

Assisted Reproductive Technology: Techniques and Limitations

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Summary:

Infertility is a source of social and psychological suffering for both men and women and can place great pressure on the relationship within the couple. One in six couples of any society remains infertile and 10% of them need help of assisted reproductive technology (ART). ART refers to all technology where gametes are manipulated outside the body. In-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the commonest of all type of ART. Bilateral tubal block, endometriosis, severe oligospermia, and azoospermia are the commonest indications for ART. Whoever is responsible for infertility women are usually treated for superovulation, which

Introduction:

Infertility is the central issue in the lives of the individual who suffers from it. It is a source of social and psychological suffering for both men and women and can place great pressure on the relationship within the couple. For continuation of genesis procreation is human right. Based on the note from UN declaration of human rights Article 16: 1 it is said that men and women of full age, without any limitation due to race, nationality or religion have the right to marry and to find a family¹. But unfortunately 1 in 6 couples of any society remains infertile, and 10% of them need the help of assisted reproductive technology².

The announcement of birth of Luise Brown in July 1978 was not the beginning of the end of in vitro fertilization (IVF), but an important milestone along the way to what is now an important and internationally recognized treatment option for some infertile couples. The birth of Luise Brown occurs exactly 100 years after first attempts of in vitro fertilization of mammalian eggs which was made by the embryologist, Schenk in 1878. Since then significant contribution and refinements in the knowledge of reproductive biology and biotechnological science have been adding till date.

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sometimes involves risk of the patient. Collection of oocyte is also invasive. Result of treatment in terms of pregnancy is not very satisfactory. Average pregnancy rate is 30%-34% worldwide. Abortion and congenital anomaly rate is a bit higher than normal population, which is related to age of the female partner not related to the procedure. There are a number of barriers in this treatment like high cost of treatment, poor result, social stigma and superstitious believe. In addition to creating family by means of assisted reproductive technology, it has made a new dimension for distressed infertile couple.

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Assisted reproductive technology (ART) refers to all technology where gametes are manipulated outside the body. It does not include where only spermatozoa are manipulated like intrauterine insemination (IUI). The first and the most common procedure is in vitro fertilization (IVF), but there is an ever increasing list. The various procedures are as follows

- In-vitro fertilization and embryo transfer (IVF & ET)
- Gamete intrfallopian transfer (GIFT)
- Pronucleate or Zygote intra-fallopian transfer (PROT, ZIFT)
- Intracytoplasmic sperm injection (ICSI)
- Round nuclei injection (ROSNI) or spermatid injection
- Assisted hatching

Among this list most commonly practiced procedures are IVF & ET and ICSI.

In-vitro fertilization (IVF)

In-vitro fertilization involves fertilization outside the body in an artificial environment. This procedure was first used for infertility in humans in 1977 at Bourne Hall in Cambridge, England. To date millions of babies were born world wide as a result of IVF treatment. The commonest reasons are bilateral tubal damage or block or the sperms are too few or of too poor quality to fertilize an egg. IVF offers an

opportunity to avoid such problems by allowing fertilization to occur outside the body in a glass dish, hence the use of the Latin words “in vitro” which literally means “in glass”.

Indications of IVF

- Absent fallopian tubes or bilateral tubal block or disease that can not be treated successfully by surgery.
- Endometriosis that has not responded to surgical or medical treatment.
- A male factor contributing to infertility, in which sperm counts or motility are low but there are enough active sperm to allow fertilization in the laboratory.
- Unexplained infertility that has not responded to other treatments.
- Infertility secondary to sperm antibodies.
- Genetic disease that result in miscarriage or abnormal births.

Intracytoplasmic Sperm Injection (ICSI)

Injection of single mature immobilized normal spermatozoa into the cytoplasm of a mature metaphase II oocyte is known as intracytoplasmic sperm injection (ICSI). Since the introduction of ICSI, it has revolutionized the treatment of male factor infertility and excellent pregnancy and implantation rates achieved in couples for whom there were no treatment option except donation or adoption. ICSI was first used successfully in patients whose oocytes had failed to become fertilized after insemination with motile spermatozoa³⁻⁵. Then it became evident that ICSI might equally be well applied in couples with too few motile spermatozoa for conventional IVF⁶⁻⁹. Finally researchers tried for azoospermic men by injecting sperm, which obtained from epididymis (obstructive azoospermia, OA) and testes (non obstructive azoospermia, NOA). It was also successful in terms of normal fertilization, embryo development and implantation rates as well as birth of healthy offspring¹⁰⁻¹². Before 1992 the majority of severe male factor infertility were virtually untreatable. Due to establishment of ICSI as a routine it is now possible to treat the whole spectrum of male infertility from such optimal ejaculate samples or ejaculatory failure to obstructive and non-obstructive azoospermia.

