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### *In vitro* fertilization: Facts in medical sciences

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#### ABSTRACT

*In vitro* fertilization (IVF) is one of the assisted reproductive technologies in the field of medical sciences. Fusion of collected egg from female and sperm from male done in a culture media under aseptic condition in laboratory is called as IVF. This technique is one of the gifts of science towards the human society and mainly those who are facing problem in reproduction due to having either male or female defective reproductive systems. In this present study, factors to consider during IVF, steps followed in this technology, need of IVF are discussed. Risk of multiple birth and phenotypic changes to the newborn due to IVF are also well reviewed. And different types of instruments used during this process are focused. Few ethical and legal issues arising during this IVF process are shown as well. We also emphasize that part of IVF.

## 1. Introduction

*In vitro* fertilization (IVF) is a complex and expensive process which results in the birth of more than 200 000 babies. IVF was previously coined as “test tube babies”. IVF involved the fertilization of eggs and sperms outside the body in a laboratory and embryos are formed which are then placed in the uterus. IVF, a treatment is done by only 5% of infertile couples[1].

Infertility affects more than 10% couples worldwide. It leads to depression and it is physiologically stressful. To overcome this painful situation, IVF was introduced to the world like a boon. Infertility is of two types, one is female infertility and another is male infertility. Female infertility is often due to the damage in fallopian tubes, and male infertility is due to the less sperm count. Infertile couples want to have a child who is related to them biologically. So, they want to go for IVF, which is a process of fertilizing an egg in laboratories[2].

The culture of the egg which is fertilized (zygote) is kept in the growth media for 2-6 days, then it is transferred to the woman's uterus for implantation. At a time, in the woman's uterus 2-4 eggs were placed and this attempt was called a cycle. “Test tube baby” is the term used to refer children who are conceived by this technique. Louise Brown was born in England in 25 July, 1978 who was the first test tube baby[1].

There are factors to consider in IVF: (1) Age: The success rate is decreased according to the age of the woman. Woman whose age is less than 35 has more chances of success by implementing this technique; (2) Multiple births: It is an important factor in this technique. About 63% of the babies are single, 32% of the babies are twins, and 5% of the rest are triplets or more; (3) Less need of surgery: As we know, many women suffering from blocking of

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fallopian tube prefer IVF, so it can reduce surgery rate.

The various techniques that increase the chance of success are as follows: (1) Intra-cytoplasmic sperm injection (ICSI): In this technique, the sperm is directly injected into the ovum to increase the success rate. For a better solution of male infertility in 1992, this technique was introduced. Nearly 50% of ICSI conceptions in humans were successful, and from the studies related to animals, abnormalities were indicated which are calcium oscillations alterations, and delay in sperm chromosomes decondensation[3]; (2) Endometrial receptivity array: This technique determines the best time for implantation of the embryo. One-third of the failure of IVF is due to failure in implantation and these techniques reduce the failure rates. It is thought that the specific time window opens before 4–6 days and closes after 9–11 days of progesterone administration, creating a physiological window which is receptive for limited days, *i.e.* 19–24 days of the menstrual cycle in women[4]; (3) Preimplantation genetic diagnosis: It was introduced at the beginning of the 1990s for the genetic diagnosis of embryos whose parents have genetic disorders. This increases the chances of genetic disease-free child[5]; (4) Embryoscope: This technique allows the embryologists to watch the development of embryo in the incubator with time-lapse technology. The evaluation of the embryo could be performed with this kind of system where the embryos are not eliminated from the incubator, decreasing the risks of stress due to variations in temperature, exposure to light, high exposure to oxygen and changes in pH in the medium of culture[6]; (5) Endometrial scratch: Endometrial scratching, also called as endometrial injury, is now a technique used to increase the implantation probability in women who are conceiving through IVF. The luteal phase is the common time period for endometrial scratching before the embryo transfer cycle. This technique is used to improve success rates in couples with repeated implantation failures[7].

## 2. Steps of fertilization

### 2.1. Step 1: Gonadotropin-releasing hormone (GnRH)-agonist protocol

The GnRH agonist protocol is designed to suppress the release of pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH) by inactivating the pituitary receptors[8].

