# Chapter 9

# In-vitro Fertilisation

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## 10.1What is IVF Technology?

In-vitro fertilization popularly known as IVF technology is one of the most successful forms of assisted reproductive technologies that are available today to assist couples who are finding it difficult to have children. It is a simple process in which the egg of a woman is fertilised with a sperm in a medically controlled laboratory under artificial conditions (literally in a glass). The fertilised embryo is then transferred into the uterus for growth. There is a systemic method for carrying out this process in numerous hospitals all over the world.

The term *In-vitro,* from Latin word meaning in glass is used, because early biological experiments involving cultivation of tissues outside the living organism, from which they came, were carried out in glass containers such as beakers, test tubes or Petri-dishes. Today, the term *in vitro* is used to refer to any biological procedure that is performed outside the organism it would normally be occurring in, to distinguish it from an *in vivo* procedure, where the tissue remains inside the living organism within which it is normally found.

## 10.2 Conditions that might require IVF treatment

### 10.2.1 Infertility

Infertility is usually defined as the inability to get pregnant after trying for one year of regular sexual intercourse without the use of contraceptives. Or not being able to carry a pregnancy to term and have a baby. This could be as a result of both male and female factors as indicated by Chan and Tucker (1991).

### 10.2.2 Tubal Blockage or failed tubal reversal

Tubal blockage or tubal occlusion (the medical term) is defined as the mechanism by which tubal ligation procedures prevent pregnancy.

Tubal blockage prevents sperm from being able to reach an egg and also prevents eggs from being able to reach the uterus. When tubal sterilization is performed, tubal blockage is intentional. Tubal blockage also occurs due to disease conditions and results in involuntary infertility. Whether intentional or resulting from disease, tubal blockage can often be corrected with reconstructive tubal surgery (Fishel and Malcolm, 1986). http://www.tubal-reversal.net.

### 10.2.3 Endometriosis

Endometriosis is defined by Live science as a disorder in which the tissue that lines the uterus, the endometrium, grows outside of the uterus. The cause is not known, but there are postulations. The condition leads to pain, internal scar tissue and infertility problems (Cheng et al., 2008). http://www.myhealthnewsdaily.com

### 10.2.4 Cervical Factor

Cervical disease occurs when the cervical mucus is insufficient, too thick or thin, or hostile to sperm. When anti sperm antibodies are present in the cervical mucus, they can incapacitate or destroy sperm. Anti sperm antibodies can also be produced by a male to his own sperm. Cervical factors could either be physical problems or mucus related problems. The physical problems of the cervix are more related to recurrent miscarriages than a cervical evaluation. The mucus related problems usually involve three possible issues

1) There is not enough mucus for the sperm to survive.
2) The mucus is too thick for sperm to survive.
3) The mucus contains sperm antibodies.

http://www.seattleivf.com/cervical-factor-infertility.html

### 10.2.5 Pelvic Adhesions

Pelvic adhesions can also be commonly referred to scar tissue. Adhesions are areas of fibrous tissue that are formed as a result of a healing process which remained after the original inflammation or trauma has healed (Metwally et al., 2008). Adhesions can cause different organs in the abdomen or pelvis to become stuck together thus distorting the normal pelvic anatomy or cause decreased mobility and function. Therefore, pelvic adhesions can be a cause of infertility. For example, an adhesion may affect the chances for a woman to get pregnant if it becomes more difficult for the egg to enter the fallopian

tube at the time of ovulation. Adhesions will sometimes form a barrier between the ovary and the fallopian tube (Kodaman and Arici, 2007). http://www.ivf1.com/adhesions-infertility

### 10.2.6 Male Factor

Infertility in men is often caused by problems with making enough normal sperm or getting the sperm to reach the egg. Problems with sperm may exist from birth or develop later in life, due to illness or injury. Some men produce no sperm, or produce too few sperm ( *oligospermia*).