Indications of intracytoplasmic sperm injection (ICSI)

- Couples who have suffered from recurrent failure of fertilization due to disorder at functional level of gametes. There might be a barrier at the level of acrosome reaction, zona binding or interaction, zona penetration or fusion with oolema. In ICSI all these steps are bypassed and only requirement is the decondensation of spermatozoa inside the oocyte.
- Severe oligospermia where sperm count is less than 5 million/ml. Severe oligospermia with high FSH is the indicator of compromised spermatogenesis and imminent testicular failure. Oligospermia due to hypogonadotropic hypogonadism, environmental factors, drugs or due to any disease can be corrected by behavioral changes and specific treatment. Otherwise repeated low sperm count with high FSH and without any specific reason (idiopathic) or Y chromosomal microdeletion are the candidates for ICSI.
- Severe asthenospermia including patients with ultra-structural abnormalities such as kartagener’s syndrome.
- Teratospermia where >70% sperms are morphologically abnormal.
- Obstructive azoospermia due to congenital absence of Vas deference, vasectomy or post inflammatory obstruction of the vas deference. Sperm can be retrieved by per epididymal sperm aspiration (PESA), testicular sperm aspiration (TESA) or testicular sperm extraction (TESE).
- Non-obstructive azoospermia. Sperm can be retrieved by TESA, TESE or open biopsy of the testis.
- Ejaculatory dysfunction such as retrograde ejaculation.
- Paraplegic male if electroejaculation is not satisfactory then TESE and ICSI can be done.
- Immunological factors-Antisperm antibody in both male and female partner.
- Frozen semen sample in patients having chemotherapy and radiotherapy. Testicular biopsy specimen may also be cryopreserved as backup where quality of ejaculation is inadequate for freezing.

For pre-implantation genetic diagnosis when PCR is used. ICSI should be used as the means for fertilization to prevent sperm contamination of the sample.

Rescue ICSI in same cycle on D2 when there is fertilization failure.

Patient selection for IVF and ICSI

Initially, indication for ART was considered for irreversible tubal damage, but since early 1980s the treatment has been extended to individuals with male factor infertility, unexplained infertility, endometriosis and immunologic causes of infertility. For ART it is essential to have

- a healthy uterus
- source of eggs and
- source of sperms

Age of female partner and decreased ovarian reserve are the most important determining factors for success of ART. More the female partner's age less is the chance of success. Age, basal D3 levels of FSH and oestradiol (E2) are significant markers of ovarian reserve¹³. Ovarian responsiveness to gonadotrophin stimulation is expected to be poor in women not only with higher basal levels of FSH but also with elevated basal levels of E2. High basal E2 level can artificially suppress FSH and therefore a normal basal FSH may sometimes be misleading if the E2 level is not measured simultaneously.

Steps of ART

Preparation of female partner

For both IVF and ICSI preparation of female partner for egg retrieval is same.

Step 1

Down regulation:

A drug is given for temporary switching off the message going from the brain to the ovaries telling them to produce an egg on a monthly basis. Gonadotrophin releasing hormone (GnRH) agonists are used for this purpose to create a state of reversible medical hypophysectomy, suppressing the greatest part of endogenous follicle stimulating and luteinizing hormone (LH) secretion^{14,15}. It takes 2-3 weeks to achieve down regulation, which is to be assessed by measuring serum E2 and LH level and by

observing thinness of uterine lining and ovarian quiescence. After proper downregulation gonadotrophin is used to stimulate folliculogenesis. The use of GnRH analogue together with gonadotrophins makes it possible to conduct follicular and oocyte maturation under exogenous influence only with no risk of interference from possibly detrimental endogeneous phenomenon. High level of LH is detrimental for oocyte development. So use of GnRH agonists has been advocated to prevent high level of LH during folliculogenesis and any inadequate LH surges before hCG administration. GnRH antagonist also is used for downregulation.

Down regulation protocols

Agonist protocol

For GnRH agonist there are mainly two different protocols

1. Long protocol
2. Short protocol

In the long protocol the basic principal is to conduct the complete period of folliculogenesis with the lowest possible LH. The GnRH agonist is given either at D2 or D21st of cycle¹⁶. After 2-3 weeks of administration when hypophyseal desensitization is complete, follicular growth and maturation are induced by exogenous gonadotrophins while GnRH agonist is continued to prevent any premature LH rise. The administration of GnRH discontinued at the same time as gonadotrophin administration is stopped.

In the short protocol the immediate stimulatory action of the GnRH agonist serves as initial stimulus for follicular recruitment. Administration of GnRH agonist is begun on the first or second day of cycle with simultaneous use of gonadotrophin. Besides these two protocols, an ultra-short protocol has been described in which the agonist is used only during the first 3 days of ovarian stimulation¹⁷.

