Reproduction

Reproduction is one of the fundamental characteristics of living organisms. All living organisms produce new individuals of the same species from the existing individuals in this process which ensures the perpetuation of species.

Asexual and sexual are the two main known methods of reproduction. Asexual reproduction is quite simple and an individual can reproduce without involvement with another individual of that species, e.g. the division of a bacterial cell into two daughter cells occurs asexually. Sexual reproduction typically requires the involvement of two individuals and the union of two special sex cells called gametes, one each from opposite sex.

Sexual reproduction starts with the gametogenesis - the process of formation of haploid gametes from diploid germ cell. Gametogenesis takes place in the special organs called gonads; testis is a male gonad and ovary is a female gonad.

Spermatogenesis

Spermatogenesis or sperm formation is a complex, multistage process takes place in the testes. It is characterized by proliferation, meiosis and spermiogenesis. Spermatogonia are diploid cells derived from embryonic germ cells divide by mitosis to produce more spermatogonia. At puberty, male hormone testosterone stimulates the spermatocytes to begin meiosis. Primary spermatocyte generates two secondary spermatocytes at the end of first meiotic division. Each haploid secondary spermatocyte then undergoes the second
meiotic division each producing two haploid spermatids. Thus four haploid spermatids are formed at the end of meiosis as shown in Fig. 2. These spermatids undergo extensive differentiation and morphological changes to eventually mature into a spermatozoon. One diploid spermatocyte gives rise to four haploid sperm cells. Primary spermatocytes are produced throughout the lifetime of the male. The sperm cells produced in the testis are stored in the epididymis and complete their maturation to functional sperm.

**Oogenesis**

Oogenesis is the process of formation of ovum in Graafian follicle of ovary. Humans as well as other mammals are born with a defined pool of primordial oocytes, which are arrested at the diploten stage of meiosis I. In sexually mature women, Follicle Stimulating Hormone (FSH) signaling and other factors stimulate the maturation of individual follicles on a monthly basis, generating primary and secondary follicles. A cascade of events is initiated, by rapidly increased concentration of luteinizing hormone, including further oocyte growth and meiotic resumption. Reactivation of meiosis results in segregation of first meiotic chromosome and one set of chromosomes that become arrested at the metaphase stage of meiosis II, and a second set of chromosomes that are discarded as the first polar body. Following this, the mature follicle ruptures and ovulation ensues, i.e. the egg is released from the ovary near the open end of the fallopian tube. This secondary oocyte is carried down the fallopian tube by ciliary movements of its cells; the oocyte begins to enter meiosis II, again meiosis II get arrested at its metaphase until a sperm enters the secondary oocyte.
Fig. 2 Gametogenesis

Spermatogenesis

Oogogenesis

Spermatogonia

Oogonia

Primary spermatocyte

Primary oocyte

Meiosis I

Meiosis I

Secondary spermatocyte

Secondary oocyte

Meiosis II

Meiosis II

Spermatid

Meiosis I

Polar body

Cell differentiation

Meiosis II

first Polar body

mature sperm cells

second polar body

ovum
Fertilization

Sperm, entering from the uterus, will move towards the released egg from the opposite end of the fallopian tubes. Interaction of sperm and egg results in a fertilization. Fertilization is an exceptional event in which male gamete fuses with female gamete only if they meet during their fertilizable life span. Successful penetration of a single sperm through the egg coat will initiate a set of sperm-egg interaction events that relieves the meiosis II arrest of the oocyte. This results in the formation of diploid zygote, the first cell of the new life.

Fig 4. Fertilization

Following fertilization, embryos undergo a series of mitotic cell divisions i.e. cleavages.

Naturally, fertilization and embryo development occurs during the journey of the oocyte through the fallopian tube to uterus. Once in the uterus, the embryo implants into the wall of the uterine lining, called the endometrium where its further development into fetus and baby till child birth takes place. The uterus provides a conducive environment for this development.

Dysfunction at any of these points may lead to infertility.

