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Update on Human Assisted Reproductive Technology (ART)

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Introduction

Assisted Reproductive Technology (ART) includes a diverse group of medical procedures that allow infertile couples to be pregnant. According to the guidelines established by the American Society of Reproductive Medicine, infertility is defined as the inability to become pregnant after 1 year of unprotected coitus. The incidence of infertility is higher than most people realize:

15-20% of all couples of reproductive age are infertile

40% of the infertility due to male factors
60% of the infertility due to female factors
<u>20%</u> of the infertility due to male and female factors
120%

The major etiologies for infertility include:

1. Ovarian Causes (30%)
   - Abnormal ovulation, CL, hormone production
   - Premature ovarian failure

2. Tubal Causes (30%)
   - Occlusion
   - Inflammation (PID)
   - Adhesions

3. Uterine Cause (10%)
   - Uterine anomalies
   - Leiomyomata

4. Cervical Causes (15%)
    - Stenosis of cervix
    - Hostile cervical mucus

5. Male Factor (40%)
    - Oligozoospermia
    - Asthenozoosprmia
    - Teratozoospermia
    - Endocrine disorders
    - Vas obstruction
    - Agenesis of the Vas

6. Other Causes (5%)
    - Pituitary disorders
    - Psychological disorders
    - Metabolic disorders
    - Unknown causes

The major ART procedures include:

1. IVF – In Vitro Fertilization
2. GIFT – Gamete Intra-Fallopian Tube Transfer
3. ZIFT – Zygote Intra-Fallopian Tube Transfer
4. CRYO - Cryopreservation of Gametes
5. Micromanipulation
    - ICSI
    - AH
    - De-fragmentation

History of ART

- 1790 – 1st birth from AI (UK)
- 1890 – 1st reported birth from AI in USA
- 1959 – 1st IVF in mammals (rabbit; USA)
- 1968 – 1st fertilization of a human oocyte (UK)
- 1978 – 1st baby, Louise Brown (UK)
- 1981 – 1st IVF baby in USA
- 1984 – 1st surrogate embryo transfer (USA)
- 1984 – 1st donor embryo baby (Australia)
- 1986 – 1st baby from a cryopreserved embryo (Australia)
- 1991 – 1st ICSI baby (USA)

The procedural steps involved in an ART procedure include:

1. Ovarian Stimulation
2. Collection of Oocytes
3. Collection of Sperm
4. Insemination
5. Inspection for Fertilization & Development
6. Embryo Transfer
7. Obstetrical Follow-up

Ovarian Stimulation

The agents used in ovarian stimulation/ovulation are:

1. HMG – Human Menopausal Gonadotropins
2. CC - Clomiphene Citrate
3. rFSH - Recombinant Follicle Stimulating Hormone
4. rLH – Recombinant LH
5. GnRH – Gonadotropin Releasing hormone
6. GnRH agonist

Folliculogenesis is monitored by:

1. U/S
2. Systemic Estradiol

In human ART, ovarian folliculogenesis is first shut down with the administration of GnRH agonist. This allows for complete control of the subsequent hormonal ovarian stimulation and prevents an unplanned endogenous LH surge. When the ovary is suppressed (i.e., low estradiols), the patient is given daily gonadotropin injections to stimulate folliculogenesis. HCG (human chorionic gonadotropin) is given when the lead follicles are > 18 mm and the estradiol concentration is >200-300 pg/ml/follicle.

Notes:

Collection of Oocytes

Oocytes are collected by U/S guided collection through the vagina. The collected oocytes are washed in culture media and placed in the CO_2 incubator until insemination. The collected oocytes can be at varying stages of development. The three major stages of oocyte maturation seen at the time of collection are:

1. Immature oocyte (condensed cumulus GC w/ a GV)
2. Intermediate oocyte (partially condensed cumulus GC; MI)
3. Mature oocyte (expanded cumulus GC w/ polar body; MII)

Notes:

Collection of Sperm

Sperm are collected by the patient in an appropriate container. The semen sample is evaluated and the motile, normal morphology sperm are isolated by:

1. Gradient centrifugation separation
2. Swim up isolation
3. Sperm wash

Notes:

Insemination

The three forms of insemination include:

1. Conventional – Addition of 50- 200,000 sperm to a 1.0 ml, double well dish containing 1-5 oocytes
2. Microdrop – Addition of sperm (20,000 sperm /oocyte) to 30-50 µl drops under oil containing 1-4 oocytes
3. Micromanipulation – Direct insertion of sperm into the oocyte using a micro-injector

Notes:

