AN UPDATE ON TECHNIQUES OF IN-VITRO FERTILIZATION (IVF)

ABSTRACT:

The modern age has witnessed an enormous progress in terms of industrialization, technology development and civilization. The area of this progress has reached to grass root level and most of the population on the earth is part of this race. The increasing industrialization has produced an impact on human health in terms of stress and pollution. One of the issues closely associated with stress and bothering the human society is infertility. Increasing number of young couples are facing problem in conceiving the baby at inappropriate age. This problem has been addressed by the advancement in technology introducing *In-vitro* fertilization technique. It involves fertilization of egg with the sperm outside the body in *in-vitro* condition. It is crucial to educate the people regarding the advancements in Science and technology like IVF, in order to overcome the challenges like infertility in them. Thus, this review focuses on techniques and innovations in IVF in detail.

KEYWORDS: IVF/ ICSI/ ovarian stimulation/ embryo transfer/ risk factors/ innovations/ updates

ABBRIVIATION: ART: Assisted Reproductive Technology

IVF: In-vitro fertilization

ICSI: Intracytoplasmic sperm injection

LPD: Luteal Phase Deficiency

FSH: Follicle Stimulating Hormone

ET: Embryo Transfer

OHSS: Ovarian hyperstimulation Syndrome

AMH: Anti-Müllerian Hormone

PCOS: Polycystic Ovarian Syndrome

HCG: Human Chorionic Gonadotropin

FET: Frozen-thawed embryo transfer

ZP: Zona Pellucida

AH: Assisted Hatching

GnRH: Gonadotrophin Releasing Hormone

INTRODUCTION:

Infertility, according to NICE (National Institute of Health and Care Excellence), can be defined as: A woman of reproductive age who has not conceived after 1 year of unprotected sexual intercourse. **(Tom Treasure *et. al.,* February 2013).**

It stands as second most common problem in women of age ranging from 20 to 45 to visit a gynecologist after pregnancy. Conceiving problem is experienced by every one in seven women. About 85% of women (aged 38 and above) conceive within a year and 92% within two years, the conception rates drastically decreases to 77% within 3 years. As the age of childbearing is getting higher and more couples are delaying to start a family, infertility has become a crucial health issue. **(Caroline Lafarge, Pauline Fox, 2012)**

An increase in the number of couples seeking for the treatment for infertility is reflected by the rising number of children born after Assisted Reproductive Technology, generally termed as A.R.T. **(Jin Liang Zhu *et. al.*, 2006).**

One of the most utilized and successful A.R.T. technique is In-vitro Fertilization (IVF). In-vitro fertilization, commonly known as 'test-tube baby', is a process by which the ova are fertilized by sperms outside the body (generally in a *petri* plate). This process involves monitoring and stimulating woman's ovulatory process, removing egg or ova from the ovaries and letting sperm fertilize them in a fluid medium in the lab. These fertilized eggs are then placed back in the woman's uterus and the growth of the embryo is monitored.

In vitro fertilization (IVF) is quite common these days, with over 4 million children born following IVF treatments worldwide and it is generally regarded as a safe technique **(Is IVF safe-A review of current evidence, 2010)**

ICSI is an alternative to the IVF process. ICSI stands for Intracytoplasmic sperm injection in which a single sperm is isolated via needle and is injected into the egg which is held via pipette. ICSI is performed when the sperm count is low, or the sperm morphology is unusual.

FACTORS CAUSING INFERTILITY

Couples (about 10-20%) who are trying to conceive have a waiting period to pregnancy longer than 1 year, is the clinical definition of infertility in most developed countries **(Jin Liang Zhu *et. al.,* 2006)**. In short, the fertility factor in the individual is absent or lost. About 1 of 7 heterosexual couples is affected by infertility. The main causes of infertility are (percent figures indicate approximate prevalence):

- **Ovulatory disorders (25%)**

  The ovulatory disorders occur when a woman stops ovulating. The major symptoms of the disorder include amenorrhea i.e. cessation of period and unbalanced or irregular periods. However, in some cases, a woman will continue to have regular periods even though she is not ovulating. For these women, having troubles conceiving is their only signal of a problem.

1. **Polycystic ovary syndrome (PCOS):** In PCOS, complex changes occur in the hypothalamus, pituitary gland and ovaries, resulting in a hormone imbalance, which affects ovulation. PCOS is associated with insulin resistance and obesity, abnormal hair growth on the face or body, and acne.
2. **Hypothalamic dysfunction:** The two hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are responsible for stimulating ovulation, are produced by the pituitary gland during the menstrual cycle in a particular pattern. This pattern can be disturbed by excess stress, a very high or very low body weight, or a recent substantial weight gain or loss. This disturbed pattern affects ovulation. The major symptoms are irregular or no periods.
3. **Premature ovarian insufficiency:** This disorder usually occurs when ovarian tissues are mistakenly attacked by autoimmune response or when premature loss of eggs from your ovary occurs due to genetic or environmental issues such as chemotherapy. It results in the loss of the ability of the ovary to produce eggs, as well as a decreased estrogen level under the age of 40.
4. **Excess of prolactin:** Less commonly, the pituitary gland can cause excess production of prolactin (hyperprolactinemia), resulting in reduced level of estrogen production and which may cause infertility.