Other problems include:

- Sexual dysfunction.
- Hormonal or endocrine problems.
- Infection.
- Congenital problems. Birth defect, such as absence of the tubes that carry the sperm ( *vas deferens*).
- Genetic/chromosomal problems.
- Anti sperm antibody problems.
- Retrograde ejaculation (sperm going into the bladder).
- Varicoceles, spematoceles, or tumors of the testicles.
- Lifestyle can influence the number and quality of a man's sperm.
- Alcohol and drugs can temporarily reduce sperm quality.

2

- Environmental toxins, including pesticides and lead, may cause some cases of infertility in men. All these factors have been discussed extensively by workers such as Cooper et al., (2010), Cobo et al., (2012), Wainer et al., (2004) as well as earlier reports by Kruger et al., (1986, 1987) and WHO (1999, 2010).

### 10.2.7 Unexplained Infertility/failed conventional therapy

After a standard fertility work up fails to provide an explanation for a couple's infertility the diagnosis is referred to as unexplained. By definition, these couples have unexplained infertility. If you've been through a number of tests for infertility, yet there's no conclusive answer to why you and your partner haven't managed to get pregnant, you may well be given the diagnosis of unexplained infertility.This means that either nothing really is wrong with you or your partner – or that the current available tests are not good enough to pinpoint the problem that is preventing you from falling pregnant

### 10.2.8 Pre-implantation Genetic Diagnosis (PGD) for Single Gene Defects

This technique is used by most IVF providing clinics to test an embryo without damaging it. This is done by first removing one cell (blastomere) using a technique known as embryo biopsy. The gene of interest is copied many times using the polymerase chain reaction (PCR) and then genetic probes are used to explore the genetic makeup of the embryo. Li et al., (1988).PGD is the only reliable method for family balancing / gender selection, detecting certain genetic disorders such as haemophilia A and muscular dystrophy, both of which are linked to X chromosome. PGD technique can be used to detect possible reasons for multiple miscarriages. Handyside *et al.*, (1990), Handyside *et al.*, (1992). PGD is an early form of prenatal diagnosis and determines the genotype of an embryo before implantation takes place to avoid the implantation of disease embryos.

## 10.3 A Brief History of IVF

Chang (1959) was first to make an IVF attempt with animal model. It however took another seven years for Edwards et al., (1966) to publish successful human Oocyte fertilization (Thomas, 2010). The first ever pregnancy conceived with assisted reproductive technologies (ART) in humans was achieved in Australia (De Kretzer *et at.*, 1973). This pregnancy however resulted in miscarriage. After an ectopic pregnancy (Steptoe and Edwards, 1976) Steptoe and Edwards (1978) succeeded in a subsequent attempt to generate an offspring from in vitro fertilized human eggs. The first IVF baby was Louise Brown, born at 11:47 p.m. on July 25, 1978 at Oldham General Hospital, Oldham, England through a planned caesarean section. She weighed 5 pounds, 12 ounces (2.608 kg) at birth. Dr. Patrick Steptoe, a gynaecologist at Oldham General Hospital, and Dr. Robert Edwards, a physiologist at Cambridge University, had been actively working on finding an alternative solution for conception since 1966.

## 10.4 Basic steps involved in In-vitro fertilization

### 10.4.1 Ovarian Hyper stimulation

**10.4.1.1. Super ovulation**:

The ovaries are stimulated for egg production. Ovarian hyper stimulation (also called controlled ovarian hyper-stimulation)is where a regimen of fertility medications is used to stimulate the development

of multiple follicles of the ovaries in one single cycle, resulting in super-ovulation (release of a larger-than-normal number of eggs). It may be used as a part of *in vitro* fertilization (Pauli et al., 2009). Treatment cycles are typically started on the third day of menstruation.

**10.4.2 Oocyte / egg retrieval**.
Once the follicles appear mature and are ready for ovulation, 5000 unit of human chorionic gonadotropin (H.C.G) injection is administered. Eggs are retrieved between 33 – 35 hours after the administration of the injection (Pauli et al., 2009). Two methods are used for egg retrieval.