Infertility:

“Infertility (clinical definition): a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse “ (Human Reproduction, Vol.24, No.11 pp. 2683–2687, 2009, Advanced Access publication on October 4, 2009). The Infertility primarily refers to the biological
inability of a person to contribute to conception. Infertility may also refer to the state of a woman who is unable to carry a pregnancy to full term.

Primary infertility is infertility in a couple who have never had a child. Secondary infertility is failure to conceive following a previous pregnancy. In some cases, both the man and woman may be infertile or sub-fertile, and the couple's infertility arises from the combination of these conditions. The cause of infertility may be immunological or genetic; it may be that each partner is independently fertile but the couple cannot conceive together without assistance.

Factors that contribute to infertility are---

1. **Ovulatory dysfunction**: It is a condition where ovulation is irregular, abnormal or absent.

2. **Premature ovarian failure**: The ovarian reserve starts to decrease at age of 30 or even earlier and falls rapidly due to various reasons.

3. **Polycystic ovary syndrome (PCOS)**: Patients whose ovaries contain many small cysts due to hormonal imbalances and do not ovulate regularly. Also called as Stein - Leventhal syndrome. The ovaries function abnormally with abnormally high levels of androgen.

4. **Hyper-prolactinemia**: High prolactin levels affect ovulation and fertility.

5. **Poor egg quality**: Eggs that become damaged or develop chromosomal abnormalities and cannot sustain a pregnancy. This problem is usually age-related, egg quality declines significantly in the late 30s and early 40s.

6. **Overactive/Underactive thyroid gland**

7. **Tubal dysfunction**: Blocked or damaged fallopian tubes or epithelial dysfunction. This prevents oocyte entry into the uterus and sperm from getting to the oocyte. Leading causes include pelvic inflammatory disease, sexually transmitted diseases such as chlamydia, pelvic or genital tuberculosis and previous sterilization surgery or ruptured appendices. Ectopic pregnancy in which an egg get fertilized but, due to the damaged cilia, unable to travel to the uterus instead starts growing in the wall of the fallopian tube. This condition can result in rupture, internal bleeding and further tubal damage leading to infertility.

8. **Endometriosis**: Is a major cause of infertility in women. This is a condition, in which endometrial tissue (the uterine lining that sheds with each monthly period) grows outside the uterus.
9. **Cervical factor:** Cervical narrowing or blockage which prevents sperm entry to the uterus. Abnormal cervical mucus may result in destruction sperm or inhibition of sperm penetration. In rare cases mucus contains antibodies against sperm.

10. **Male tube blockages:** despite of normal spermatogenesis, any obstructions in the vas deferens or epididymis (the tubes that transport fertile sperm) results in problem with sperm emission. Most common cause of male tube blockages is a.) Varicoceles i.e. varicose veins in the testicles and b.) Infection - Onset of Sexually transmitted diseases, such as chlamydia or gonorrhea are also responsible for tube blockage.

11. **Sperm disorder:** Absence of sperm in semen sample, very low sperm concentration, poor sperm motility, and morphologically abnormal sperm cells can all cause infertility. An inadequate quantity or poor quality of sperm is caused by defective spermatogenesis which may occur due exposure to toxins, certain drugs, and smoking habits or as a result of endocrine, genetic or genitourinary disorder.

12. **Sperm allergy:** This is an immunological condition observed in infertile women and men in which anti-sperm antibodies are generated. In men, this is most common after a vasectomy.

13. **Unexplained infertility:** It is the condition when abnormalities are likely to be present but not detected after a full series of investigations.

14. **Combination infertility:** The condition where both male and female partner contributes to infertility or one partner has more than one fertility problems.