- **Tubal damage (20%)**

  When fallopian tubes become damaged or blocked, they block the path of the fertilized ova into the uterus or keep sperm from getting to the egg.

  Fallopian tube damage or blockage can be caused by:

1. Pelvic inflammatory disease, where infection occurs in the uterus and fallopian tubes due to *Chlamydia*, gonorrhea or other sexually transmitted infections
2. Previous surgery in the abdomen or pelvis, including surgery for ectopic pregnancy, in which a fertilized egg becomes implanted and starts to develop in a fallopian tube instead of the uterus.

- **Factors causing infertility in male (30%)**

  The task of deciding whether or not the semen analysis is "normal" may seem simple, but in reality it is often hardly clear-cut. For one thing, a man's sperm parameters can change on a daily basis, so one borderline normal or abnormal result may not tell the whole story. For this reason, a fertility specialist will sometimes requests the male partner to produce a second sample before deciding whether or not a man has a meaningful abnormality. Many specialists will recommend a 6-10 weeks waiting period before repeating the semen analysis. This is because the life cycle of a man's sperm is approximately 60-80 days, such that two abnormal semen analyses spaced by this time provides stronger evidence of a "true" (i.e. persistent, as opposed to transient) problem **(Klein, 2012)**.

  Teratozoospermia is the condition where the sperm are of abnormal morphology. As sperm morphology did not appear to influence blastocyst development or blasocyst morphology. Microscopic selection of sperm with "normal" morphology during the ICSI procedure allowed excellent outcomes even in samples with severe teratozoospermia **(French *et. al.,* 2010)**.

- **Uterine or peritoneal disorders (10%).**

  Endometriosis or adnexal adhesions may cause or contribute to infertility. History and/or physical examination findings may cause suspicion but these are not sufficient for diagnosis. In women with unexplained infertility, prenatal factors should also be taken into consideration.

- **Unexplained infertility (no identified male or female cause) (25%)**

  In some cases, a cause for infertility is never found. It's possible that a combination of several minor factors in both partners underlie these unexplained fertility problems.

1. In the case of unexplained infertility, the oral ovarian stimulation agents such as clomifene citrate, anastrozole or letrozole should not be offered to the woman and if she does not get pregnant even after 24 months of unprotected sexual intercourse, she should be offered with In-vitro Fertilization (IVF) treatment **(Tom Treasure *et. al.,* February 2013).**

Cases disorders found in both, the male and the female, are about 40%. Some of the disorders may include uterine or endometrial factors, gamete or embryo defects, and pelvic conditions such as endometriosis. According to the given range of causes of infertility problems, the stipulation of appropriate investigations is critical. These investigations involve semen analysis; assessment of ovulation, tubal damage and uterine abnormalities; and screening for infections such as *Chlamydia trachomatis* and susceptibility to rubella. **(Tom Treasure *et. al.,* February 2013).**

HISTORY OF IVF

Working of at least one fallopian tube is essential for the natural fertilization of an egg and a sperm *in vivo*. Non-functioning of both the fallopian tubes results in infertility in women. In late 1970s Lasley Brown, a patient with the history of nine years of primary infertility secondary to tubal occlusion came in assistance with Patrick Steptoe and Robert Edwards. It was the era where IVF was just experimental and was unsuccessful leading to miscarriage and unsuccessful pregnancy**.** Natural IVF cycle was performed on Lesley Brown. She underwent laproscopic egg retrieval, with only one egg fertilized *in vitro,* it was transferred back to her uterus. The first successful pregnancy was recorded through the IVF technique were a girl child Louis Brown was born in year 1978 **(Wang and Sauer, 2006).**

Robert Edwards was awarded Nobel Prize for Physiology or Medicine (2010) for developing IVF and embryo transfer (IVF/ET) to treat sterility in women with non-functioning oviducts.

After three months of the birth of Louis Brown, **Durga** was the first Indian and world's second human test-tube baby, born in October 1978 in Kolkata, under Dr. Subhash Mukhopadhyay.

Now, after a phase of about 37 years, more than 4 million babies have been born using IVF, and a new specialty of ART has been established with its own professional societies.