**10.4.2.1.** ***Laparoscopy:*** Under general anaesthesia, the eggs are retrieved from the follicles using a fine suction needle under laparoscopy (Rutherford et al., 1988).

**10.4.2.2.** ***Trans-vesical Ultrasound Directed Oocyte Recovery (T.U.D.O.R.).*** Here eggs are retrieved from the follicles under local anesthesia. The suction probe is guided using ultrasound through the bladder. The advantage of this method is that eggs can be retrieved from an inaccessible ovary even if it is covered by adhesions.

## 10.5 Egg and sperm preparation
In the laboratory, the identified eggs are stripped of surrounding cells and prepared for fertilisation. An oocyte selection may be performed prior to fertilisation to select eggs with optimal chances of successful pregnancy. In the meantime, semen is prepared for fertilisation by removing inactive cells and seminal fluid in a process called sperm washing. If semen is being provided by a sperm donor, it will usually have been prepared for treatment before being frozen and quarantined, and it will be thawed ready for use.

**10.5.1 Sperm retraction**
**10.5.1.1Testicular sperm extraction (TESE)**
Testicular Sperm Extraction (TESE) is usually performed by a Urologist who specializes in Male Fertility. Approximately 40% of men who produce no sperm have some sort of tubal obstruction usually in the Vas Deference. Men who have had hernia repairs are usually at high risk for having obstructions created from surgery.

Some men, especially those with mild forms of Cystic Fibrosis are born without the Vas Deference. In these cases, there is usually sample sperm in the testis (epididymis). An urologist can simply extract sperm directly from the testis with a special syringe. The eggs extracted from IVF can then be fertilized via Intra-cytoplasmic Sperm Injection ICSI using these sperm. ICSI (Intra Cytoplasmic sperm injection) involves injecting a single sperm directly into an egg to fertilise it

## 10.6 Fertilization

The sperm and the egg are incubated together at a ratio of about 75,000:1 in the culture media for about 18 hours. In most cases, the egg will be fertilised by that time and the fertilised egg will show two pronucei. In certain situations, such as low sperm count or motility, a single sperm may be injected directly into the egg using (ICSI). Intra-cytoplasmic Sperm Injection (ICSI) is a procedure in which a single sperm is isolated and directly injected into the egg, creating fertilization. This technique was revolution when finally mastered in the late 1990s. Lanzendoff et al. (1988), Ng et al., (1991), Yanagida et al., (1999) and Yanagida et al. (2001). Prior to this, men who produced few sperm or sperm with low morphology (shape) or motility (swimmers), required donor sperm since their sperm may not have had the capacity of burrowing through the egg's shell appropriately. With ICSI, we do not have to worry about burrowing through the egg's shell, as a syringe will contain a single sperm and this is directly injected into the egg and as stated above, fertilization is achieved.

## 10.7 Reasons for ICSI

- Sperm completely absent from the ejaculate (azoospermia)
- Sperm present in low concentrations (oligospermia).
- Poor sperm motility (asthenospermia)
- Poor sperm morphology (teratospermia)
- Sperm retrieved by surgical techniques (for example TESA, TESE)
- Problems with sperm binding to and penetrating the egg

Antisperm antibodies

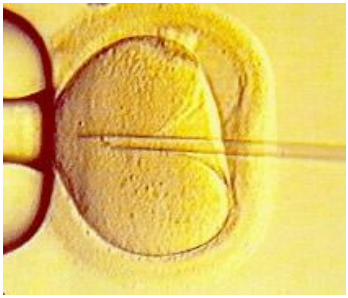

**Figure 1:** injection of a single sperm into an oocyte (ICSI).
ICSI: Image taken by Andy Glew. Essex Fertility Centre, United Kingdom. www.ivf.net

## 10.8 Embryo culture

Typically, embryos are cultured until having reached the 6–8 cell stage three days after retrieval. Embryos are placed into an extended culture system with a transfer done at the blastocyst stage at around five days after retrieval, especially if many good-quality embryos are still available on day 3. Blastocyst stage transfers have been shown to result in higher pregnancy rates.