### 2.2. Step 2: Retrieval of egg

Retrieval of egg is done in an operating room under the guidance of the embryologist. With the help of trans-vaginal ultrasonography, ovarian follicles are aspirated by a needle. The embryologist scans the follicular fluids to locate the eggs, which are then kept in an appropriate media and cultured until insemination in an incubator.

### 2.3. Step 3: Fertilization

Standard insemination is a process of transfer of motile sperms (50 000-100 000) to a plate having eggs. In case the sperm parameters are not normal, ICSI technique is then used for the fertilization of the mature egg. In the ICSI technique, with the help of high power microscope, the embryologist transfers single spermatozoa to the egg cytoplasm by using fine glass micro needle.

### 2.4. Step 4: Culture of embryo

After 16-18 h of insemination zygotes are formed, they are cultured in a special formulated culture media. When sufficient number of embryos are good in developing stage, they are selected for blastocyst formation in a specially designed culture media. Higher potential for implantation develops at this stage. To prevent multiple pregnancies on day 5, few in number of embryos are transferred. If the growth of embryos is poor, then they are transferred on day 3 as the chances for good blastocyst development is reduced.

### 2.5. Step 5: Quality of embryos

The embryologist and the physician select the best embryo for transfer. The transfer of the embryo is on the basis of the appearance and development of the embryos that are analysed and recorded by the embryologist.

(1) Day 3 embryo transfer — Cleavage stage embryos are the embryos having 4-8 cells after oocyte retrieval. The embryologist scans the number and symmetry of the cells. The embryo is transferred if there is no fragmentation.

(2) Day 5 embryo transfer — It is also called as blastocyst embryo. At blastocyst stage, the embryos are well formed resulting in many cells containing fluid inside it. The quality of the embryos depends on their expansion. More expanded better is the quality of the embryo[9].

### 2.6. Step 6: Embryo transfer

Embryo transfer depends on the time relationship between the donor cycle and the recipient cycle. Synchronous transfer occurs when the interval between the ovulation and recovery in the donor and ovulation and transfer in the recipient is similar[10,11].

## 3. Why should people go for IVF?

People go for IVF because of infertility related problems.

### 3.1. Female infertility

Causes of female infertility vary from one place to another. According

to World Health Organization, causes of infertility in females are: tubal factors, polycystic ovarian disease, endometriosis, and chromosomal abnormalities[12].

### 3.2. Male infertility

Male infertility is triggered by the following causes: defective sperm, azoospermia or primary spermatogenic failure, hormonal disturbances, and poor semen quality. However, the main causes of male infertility are: microdeletions of the Y chromosome, Klinefelter syndrome, and mutations of cystic fibrosis transmembrane conductance regulator gene, but majority of cases remain idiopathic[13].

There are two ways for diagnosis of male infertility: (1) Damage of the DNA of the sperm has been recognized as important biomarker of male infertility. Sperm chromatin dispersion test in semen, the most useful test is adopted for determining sperm DNA damage in male[14]; (2) Scrotal sonography, hormone and semen measurements are useful in diagnosis of male infertility[15].

## 4. Final oocyte maturation for GnRH agonist in GnRH antagonist IVF treatment cycles

GnRH antagonist has been used for pituitary desensitization in IVF treatment cycle. Due to some particular activities of GnRH antagonist, quick and reversible response, GnRH agonist as a mid-cycle bolus dose varying from 0.1-0.5 mg and human chorionic gonadotropin (hCG) administration could be used to induce oocytes final maturation triggering.

HCG administration is an effective trigger for maturation of final oocytes in antagonist of GnRH which is co-treated with IVF treatment cycles than GnRH agonist. Some investigators suggested that to reduce ovarian hyperstimulation syndrome without compromising pregnancy rates, GnRH agonists should be administered rather than hCG, triggering their oocytes for final maturation.

GnRH agonist induces endogenous LH and FSH surges which might stimulate the natural mid-cycle LH surge. GnRH agonist is used for the maturation of oocyte triggered in those women who are donating the oocytes to the recipient and also in women who freeze their eggs according to their wish for fertility preservation[13].

## 5. Ovarian response for hyperstimulation during IVF and its effect on salpingectomy

Salpingectomy is a treatment operation in case of hydrosalpinx and ectopic pregnancy. Because of the close relationship between the mesosalpinx and ovarian blood supply, salpingectomy may compromise ovarian response.