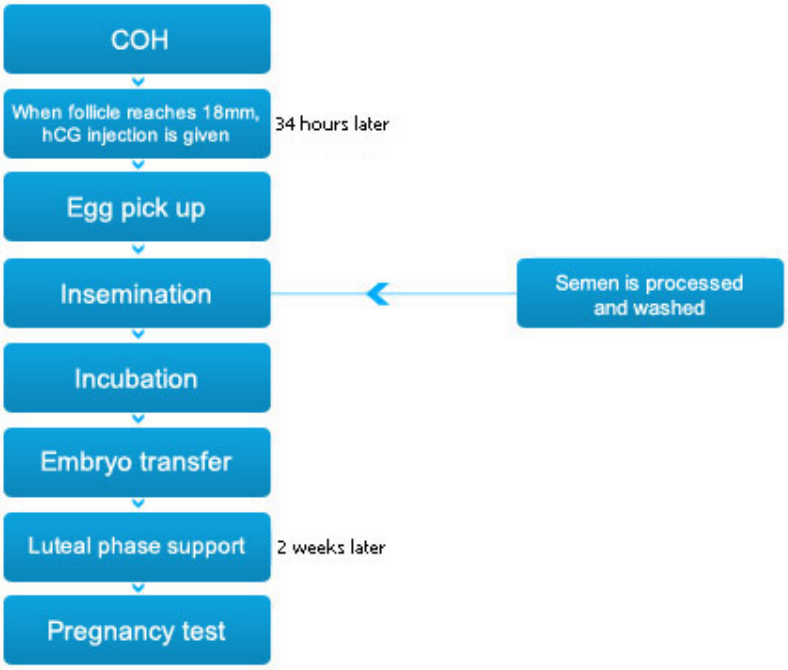
Different improvements in technique have occurred and thus the proposal has been made that IVF/ET and surgery should be regarded as complimentary techniques for the treatment of tubal infertility**. (John D Biggers, 2012).**

METHODOLOGY

IVF technique involves:

1. Ovarian Hyperstimulation
2. Egg retrieval
3. Fertilization
4. Embryo transfer
5. Luteal Phase Control

The methodology of IVF is briefly explained in the flow picture below:



(source: metro.com.my) (COH- Controlled Ovarian Hyperstimulation)

The IVF cycle.

The IVF process generally starts from the 3rd day of menstrual cycle. The IVF cycle can be carried out with or without ovarian stimulation. Usually the dosage varies with reference to the health of female.

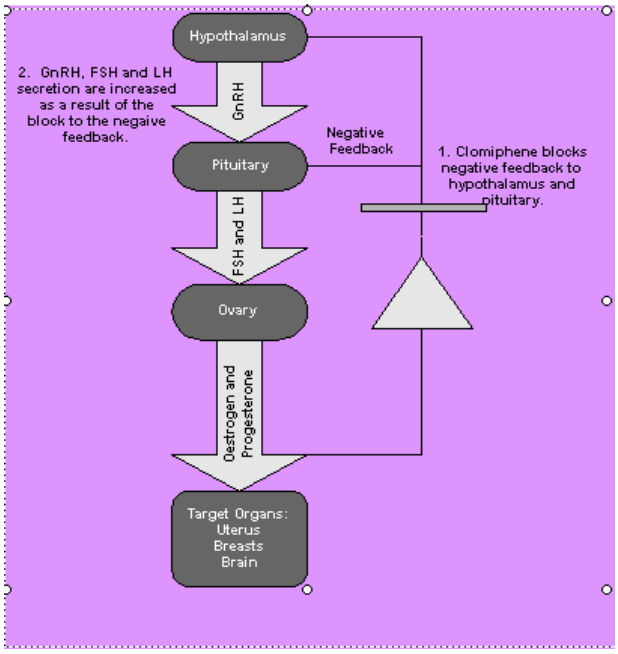
1. Ovarian hyperstimulation

Ovarian hyperstimulation is the process where the ovaries are stimulated in by hormones in order to produce more number of eggs than the normal range. As the chances of successful

IVF increases with the increase in number of ova produced, this step is taken into consideration. Hormones like follicle stimulating hormone (FSH), gonadotrphins releasing hormone and its alternatives are used **(Verberg *et. al.,* 2009).** These hormones are provided to the patient in order to provide large number of oocytes in single cycle. The hormonal injections of FSH are given every day to the patients for approximately 10 days and then transvaginal ultra sonography is performed to check the size of the follicles. If the follicle reaches required size, then the process of egg retrieval takes place.

Ovarian hyperstimulation is not necessarily required for the IVF. For instance, natural IVF cycle was performed on Lesley Brown where no hormonal stimulations were provided in order to retrieve large number of eggs. Only one egg was isolated during egg retrieval process and it was placed back in the uterus after its in-vitro fertilization **(Wang and Sauer, 2006)**, while mild ovarian stimulation requires small amount of stimulation to produce ova.

Mild ovarian hyperstimuation is another term which can be defined as the supervision of small doses for fewer days of exogenous gonadotrphins, and/or oral compounds like anti-estrogen or aromatase inhibitors for ovarian stimulation for IVF, aiming to bound the number of oocytes obtained to less than eight **(Fauser *et.al.,* 2010).**



<u>THE PATHWAY BY WHICH THE HORMONES ACTS IN THE FEMALE BODY (pics9.imagezone.org)</u>

Mild ovarian stimulation is known to be cost-effective, patient-friendly and which balances outcomes and risks of treatment **(Verberg *et. al.,* 2009).**

The advantages of mild ovarian hyperstimulation compared to that of normal ovarian hyperstimulation are stated below.