**10.8.1 Assisted Hatching (AH)** - Embryos sometimes have shells that surround them that look very thick. These thickened shells may not allow the embryo to "hatch", which wouldnot allow for implantation and pregnancy. These thickened shells are notoriously encountered in Poor Responders and in women over the age of 38. The shell can be thinned out either with a laser or an acid solution to make it easier for the embryo to hatch.At early stages of development the human embryo is protected by a surrounding two layer coating – the zona pellucida. As the embryo develops further, this coating becomes thinner and at the blastocyst stage the thin coating is broken off and the embryo "hatches" such that it can implant into the uterine wall. Madelon and Fulco (2011). Cultured embryos are known to hatch and implant at a lower rate than naturally developed embryos in vivo. This lower hatching rate could be attributed to thicker zone pellucida. It is therefore suggested that making a hole in or thinning this outer layer may help the embryo to "hatch" thus increasing the rate of implantation. In 1990, (Cohen 1991) such an assisted hatching

procedure was developed. A review of hatching procedures was reported by Das et al., (2009).

### 10.8.2 Embryo transfer

The embryos are usually selected on day 3 or day 5 after retrieval. The number of embryos chosen for transfer will depend on a number of factors such as the maternal age, quality of embryos and results of previous cycles. After the transfer is complete the catheter is checked to ensure that none of the embryos are left behind. Embryo transfer can cause mild cramping. After transfer, the woman may get dressed and leave after a brief recovery period. A pregnancy test will be done twelve to fourteen days after the transfer, regardless of the occurrence of any uterine bleeding. The transfer of several embryos increases the probability of success. A multiple embryo transfer also increases the risk of a multiple pregnancy. Any multiple pregnancies carry an increased risk of miscarriage(s), premature labour and premature birth as well as an increased financial and emotional cost.

## 10.9 Pre-implantation Genetic Diagnosis (PGD)

This is a procedure where a specific genetic defect can be determined in embryos. A Day 3 embryo usually has 8 cells and one of these cells is extracted and analyzed for the specific defect. For example, a couple if both are carriers for Cystic Fibrosis (CF), meaning they are at high risk for passing CF to their children. The cell that was extracted from the embryo is analyzed for CF, and if the cell does not have this genetic defect, it is assumed the embryo does not and vice versa. Only those embryos that had the normal cells would then be transferred back into the uterus to allow implantation and pregnancy to occur with a non-afflicted baby.

## 10.10 Pre-implantation Genetic Screening (PGS)

This is a procedure used when the couples simply want to make sure the embryos being transferred back are chromosomally normal. The biopsy can either be performed on Day 3 or Day 5 blastocysts (embryo). The advantage of doing a day 5 trophoectoderm biopsy is that multiple cells (not just one that is done on Day 3) can be biopsied, usually from the area of the blastocysts that is destined to be the placenta. Trophoectoderm biopsy is a new technique for genetic screening of embryos. The technique involves taking cells from the

outer layer of the embryo on day 5 or 6 of development. Successful pregnancies after this technique have been reported by some workers such as Dokras et al., (1990), Anderson et al., (2007). Presently, there are two ways to screen embryos, either through Flourescent In-Situ Hybridization (FISH) or Comparative Genetic Hybridization (CGH). FISH involves involves a single cell biopsy, cell spreading and fixation of the nucleus on a slide, followed by probe hybridization for a limited number of chromosomes. Therefore, FISH cannot check all of the chromosomes, so only a few are checked. CGH can however, check all 46 (including sex) chromosomes and is becoming commercially available. Both of these procedures only check for the number of chromosomes, which can avoid genetic defects such as Down's syndrome. With a Day 3 transfer, the resulting fresh blastocyst can be transferred back fresh on Day 5. If one desires to do CGH, the biopsied embryo must presently be frozen because it takes a few weeks to get the results back. Then, the normal embryos are thawed and transferred back. As several articles are pointing out however, the biopsied cells that are being screened with these two technologies are not necessarily representative of the cell itself. Wells and Sherlock (1998), Wells et al., (1999), Werlin et al., (2003), Wells et al., (2008), Wilton (2005) and Wilton et al., (2009). Some "abnormal" embryos seem to "self-correct" and become normal babies and vice versa. Pregnancy rates also appear to be lower when the embryos are biopsied like this. Although CGH is more complete than FISH for checking chromosomes, CGH is in its infancy and requires at present the freezing of the biopsied blastocyst with eventual thawing of the "normal" blastocyst for transfer. At present, PGS remains controversial in the fertility world, and is not presently recommended by the community for simple screening purposes.