Ovarian response of salpingectomy group compared with that of

the control group demonstrated that the total dose of gonadotropin used after salpingectomy[16] was increased significantly compared with the control group.

Ovarian responses of the unilateral salpingectomy group compared with that of the bilateral salpingectomy group did not show a statistically significant difference in the total stimulation dosage.

The ovarian response comparison between ipsilateral ovary and also contralateral ovary in unilateral salpingectomy showed that more oocytes retrieved showed a significant decrease and also had a substantial evidence of heterogeneity.

The result of evidence-synthesis suggests that salpingectomy impacts the response of ovary during its more stimulation in IVF. Ovarian blood circulation can be a theoretical decrease in ovarian blood perfusion after salpingectomy, since uterine arteries are taken through the branches and vascular arcade of mesosalpingeal[16].

## 6. Sperm and its characteristics varying concomitantly after scrotal heat stress and its specific role in IVF and development of embryo

The scrotal isolation miniature is a valuable developmental tool with the ability to be implemented to many areas of research. Thus, the thermic insult generates a variety of morphological modifications of sperms which may be related to performance of the male reproductive system. Many oocytes were subjected to IVM which were monitored with semen and the degree of development of cleavage and blastocyst were correlated with pre-insulation duration and the control group. The mean percentage of cleaved oocytes were diminished when they were compared with the pre-insulation duration after scrotal insulation between 28 and 42 days. When compared with control group, the cleavage rates were lower. The change in sperm-head morphometric measures were induced by scrotal heat stress at fluctuating times after insulation and chromatin integrity. The roundness of the sperm cells is due to changes in organization of chromatin which are induced by heat stress. Sperm and its traits were altered simultaneously and these played a specific role in IVF and production of blastocyst[17].

## 7. Temperature of embryo *in vitro*

It was reported that the pregnancy rates were increased when the temperature of the incubator was cooler than 37 °C. At a temperature, lower than 37 °C the fertilization rates were higher[18].

## 8. Recombinant FSH and its ovarian response

The purpose of controlled ovarian stimulation with gonadotropins for IVF/ICSI is to get a proper number of competent oocytes with less risk for women. The present study demonstrates the relationship

of anti-muller tube hormone (AMH)-dependent dose-response between number of oocytes retrieved and exogenous FSH for IVF.

The present-day study also suggests that there is an inverse relationship between the FSH dose and fertilization and blastocyst-to-oocyte rates, for both low and high AMH stratum in women.

The high AMH stratum in women had more significant blastocyst available for transfer than women with low AMH stratum. The increased yield of oocyte, in not either of the stratum at higher gonadotropin, results in parallel increase in blastocyst number[19].

## 9. Angiogenic markers in pregnancies conceived through IVF

Many studies suggest that pregnancy conceived through IVF carries higher risk for obstetric complications. The reason for obstetric complications is not clear, but it has been suggested that aberrant placentation may be pathogenic at initial stage. IVF placentas have also been shown to have altered glucocorticoid metabolism and expression of placenta genes when compared to spontaneous conceptions. Previous research has demonstrated that preeclampsia and other pregnancy-induced hypertensive disorder are associated with alternation in angiogenesis, which is detected by changes in the levels of circulating and angiogenic and antiangiogenic protein. Angiogenic markers are used as predictors of preeclampsia and other disorders of placental dysfunction.

Compared with spontaneous conception, IVF pregnancies have altered levels of placental growth factor and soluble fms-like tyrosine kinase-1. Angiogenic markers in pregnancy that were conceived through IVF are altered when compared with those pregnancies which were conceived spontaneously. Antiangiogenic markers are fast becoming an important part of targeted therapy to treat disorders of placental dysfunction[20].

## 10. Negative effect of endometrioses on IVF

In severe endometrioses, cycle of cancelation rates is higher. Retrieved rates, number of embryos, implantation rates is lower. Thus, with increase in endometrioses, the rate of IVF decreases. Ovarian reserve, ovarian response during gonadotropin stimulation to implementation and pregnancy rates are negatively influenced by severe endometrioses[21].

## 11. Risk of multiple birth through IVF may caused by donor eggs

Adverse foetal and infant outcomes are associated with multiple-gestation pregnancies. These also lead to the increased risk of maternal mortality and morbidity. The association between lower risk of multiple birth and maternal age are impelled by factors

which are accompanied with the uterus, the egg, or both uterus and egg; and these factors are also eliminated at the initial stage of embryo implantation. Many studies have concluded that the age of the mother was not linked with multiple births. The risk of multiple births is more due to egg factors than uterine factors[22].