<u>Considerations related to different approaches in ovarian stimulation.</u>

Current ovarian stimulation approaches

- aiming for maximum number of oocytes
- Time consuming and complex stimulation regimens
- High costs
- Much patient discomfort
- Short-term complications—ovarian hysterstimulation syndrome (OHSS)
- Long-term health consequences uncertain
- High drop-out rates
- Supraphysiological steroid levels with possible implications
- Emphasize additional pregnancy chances from cryopreserved embryos
- Emphasize maximizing pregnancy rates per cycle**(Verberg *et.al.2009*)**

Mild stimulation approaches

- Less complex
- Less time consuming
- Cheaper (making IVF more accessible for a broader patient population)
- Reduced chances for complications
- Reduced chances for discomfort
- Reduced chances for drop-out
- Effects on oocyte quality
- Effects on endometrial receptivity
- Emphasize maximizing chances for healthy children born per started treatment at reasonable cost, patient discomfort and chances for complications **(Verberg *et.al.2009*)**

The protocol and dosages of ovarian stimulation varies with the result predicting poor or hyper-response to ovarian hyperstimuation. The age, antral follicle count and Anti-Mullerian hormone (AMH) level is taken into consideration to decide the dosage of hormone.

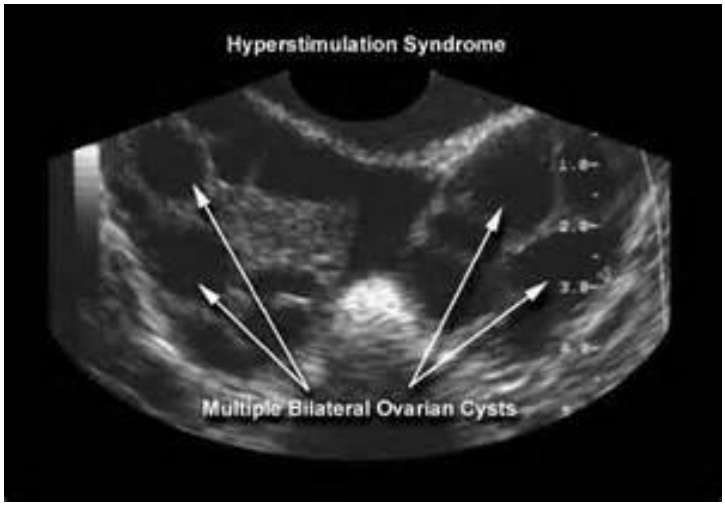
Antral follicle count is the number of antral follicles, generally in both the ovaries taken together. It can be determined by transvaginal ultra sonography. The follicles of diameter 2-6mm are counted. When there is low antral follicle count, high dosage of hormonal injections are required.

Anti-Mullerian hormone, AMH, is secreted by both, male and female. In males, they are secreted by the sertoli cells of testes during embryogenesis of fetal male and in female, it is secreted by granulose cells of ovarian follicles.

AMh level less than or equal to 0.8ng/ml predicts low response to ovarian hyperstimulation while level greater than 3.6 ng/ml predicts high response. High level of AMH creates the good chances of live birth after IVF, even in over age women.

Measuring only AMH level can however be misleading as it occurs in high levels in the conditions like Polycystic Ovarian Syndrome (PCOS). Hence transvaginal scan should also be performed to access antral follicle count **(Marca *et. al.,* 2010).**

The hyperstimulation of ovaries can also have side effect. High dose may lead to Ovarian Hyperstimulation Syndrome (OHSS). It's the syndrome in which multiple bilateral ovarian cysts are produce due to high level of hormone. It occurs when the dosage level needed for the production of eggs is more than that of the required level. It is generally mild and rarely severe **(Verberg *et.al.,* 2009).**

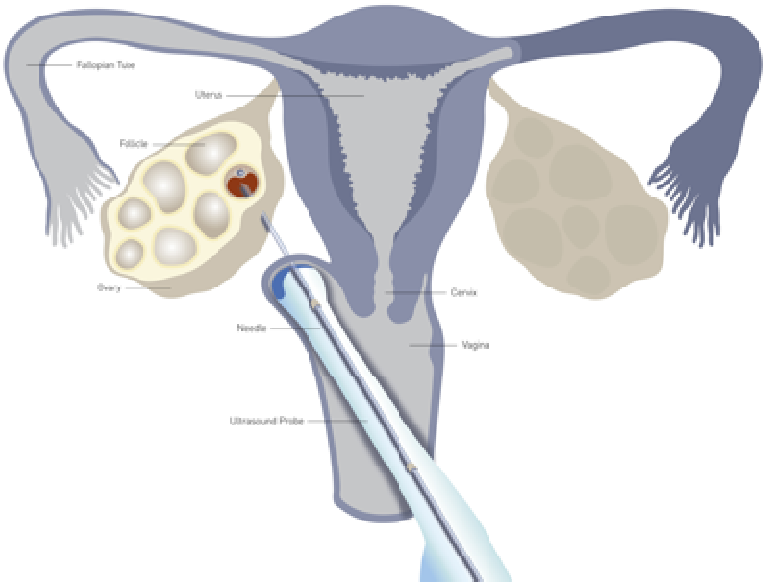


OVARIAN HYPERSTIMULATION SYNDROME (OHSS) SONOGRAPHY SCAN (trialx.com)

2. Egg retrieval

After all the required dosage of ovarian stimulation, the patient is provided with the final dose of human chorionic gonadotropin (HCG) is given to the patient. After 38-40 hours of this dose, ovulation occurs in patient. Hence, egg retrieval procedure is performed in 34-36 hours after HCG injection in order to get complete mature eggs which are yet to ovulate. Gonadotrophin releasing hormone (GnRH) agonist can be used as an alternative which eliminates the risk of OHSS, but the delivery rate decreases approximately by 6%.

