however there is scarcity of egg donors since the treatment requires that the donor undergoes IVF treatment herself. More recently egg share scheme have developed whereby an infertile donor is prepared to donate half of her oocytes from an IVF cycle to a recipient who pays the
cost of both of their treatments. The donor undergoes conventional IVF treatment up to the point of oocyte collection. The oocytes collected from the donor are then fertilized with sperm from recipient’s partner and the embryos are transferred as normal.

v. 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.

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

**Indications for oocyte or embryo donation**
1. Gonad dysgenesis
2. Premature ovarian failure
3. Iatrogenic (due to ovarian surgery or radiation, or chemical castration) ovarian failure
4. Women who have resistant ovary syndrome, or who are poor responders to ovulation induction.
5. Women who are carriers of recessive autosomal disorders. Women who have attained menopause.

x. In Vitro Culture Media: There has been a spurt of new media introduced for in vitro culture of gametes and embryos. These products have evolved and modified over the years. Most of the manufactures deny giving the exact composition of their media stating the reasons of patent protection or trade secret.

y. Cloning: Cloning is a laboratory phenomenon and the word “clone” designates a “viable human or animal generated from a single parent”. A clone is a twin of the individual cloned with a time gap. Cloning is done by a procedure called “Somatic Cell Nuclear Transfer”. Different types of somatic cells can be used for nuclear transfer, provided they are in resting state. The nucleus of these cells are transferred
to the cytoplasm of a mature oocyte, i.e., an oocyte which is ready to be fertilized and has geared up the biochemical pathways for reprogramming and cleaving. Before bringing the somatic cell into with the oocyte, the nucleus of the oocyte has to be removed. Assisted Reproductive Technologies have not only enhanced the possibility of pregnancy but also have made women conceive in situations where this would not have been possible a decade ago. However, these techniques require enormous technical expertise and infrastructure and the success rate is often below 30%. New scientific developments, like ART that has wide application and those impinge on human life, thereby raises great public concern on ethics and safety standards. The next Chapter thereby explores into the socio legal implications arising by Assisted Reproductive Techniques.

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i The Concise Oxford Dictionary Ninth Edition Oxford University Press; pgs 556, 977
1336 (1997)

ii Indian Council of Medical Research, National Guidelines for Accreditation, Supervision and Regulation of ART Clinics in India; pgs 18, 19, 58, 59 (2000)

iii Winston Richard; IVF Revolution; pgs 79, 80, 82 (1999)