Antagonist protocol:

One of the disadvantages of use of GnRH agonists for downregulation is the length of time required for the effect to occur and the need for an increased gonadotropin dose for achievement of an adequate response. Antagonists have high-affinity binding to the GnRH receptor without any agonistic properties.

Addition of antagonist during late follicular phase postpones the LH surge and abolishes the positive feedback of oestradiol during the preovulatory period¹⁸⁻²⁰. It is given for 3-6 days in the late follicular phase till day of hCG.

Step II

Ovarian stimulation:

Gonadotropins are given in the form of daily injections to stimulate the ovaries to produce multiple eggs. Either HMG or rFSH can be used. According to need of the patient 150-450 IU daily for 10-12 days is required to get mature eggs. When 3 or more follicles attain a size of 18 mm or more it indicates follicular maturity. At that point both GnRH agonist and gonadotropins stopped and injection hCG 5000 – 10000 IU is injected to trigger ovulation. In antagonist protocol both antagonist and gonadotropin continued till the day of hCG. After 36 hours of hCG injection ovum pick up is scheduled, which is done under the guidance of transvaginal ultrasonography

Monitoring of ovarian response:

The ovarian response to stimulation is monitored mainly by three parameters. Steady synchronous increase of at least three follicles with diameter increasing roughly 2mm per day. Steady increase in serum E2 level leading to approximately 200pg/ml per follicle larger than 14mm in diameter and thickness of the endometrial bed 8mm or more on the day of hCG administration generally denote appropriate response to stimulation. Cancellation and avoidance of hCG injection are to be considered if the ovaries are markedly hyperstimulated (more than 25 follicles and /or E2 more than 4000pg/ml on day of hCG).

Step III

Egg retrieval

After 36 hours of hCG injection the ripe eggs are collected. This is done usually under deep sedation or general anaesthesia, which takes between 10-30 minutes depending upon the number of follicles that have grown in response to the drugs. A thin needle is passed through the vaginal wall into the ovaries while they are scanned on ultrasound. The fluid within each follicle is sucked out and given to the embryologist for them to search for the eggs using a microscope.

Women are usually recovered fully within short time and can go home after a few hours.

Step IV

Insemination/ICSI and fertilization:

Before egg retrieval the woman's partner is asked to produce a semen sample, which is washed and prepared in such a way that a concentrated collection of the most vigorous and active sperm is produced. Each oocyte is inseminated with 50,000 to 100,000 motile sperms selected by percol gradient or swim up technique. Sperms and eggs are put together in a CO2 incubator overnight in a dish containing a special fluid that provides them with all the nutrients to allow fertilization to occur. In case of ICSI sperm is collected either by ejaculation from normospermic and oligospermic men or by PESA/TESA/TESE from azoospermic men.

Per epididymal sperm aspiration (PESA): It is done using a small needle under local anaesthesia to aspirate sperm from proximal to the obstruction.

Testicular sperm aspiration (TESA): Testicular sperm is aspirated from non obstructive azoospermic men by a syringe or butterfly needle.

Testicular sperm extraction (TESE): If spermatozoa is unavailable after PESA or TESA testicular tissue is taken under local or general anaesthesia.

All sample either ejaculated or aspirated is needed to be prepared by percol gradient or swim up method. From prepared sperm one sperm is injected within a denuded metaphase II egg. Injected egg is kept in the CO2 incubator overnight in a dish containing culture media to allow fertilization to occur. In next day evidence for fertilization is examined. After another 24 hours evidence for cleavage is examined.

Step V

Embryo transfer:

Embryos may be transferred at any stage between pronucleate to blastocyst stage. Usually embryo is transferred on D2 or D3 at 4-8 cell stage. Two to three embryos are loaded in a fine catheter, which is inserted through the cervix into the cavity of the uterus. It is done in an out patient basis and takes only a few minutes to perform. Women usually go home after one to two hours of rest.

Luteal Support:

Luteal support is needed as most of the granulosa cell population are destroyed due to ovum pick up and leave a weak corpus luteum. Luteal support may be given with progesterone 50mg/day IM or 300-400mg/day in the form of vaginal pessaries or hCG 2000IU biweekly. However, it is advisable that hCG support should not be given in situations where there is a prediction of development of ovarian hyper-stimulation syndrome.

Results and safety:

Successfulness depends on the female partner's age and causes of infertility. More the age of the patient less chance of success. The effect of age appears to be due to a reduced response to the drugs that stimulate the ovaries, a smaller chance of embryos implanting and a higher rate of miscarriage.