The egg retrieval process includes ultra-sound guided needle which pierces the vaginal wall to reach the ovaries. This technique is performed with the help of transvaginal ultra sonography which guides the doctor in the process of egg retrieval. 8-10 eggs can be retrieved in 20-40 minutes, depending on the number of mature follicles. This process is performed under general anaesthesia. Ovarian stimulation can be increased in order to allow multiple oocyte retrieval in single cycle **(Santos *et. al.,* 2010).**



EGG RETRIEVAL PROCESS (www.reproductivemedicine.com.au)

3. Fertilization

The next step after the ova are retrieved takes place in the laboratory. The matured eggs are identified and surrounding cells are stripped off the cell. The ova are placed in the medium which is similar to the ovary fluid and it is incubated at 37^0C in order to keep all the parameters similar to that of a human body. Semen analysis for sperm count is performed. Semen sample is prepared by removing the inactive cells and seminal fluids. The process is known as sperm wash. During the process, the sperm sample is centrifuged and incubated for 5 minutes. The inactive sperms settles down and the upper portion is used which contains active sperm. In at least 30-40% of couples undergoing evaluation for infertility, a semen analysis will reveal some abnormality in at least one of the major categories by which the sperm are judged: concentration (how many), motility (what percentage of the sperm are swimming properly), and morphology (what percentage of sperm have a normal shape) **(Klein, 2012).**

The ratio in which the sperm and egg is incubated is 75,000:1. It is observed that the incubation period of 3-4 hours shows high pregnancy rate than 16-24 hours. In most of the cases, the eggs can get fertilized during co-incubation and will show two pro-nuclei. In low sperm count, a single sperm is injected directly into the egg using intracytoplasmic sperm injection (ICSI). The egg is placed in special growth medium, KSOM or mMTF medium, **(John Biggers, 1998)** incubated for 48 hours until the stage of 6-8 cells is seen. The media remains the same even if number of incubating sperm varies.

The petri dish consists of egg in KSOM or mMTF media. The test tube contains sperm from which they are collected and spread on the petri plate. This plate is left for incubation and daily observation is recorded. **(theaustralian.com.au)**

4. Embryo transfer

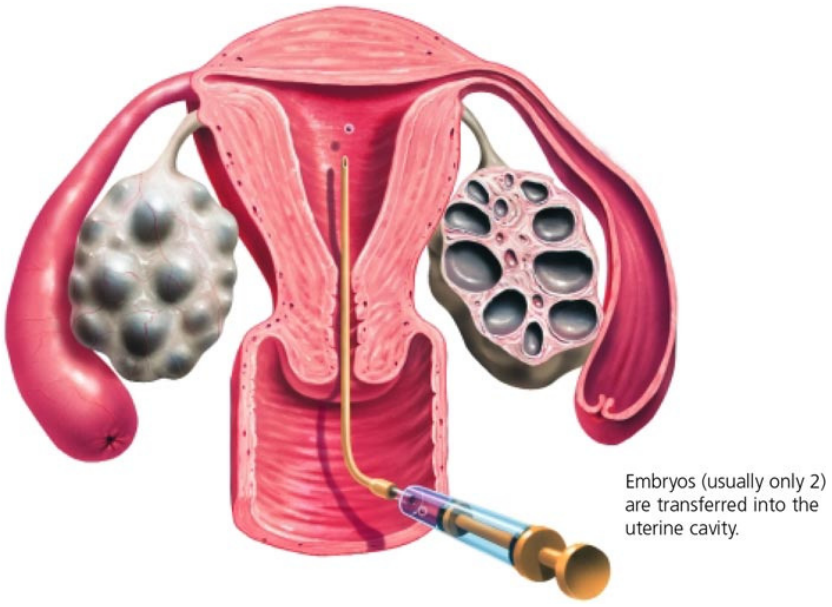
The following embryo developmental stages will be recorded in the petri plates.

Embryo development (ramsem.com)

After 36 hours of incubation, the embryo development is recorded and the embryos with blastocyst stage are removed. These embryos are to be transferred in the female uterus. If the female uterus is one of the causes of the infertility, the couple can opt for surrogacy.