## 12. Better ways to increase IVF success

Preferring the most desirable egg, sperm and embryo are important for a healthy baby through IVF. It is said that “at the end of the day, when it comes to IVF, the embryo is the main target”[23].

The embryos are being examined by experts for structural stability of cells, symmetry, and its development after two to four days of fertilization. The scientists are now focusing on the media where the embryos grow. Scientists are searching for the proteins that are secreted by the growing embryos and also measuring the amount of oxygen that the embryo consumes[23].

## 13. Instruments used in IVF

Instruments used in IVF include: (1) Sperm counting chamber: This device is used for sperm count and motility check; (2) Oxygen controller to retrofit CO<sub>2</sub> incubators: By the help of the oxygen controller, level of oxygen is controlled and this device is compatible with CO<sub>2</sub> incubator. Air quality in the IVF laboratory is very important factor. To avoid infection through air, the high efficiency particulate air filters are used; (3) Micromanipulator: It is a device which is used to manipulate the minute instruments and needle under the microscope in order to perform delicate procedures such as fertilization of the sperm and the embryo[24]; (4) Inverted microscope: The inverted microscope is used to look very closely at the eggs and embryo in the ICSI and assisted hatching micromanipulation procedures are also done on inverted microscope[25]; (6) Incubators: It is like a temporary home for the embryos before they are implanted in the uterus to continue their life[25].

Studies show the development of human blastocyst *in vitro* in steady culture conditions can be successfully supported by a micro fluidic device. Further studies are taking place in order to elucidate whether at starting stage the embryos would be benefited from in micro fluidic devices from the culture[26].

In a new strategy, the embryos of humans from day 0 can be successfully cultured in a single media without any transfer into a new media on day 3 by a time-lapse incubator with controlled air purification system. This strategy doesn't affect the development of the embryo and also gives a stable culture conditions to the embryo[27].

A valuable alternative to laparoscopic salpingectomy to improve the pregnancy rates in patients who have failed in IVF is the

hysteroscopic placement of essure microinserts. This treatment results in increase in levels of pregnancy in patients with hydrosalpinges[28]. Human pre-implantation embryos can be transported to long distances using CO<sub>2</sub> incubator which is portable. This incubator is secure and it doesn't threaten the growth of the embryo[29].

The frozen embryo transfer detects embryonic activity by the induction of positive charges due to change in hydrogen ions with by release of carbon dioxide which is dissolved during cleavage when cellular respiration activity occurs[30].

#### 14. Ethical and legal concerns in IVF

Ethical issues include: (1) Creation of life in laboratory; (2) Discarding excess of embryos; (3) The technology is very expensive and it is not affordable to common man; (4) It bypasses the natural method of conception[31]; (5) Multiple pregnancies are a major complication of IVF. It may be harmful for foetus, newborn, mothers, families and also health care system[32].

Legal issues include: (1) Donation of sperm or uterus involves adoption before birth therefore it leads to the formation of amendments for adoptions and its laws in most countries; (2) When women carry a number of embryos which could endanger her life, the termination of one or more of the developing embryos in order to avoid risk is required, hence, the problem is faced by the doctor to terminate which embryo[31]; (3) In case of women who are only able to conceive through donor eggs, there are often conflicts between the donors regarding whether the donor has legal representation[33]; (4) The infertility specialist and the analyst exhibiting IVF should be particularly concerned about the legal issues brought up by the ratification of patients and donors.

#### 15. Conclusion

IVF is emotionally and physically stressful and also may have health risk. IVF techniques lead to the evolution of new biomarkers which are hazardous to the embryos. IVF has an increased demand in modern and urban era by adopting additional techniques such as ovarian hyperstimulation to restore multiple ova, ultrasound-guided transvaginal oocyte rejuvenation directly from the ovaries followed by concoction of ovum and sperm preparation for their culture as well as choosing of resultant embryos before they are transferred into the uterus. IVF is aiming to achieve higher success rates by transferring single selected embryos with 80% success rates[1,24].

#### Conflict of interest statement

The authors declare that they have no conflict of interest.

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