Treatment of suitable patients gives a pregnancy rate of about 34% per cycle and a cumulative pregnancy rate approaching 90%²¹. The spontaneous abortion rate is 19%-22%, which is somewhat higher than for the fertile population²². There is no increase in the incidence of congenital malformation. The risk of congenital malformation in pregnancies achieved after IVF is no higher than in the general population. As majority women come for ART at their late age so age related anomaly may occur. One study reported the incidence of anomalies 8.6% in ICSI babies, 9% in IVF babies and 4.2% in babies conceived naturally²³. Sex chromosomal abnormalities were found more frequently following ICSI and that was believed to result from the increased rate of abnormalities among the fathers with low sperm and not from new mutation arising from the procedure itself. Incidence of spontaneous abortion, low birth weight, prematurity all are higher in ART pregnancies in comparison to natural pregnancies. But when it is compared with IVF and ICSI babies there is no significant difference in both the groups. Though congenital malformation is higher in ART than natural population there is no differences in IVF and ICSI pregnancies. With increasing maternal age the frequency of chromosomal abnormalities increase and maternal age is usually higher in ART pregnancies particularly in IVF groups.

Complications:

Ovarian hyperstimulation syndrome (OHSS) is a rare complication of controlled ovarian stimulation. The

incidence of OHSS is usually between 0.1 and 6.1% of all controlled ovarian hyperstimulation cycles and the severe form is seen in about 0.4% of cases [24]. Severe degree of hyperstimulation is a life threatening complication of gonadotropin stimulation. The syndrome is more common in young patient and in PCOS patients. The duration is prolonged and syndrome is severe in women who have conceived in the same cycle and who have received hCG as luteal support. The basic pathology is hyper permeability of capillaries leading to loss of fluid and protein from intravascular compartment. The net effect is hypovolemia and hypoproteinaemia. The high oestrogen is responsible for increased capillary permeability. Vascular endothelial growth factor and other vasoactive amines, which release from stimulated ovaries are also responsible for leakage of fluid from capillaries. The syndrome may partly be prevented by withholding hCG for triggering for ovulation and for luteal support when E2 level is more than 4000 pg/ml and too many follicles have appeared. Cancellation of cycle, coasting, intravenous albumin and cryopreservation are other strategies for prevention of OHSS.

Limitations of ART:

The advent of IVF in late 1970s sparked intense debate about the use of ART and the social and legal implications they were predicted to have. Some reject ART as morally unacceptable in itself that is as wrong irrespective of any of the good or bad consequences it might have. Access to ART is extremely limited in all developing countries, even access to infertility information is severely limited in developing countries. While access to ART is extremely rare, the cost is even more prohibitive. ART introduced a number of challenges with which society has to cope.

Resources:

Due to high cost of establishment of ART centres and high cost of treatment, developing countries having limited resources should not allocate resources for expensive technology that can benefit only a few. Moreover, where overpopulation is a burden, they should not prioritize infertility management, for the overpopulation poses a demographic problem for the country and for the global community. It is argued that over fertility rather than infertility should be the

focus of family planning program. Treating infertility through expensive ART cannot be justified in low resource settings where other burning problems must be given priority [25]. So it hinders the establishment of ART centre in public sector which limits the use of ART by poor resourced people. Skill development also involves high cost, which is also a limitation for poor resourced community.

Age of female partner:

Women have limited reproductive life span. With increasing age success of ART reduced. When age exceeds 35 years, number of eggs, rate of fertilization, rate of implantation all are reduced. In addition increasing rate of miscarriage with age lessens the success of ART.

Implantation failure:

Implantation is the least efficient process in reproduction and has been recognized as the rate-limiting step. In natural pregnancy about 65% of conceptions end in unrecognized loss [26]. Almost 85% to 95% of transferred embryos are not implanted in ART cycles. Satisfactory pregnancy rates are achieved only through the transfer of multiple embryos.

Religious and social bar:

In some religion ART is not an acceptable method of treatment. Islam allows ART as a treatment procedure without involvement of another person. Roman Catholics believe that "A child must be conceived through the act of love and, indeed of sexual intercourse". So child must not be born through the ART procedure [27-30]. Although, most religious community accept ART as an important tool of infertility treatment, some society due to superstitious believe and ignorance can't accept it.

Conclusion:

Every couple who experienced infertility is in a distress than the general population. Stress related to fertility problems appears to increase marital conflict. Over the past few decades, remarkable progress has been made in modern reproductive technology to solve this problem to some extent. But less success, physical and financial stress of treatment sometimes becomes a barrier of this treatment. Moreover,

creating families by means of assisted reproduction has raised a number of concerns about potentially adverse consequences for parenting and child development. As ART is a very dynamic field regarding medical improvement, new treatment modalities, ethical issues and cost-benefit analyses for allocation of resources, we hope that path of the ART will be smother in future.

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