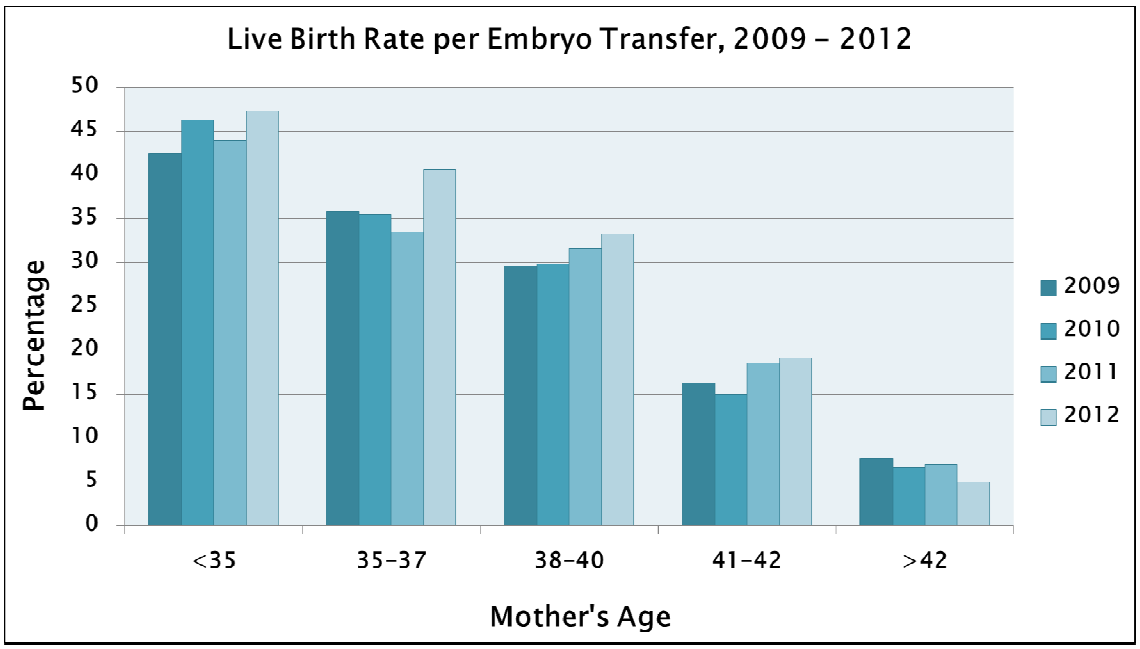
The embryo transfer is performed with or without anesthesia, depending upon the choice of the female. Generally the anesthesia is not required.

Five factors are included in order to select the embryo for transfer: early cleavage, number of blastomeres on days 2 and 3 and morphological score and presence of morula on day 3 (**Laura van Loendersloot *et. al.*, 2014**). The embryos are transferred in the uterus with the help of catheter and the female is asked to rest for few more days. **(Ahmed M. Abou-Setta *et. al.*, 2007)**



Embryo transfer technique (costalivf.com.au)

(Source: ivf.ws)



This plot shows the study of live birth rate per embryo transfer from 2009-2012

(Source: ivf.ws)

5. Luteal phase control

Broadly speaking, progesterone is the hormone that supports pregnancy. At the time of natural menstruation, as part of the exceedingly harmonized natural interaction between the ovaries and the uterus that occurs every month in order to facilitates the possibility of pregnancy, progesterone is secreted immediately after ovulation; the source of the progesterone, is actually the very follicle left behind by the egg after the process of ovulation. This structure is known as the "corpus luteum," which can usually be seen on transvaginal ultrasound after few days of ovulation. The foremost job of the progesterone at this point is to convert the cells in the uterine lining (aka endometrium) into a surface that will be receptive to the implantation of a fertilized egg (aka embryo). **(Klein, Aug 2012)**

The corpus luteum characteristically continues to produce progesterone for about 12-14 days. If no embryo has implanted, then the corpus luteum disintegrates resulting in lowering of progesterone level which leads to menstrual cycle. If, however, the egg that was ovulated is fertilized and the resulting embryo is implanted in the lining of the uterus, the *embryo*

itself produces the hormone hCG (human chorionic gonadotropin) and saves the corpus luteum. This continues the production of the progesterone. Progesterone, in turn, supports the continued growth of the pregnancy. It turns out that the pregnancy needs progesterone as much as the source of the progesterone i.e. the corpus luteum needs the pregnancy (for hCG). If the progesterone production is somehow interrupted -- for example, if the ovary containing the corpus luteum is surgically disturbed or removed during this period of time (up to about 8 weeks gestation, at which the point the placenta takes over progesterone production), the pregnancy will most likely fail, resulting in miscarriage. **(Klein, Aug 2012)**

After the study that what role of progesterone plays in sustaining pregnancy, it has long been speculated that progesterone deficiency, referred to as luteal phase deficiency (LPD), might be an central cause of infertility in couples who have no other noticeable issue. Furthermore, even if we assume LPD is real, there is little convincing evidence that treating LPD (typically with supplemental progesterone) will improve the situation. An important exemption to this statement is in IVF cycles (as opposed to non-medicated cycles or IUI cycles), when luteal function is clearly interrupted, and supplementing progesterone has been shown to be important. (Tangentially, **new data** actually questions whether we need to continue the progesterone supplementation as long as we do) **(Klein, Aug 2012)**

Care to be taken during IVF process

1. Folic acid supplementation

- Women intending to become pregnant should be informed that dietary supplementation with folic acid before conception and up to 12 weeks' gestation reduces the risk of having a baby with neural tube defects. The recommended dose is 0.4 mg per day. For women who have previously had an infant with a neural tube defect or who are receiving anti-epileptic medication or who have diabetes **(Diabetes in pregnancy, NICE clinical guideline 63)**, a higher dose of 5 mg per day is recommended.