**The consequences of infertility:** are manifold. Having a child is an important part of the life. In many cultures childless women face discrimination. It can have a highly negative impact on self-esteem. It can include societal reaction and personal suffering. Infertility may have intense psychological effects. Partners may become more anxious to conceive, increasing sexual dysfunction. Disagreement is often observed in infertile couples, especially when they are under pressure to make medical decisions. Clinical depression faced by infertile couple is comparable to have heart disease or cancer have been observed in women trying for conception. In many cultures, inability to conceive bears a stigma. In closed social groups, a mark of rejection may cause considerable nervousness and frustration.

In most of the cases infertility can be treated by medical and technical help.
There are three main strategies for treating infertility----

1) **Medication:** Fertility can be enhanced by use of certain fertility drugs. Clomiphene citrate or tamoxifen is used to stimulate ovulation in cases of irregular ovulation or anovulation. Polycystic Ovarian Syndrome is treated by metformin. Gonadotropins are in routine use to increase the chance of conception.

2) **Surgical procedures:** Most of the times infertility caused by tubal obstruction requires surgery. Laproscopic surgeries may be used to treat the cases of endometriosis, or for cystectomy. In cases of obstructive azoospermia, sperm can be retrieved surgically.

3) **Assisted Reproductive Technology (ART):** Any form of assisted conception in which the gametes are handled outside the body is called as Assisted Reproduction. ART provides options to treat infertility with its various techniques like Intra Uterine Insemination (IUI) or by In Vitro Fertilization (IVF) either by co-culturing the gametes or by Intra Cytoplasmic Sperm Injection (ICSI).

### History of IVF

Dr. Patrick Steptoe, a gynecologist at Oldham General Hospital and Dr. Robert Edwards, a physiologist at Cambridge University had been working on conceiving in vitro since 1896. In 1975 they achieved their first pregnancy by IVF which turned out in to an ectopic pregnancy. Lesley and John Brown was a young couple referred to Dr. Petrick Steptoe in 1976. Lesley Brown had blocked fallopian tubes. On November 1977 Lesley Brown underwent the experimental in vitro fertilization procedure. Dr Steptoe had retrieved an egg from Lesley Brown’s ovaries using a long, slender self-lit probe called ‘laparoscope.

Professor Edwards placed the egg with John’s oocyte in a culture medium. They placed the resulted embryo back in to Lesley’s uterus after two and half days. On July 25, 1978 Lesley delivered a girl baby, The world’s first IVF baby. Professor Edwards was awarded Nobel in Physiology and Medicine in 2010.
Subash Mukhopadyay, physician from Kolkata, had created the world's second and India's first child using in-vitro fertilization on 3 October 1978, named as "Durga" (alias Kanupriya Agarwal). However, state authorities prevented him from carrying out further work. He was also prevented from presenting his work at scientific conferences. In the absence of scientific evidence, his work is not recognized by the international scientific community. Frustrated Mukhopadhyay killed himself on June 19, 1981. These days, however, Mukhopadhyay's contribution is acknowledged in works dealing with the subject. In 2005 he was officially credited with the birth of India’s first IVF baby on 3 October 1978, just 67 days after the birth of Luise Brown for his work. Durga akise Kanupriya Agarwal, India’s first IVF baby is healthy and has completed her MBA.

Australia achieved its first IVF birth in June 1980 at the Royal Women’s hospital – third IVF baby in the world. The first IVF baby of America had born in December 1981 in the 13th attempt with the ovarian stimulation with Human Menopausal Gonadotropin (hMG).

Dr. Indira Hinduja started experiments in IVF in 1984 on animals at KEM Hospital in Parel. On December 2, 1985, her team successfully transferred an embryo prepared in a petri-dish into Harsha’s mother’s womb, the first time in India where such an attempt was made. Harsha’s mother, Maniben, had suffered from tuberculosis, which had permanently damaged her fallopian tubes. 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). Harsha India’s first IVF baby became mother by natural conception in March 2916. Harsh Chawala is working as a counselor in an IT company. She conceived naturally and delivered a baby under the treatment of Dr. Indira Hinduja.