Micromanipulation

For men with severe oligozoospermia, teratozoospermia or previously failed ART fertilization, micromanipulation of gametes can be employed. The major micromanipulation procedures include:

1. ICSI – Intracytoplasmic Sperm Injection, injection of a single sperm into the oocyte. Successful pregnancies have occurred with immotile sperm.
2. TESE – Testicular Sperm Extraction, removal of sperm from the testes of azoospermic men.
3. MESA – Microsurgical Epididymal Esperm Aspiration, collection of sperm from the epididymus of azoospermic men.
4. Assisted Hatching, thinning of the zona pellucida with acid or mechanical slicing. Thick ZP are more commonly present in older patients (>37 Y.O.) but may be present in the younger patient.
5. PGD – Preimplantation genetic diagnosis, removal of a single blastomere from an 8-12 cell embryo for assessment of chromosomal abnormalies. PGD would allow for the selection and transfer of embyos that do not contain a specific genetic anomaly.

The azoospermia of TESE and MESA patients could be due to either obstructive or non-obstructive disease. Greater pregnancy rates are found with obstructive disease.

Notes:

Inspection for Fertilization and Embryo Development

The hallmark of fertilization is the development of the pronucleus (PN). One PN is derived from the haploid sperm and the other from the haploid oocytes. The PN meet and exchange chromosomes (syngamy) to form the new diploid embryo. PN formation occurs approximately 16-18 hours after fertilization. In human work, the day of insemination is considered Day 0 of the procedure. With this in mind, the timing of embryo development is as follows:

1. Day 0 – Insemination
2. Day 1 – PN development
3. Day 2 – 2-4 Cell embryo
4. Day 3 – 6-8 Cell embryo
5. Day 4 – 32 cell to morula
6. Day 5 to 6 – Blastocyst

Oocytes and embryos are usually graded on a 4 point scale dependent upon their health and maturational status.

1. 4 = Excellent status
2. 3 = Good status
3. 2 = Fair status
4. 1 = Poor status
5. 0 = dead

The grade 3-4 embryo have a high probability of implanting while the grade 2 has a low probability dependent upon the severity of the lab's classification system. The grade 1 will not implant.

To determine oocyte/embryo grade the below scoring table is used. The below scores are subtracted from the initial score of 4.

1 Lumpy Ooplasma
1 Clear Ooplasma
1 Slightly Darkened Ooplasma
2 Dark Ooplasma
1 Uneven ZP
1 Cytoplasmic Vacuoles
1 Uneven Blasotmeres
1 Granular Cytoplasm
4 Polyspermy

Cryopreservation of Gametes

Pregnancies have occurred from cryopreserved sperm and embryos. Unfortunately cryopreservation of unfertilized oocytes has not been perfected. The most common stages of embryo cryopreservation include the PN, 2-4 and blastocyst with the PN stage being the most successful stage for cryopreservation in most labs.

Notes:

Embryo Transfer (ET)

Embryos have been successfully transferred to the patient at all stages of development, however, the most common days for ET are Day 3 (8 Cell embryo) and Day 5 (Blastocyst). The Blastocyst has the greatest chance of achieving a pregnancy, but few embryos develop to this stage. Usually only 1 to 2 blastocyts are ET to prevent multiple pregnancies. On the other hand 2-4 Day 3 embryos are usually transferred.

The ET is a non-surgical procedure. The embryos are loaded into a fine catheter in <25 μl of media and deposited mid-uterine. If the embryo implants hCG production starts within a week and can be detected systemically by day 14 post fertilization. If hCG is detected by Day 14 the patient is classified as having a chemical pregnancy. The embryo/fetus will develop a primitive heart that will beat by week 6. If a patient has an embryo/fetus with a heart beat at week 6 she is classified as clinically pregnant. If a baby is born in 9 months the patient is an official parent.

Notes:

Ethical Considerations

Since the procreation of human life is the objective of ART there are many ethical problems unique to each facet of IVF. Each religion, race or culture found in the United States has different but definite views about reproductive matters. Time does not allow for an in-depth presentation of the ethics of ART. However, just examining issues related to cryopreservation reveals how complicated ART can become.

Ethical Problems Associated with Cryopreservation of gametes

1. Use of Cryopreserved sperm/embryos after spouse/coupe are deceased.
2. Transfer of cryopreserved embryos to a sister, daughter, or the daughter's daughter.
3. Divorce or hysterectomy
4. Abandoned cryopreserved sperm/embryos
5. Lab with cryopreserved sperm/embryos destroyed by fire, hurricane, etc.

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