## 10.11 Embryo Freezing & Thawing

Embryo Thawing: Cryopreservation of embryos has always been an important tool in an IVF program (Mandelbaum, 2000). It enables a precautious policy for embryo transfer, lessening the chance of multiple pregnancies, knowing that the embryos not transferred will be available for subsequent thawed cycles (Mandelbaum, 1995). This also establishes a cumulative pregnancy rate, increasing the overall chances for patients to conceive per IVF cycle, (Queenan et al., 1997) as well as helping patient management with complications such as Ovarian Hyperstimulation Syndrome (OHSS). However, results from

frozen / thawed cycles are often disappointing with success rates usually around half that of a fresh cycle (Menezo et al., 1997).
Embryos are placed in a series of solutions to draw some of the water out by osmosis and then add a cryoprotectant. The removal of water helps prevent the formation of damaging ice crystals and the cryoprotectant protects the embryos during the freezing process. The embryo freezing process takes approximately 3 hours. The temperature is slowly decreased to $-36^0$ C and then plunged into and stored in liquid nitrogen at $-196^0$C for long-term storage.Embryo thawing is the reverse of the freezing process, and involves warming the embryos to room temperature to allow the transfer back into culture media at $37^0$C in an incubator. The embryos are then ready for transfer to the uterus. The embryos are thawed either the day before or on the day of the scheduled embryo transfer. Embryo thawing takes approximately 2 hours. Sometimes individual cells within the embryo are damaged by the freezing process. Embryos with some freeze damage can still go on to produce a healthy pregnancy however the more the embryo is damaged the less likely it will be for the embryo to develop. Sometimes all of the cells within the embryo are damaged. In this situation the embryo will not be transferred.

## 10.12 Lysed Cell Removal (LCR) after freezing - thawing

The process of freezing and thawing can be fairly harsh on the embryos and often not all of the cells or embryos survive. Lysed Cell Removal is a new technique that has been shown to dramatically increase the implantation potential of embryos that have been damaged bythawing. The technique known as Lysed Cell Removal (LCR) is giving improved results. It works by making a small hole in the zona pellucida with acid or laser then removing the cells that were damaged by the freezing which are thought to either disrupt the development of the embryo or produce negative factors as they degenerate.

## 10.13 IVF Ethics

IVF has always been controversial; Patrick Steptoe and Robert Edwards were faced with scores of people claiming things like ' they were playing God' and that 'any babies produced would not have a soul'. Fortunately when Louise Brown was born and people saw a healthy baby, many of these issues were forgotten.

However, as IVF technology develops more and more ethical questions are raised. IVF is now clearly accepted by the majority of the population and looking back on recent IVF history it does seem that ethical considerations and opinions are changing.

Who should be responsible for funding IVF? Patients? Insurance companies? Healthcare systems? How long should embryos be allowed to be frozen? If they go past that time should they be destroyed? Should frozen embryos be destroyed if patients stop paying the storage fees? Pre-implantation Genetic Diagnosis (PGD) is used to test embryos prior to transfer into the uterus. Part of the embryo is used for this procedure and is removed from the embryo. This selection of "healthy" embryos is sometimes referred to in news articles as "Designer Embryos.

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## 10.15 Further reading

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