After the first break through, there have been several significant discoveries made related to ART. Improvements in ART have overcome numerous apparently impossible barriers to allow couples the chance to have families. In the three decades following the birth of Louise Brown, enhancements in laboratory technology and clinical practice have increased
the couple’s chance of conception. Innovation and refinements in technology led to the introduction of advanced techniques like ICSI, MESA, and TESE which provided effective treatment for male infertility. More recently, for couples with sex-linked diseases and numerous genetic disorders, the advent of Pre implantation genetic diagnosis made it possible to have children free of the condition. Improving the efficiency of oocyte cryopreservation and ovarian tissue transplantation promises to provide options to women who need to delay childbearing.

Today assisted reproductive technology is available throughout world. The practice is largely different from that used during the early days.

The procedure for in vitro fertilization mainly involves ------

1. **Controlled ovarian hyper-stimulation**: The ovaries are stimulated with exogenous hormonal Injections (HMG or rFSH) to develop more number of follicles.

2. **Monitoring ovulatory process**: Woman’s follicular growth is monitored daily with vaginal ultra sound scan and the dose of hormones is adjusted as required.

3. **Ovulation induction**: When at least three ovarian follicles observed on ultrasound appear to have reached 18 mm diameter, final oocyte maturation and ovulation is induced by an injection of human chorionic gonadotropin (hCG)

4. **Ovum Pick Up or eggs retrieval**: The follicle is aspirated from the woman's ovaries under ultra sound guide by technique called trans-vaginal oocyte retrieval at 35-36h after hCG injection. Follicles are aspirated, and the follicular fluid is passed to an embryologist to identify oocyte cumulus complexes.

5. **Oocyte denudation**: The oocytes along with cumulus corona cells are isolated from the follicular fluid by screening the small portion of fluid under dissecting microscope. Surrounding cumulus corona cells are stripped of to prepare the oocyte for fertilization as described in detail in Chapter 1 Materials and Methods. An oocyte selection may be performed prior to fertilization to select oocytes with optimal chances of successful pregnancy.

6. **Sperm preparation**: In the meantime, semen sample is processed. Live sperm are separated from seminal fluid in a process called sperm washing as described in Chapter 1 Materials and Methods.
7. **Fertilization**: The sperm and the oocyte cumulus complexes are incubated together at a ratio of about 75,000:1 in a culture media for the actual fertilization to take place (conventional IVF). In certain situations, **such as low sperm count or motility**, a single sperm is injected directly into the oocyte using technique intra-cytoplasmic sperm injection (ICSI) as described in Chapter 1- Materials and Methods.

8. **Embryo culture**: The oocytes injected with sperms are cultured in special embryo culture medium at 37 °C in an atmosphere of 5 % CO₂ and left for about 48 hours until the embryo consists of six to eight cells.

9. **Embryo selection and transfer**: Good quality embryos are selected from a cohort and transferred to the patient’s uterus with the intention of establishing a successful pregnancy by Embryo transfer procedure is described in methods.

Several embryos may be passed into the uterus to improve chances of implantation and pregnancy.

Initially IVF technique was conceived to treat the patients having competent gametes and uterus but blocked or absence of fallopian tubes (Female tubal factor infertility). However, after the birth of first IVF baby, it is employed as a major method for treating male infertility i.e. below normal concentration (oligo-zoospermia), motility (astheno-
zoospermia), and morphology (teratozoopermia) of sperms or sperms having difficulty in penetrating the egg.

IVF is also employed in 1) **In egg donation program**: where the women has a competent uterus but cannot produce egg. In such cases donor oocyte is fertilized with the sperm of intended father to produce an embryo and the resulting embryo is transferred to the uterus of intended mother. 2) **Surrogacy**: where the woman has a competent egg but uterus is not competent or is absent. In such case the eggs from intended mother is fertilized in vitro by sperms of intended father, and the resulting embryo is transferred to the uterus of surrogate mother to grow till birth. 3) **Unexplained infertility**: where the women fail to conceive by conventional therapy. Unexplained infertility is a condition when abnormalities are likely to be present but not detected by current methods.