2. Alcohol

- Women should limit their drinking to 1 or 2 units of alcohol once or twice per week and avoiding intoxication, reducing the risk of harming a developing fetus.
- Men should be informed that alcohol consumption within the Department of Health's recommendations of 3 to 4 units per day for men is unlikely to affect their semen quality.
- Excessive alcohol intake in men is harmful to semen quality.

3. Smoking

- Women who smoke are likely to lessen their fertility.
- Women who smoke should be offered referral to a smoking cessation programme to support their efforts in stopping smoking.
- Passive smoking is likely to affect the chance of conceiving.
- There is an association between smoking and reduced semen quality (although the impact of this on male fertility is uncertain), and quitting smoking improves the general health.

4. Caffeinated beverages

- There is no consistent evidence of an association between consumption of caffeinated beverages (tea, coffee and colas) and fertility problems.

5. Obesity

- Women who have a body mass index (BMI) of 30 or over are likely to take longer to conceive.
- Women who have a BMI of 30 or over and who are not ovulating should lose weight in order to increase their chance of conception.
- Participating in a group programme involving exercise and dietary advice leads to more pregnancies than weight loss advice alone.
- Men who have a BMI of 30 or over are likely to have reduced fertility.

6. Low body weight

- Women who have a BMI of less than 19 and who have irregular menstruation or are not menstruating should increase their body weight in order to improve their chance of conception.

7. Tight clothing

- Men should be informed that there is an association between elevated scrotal temperature and reduced semen quality, but that it is uncertain whether wearing loose-fitting underwear improves fertility.

8. Occupation

- Some occupations involve exposure to hazards that can reduce male or female fertility and hence a specific enquiry about occupation should be made to people who are concerned about their fertility.

9. Prescribed, over-the-counter and recreational drug use

- A number of prescriptions, over-the-counter and recreational drugs interfere with male and female fertility, and therefore a specific enquiry about these should be made to people who are concerned about their fertility and appropriate advice should be offered.

10. Complementary therapy

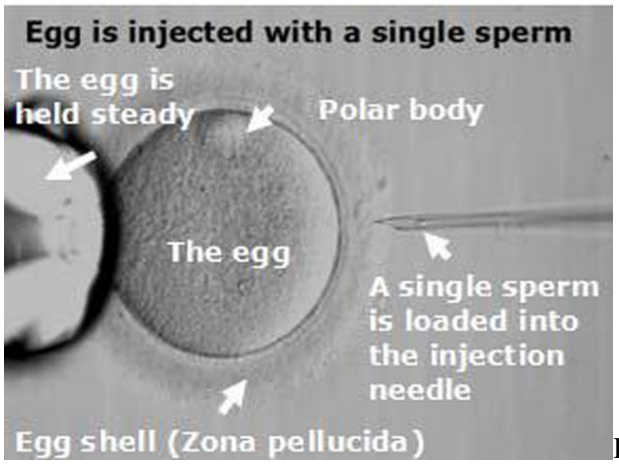
- People who are concerned about their fertility should be informed that the effectiveness of complementary therapies for fertility problems has not been properly evaluated and that further research is needed before such interventions can be recommended. **(Tom Treasure, 2013)**

Advancements in IVF

- **ICSI**

ICSI stands for Intracytoplasmic sperm injection. This procedure is recommended to the couple with male infertility in terms of speed and sperm count. Here, the egg is placed in the microscope and with the help of the projector, the screen is projected. The egg is held with the help of pipette and a single sperm is injected inside the egg via needle. This technique is recommended only when the male infertility is observed and it does not increase the chances of successful embryo development. ICSI not only overcame the fertilization barrier presented by oligo-, astheno-, and teratozoospermia, it also created a new possibility for azoospermic men to achieve fertility.

When there are no motile sperm found, they can be extracted directly from testicular tissue by either blind needle puncture or open tissue excision. The first unbeaten ICSI fertilization with testicular sperm was recorded in 1993, but no pregnancy occurred. The first successful pregnancy was reported in the very same year. With testicular sperm extraction (TESE), fertility rate as high as 70% can be achieved, despite only using a few poor-quality sperm. **(Wang and Sauer, Dec 2006)**



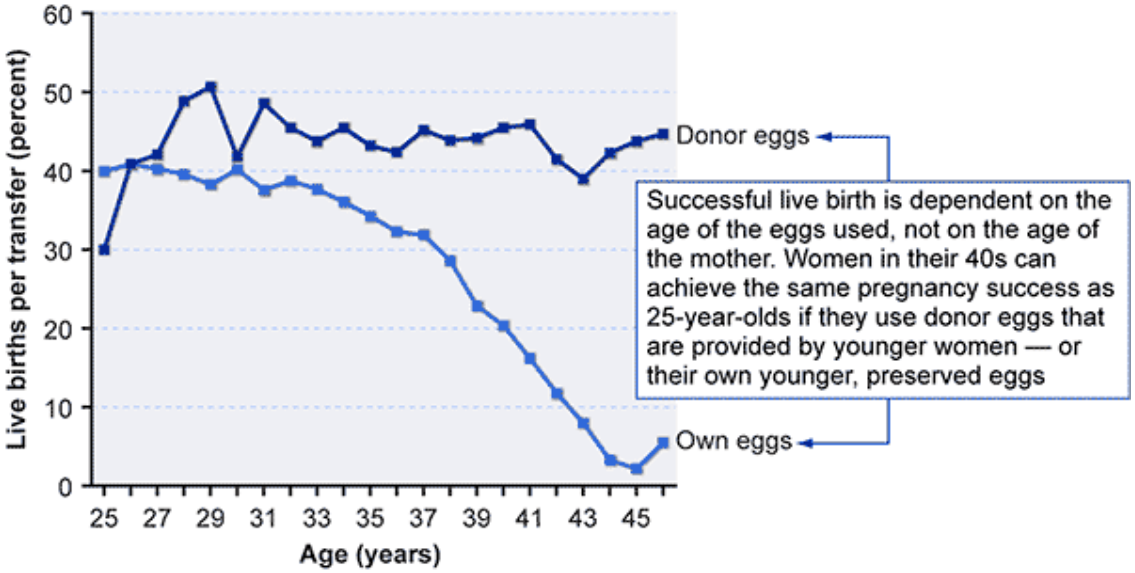
Photographic image of ICSI (bocafertility.com)

- **Egg donation**

Even with the advancements in early IVF techniques where the treatment was available for the woman with tubular damage, there was no efficient fertility treatment for those with natural or premature ovarian failure until 1983. A 25 year old patient became the first person to successfully deliver a pregnancy using a donor egg. She was suffering from secondary amenorrhea and premature ovarian failure. Dr.Peter Renou of the Monash inseminated the only ovum, which was donated by a 29-year-old patient undergoing IVF herself, with the sperm from the recipient's spouse. The embryo was transferred back into the uterus of the recipient and resulted in a successful pregnancy and live-birth **(Wang and Sauer, Dec 2006)**

Over the last three decades, the predominant indication for oocyte donation has shifted from women with premature ovarian failure to mostly women of advanced reproductive age. Factors responsible for this trend relate to the changing demographics of the population at large. More women are delaying childbearing to pursue education and careers, marriages are occurring later in life, divorce and remarriage are more common, and effective contraception and available abortion services have eliminated many unintended pregnancies. For the older patient, traditional IVF remains an option, however pregnancy rates decline precipitously after 36 years of age, mostly due to the age associated decline in normal oocytes. In contrast, pregnancy rates in women using donor oocytes are known to be as high as 50% per embryo transfer in recipients across all age groups. Indeed, women in their sixties have also given birth with donor oocytes, demonstrating that the postmenopausal uterus maintains the capacity to support pregnancies if provided adequate hormonal support. However, oocyte recipients experience increased obstetrical complications such as pregnancy induced hypertension (16%–40%), cesarean section (40%–76%), and gestational diabetes (20%) **(Wang and Sauer, Dec 2006)**

The success of IVF with donor oocytes not only crossed the traditional boundaries of ART, but also unleashed a barrage of unprecedented social, ethical, and legal concerns. Debates regarding donor anonymity, financial compensation for donor participation, the need for a registry of births from third party reproduction, and age limitation on recipients of donor gametes continue to stir controversy. Despite these unresolved issues, donor IVF remains an integral part of modern ART, and accounts for 11.6% of the IVF cycles performed in the US



(Source: ivfbaby.com)

- **Embryo cryopreservation**

Clinical and laboratory ART methodologies continued to evolve and improve, and a surplus of embryos in excess to what is used or needed for the initial IVF treatment became increasingly commonplace. During the early days of IVF, options for the patient with supernumerary embryos included discarding them, donating them to another infertile couple, or donating them for use in experimental research. Although cryopreservation of the embryos was an option, the freezing and thawing processes often caused permanent injury to the cells, and most embryos did not survive. This is best reflected in the very low rates of pregnancy seen following the transfer of frozen/thawed embryos throughout the 1980s. Intense efforts to develop various freezing/thawing techniques and cryoprotectants eventually resulted in the first reported human pregnancy from a frozen embryo in 1983, which unfortunately ended in premature rupture of the membranes and termination of pregnancy at 24 weeks of gestation **(Wang and Sauer, Dec 2006).** However, FET (Frozen embryo transfer) reduces the risk of ovarian hyperstimulation syndrome and improves outcomes for both the mother and baby. **(Jemma Evans, May 2014)**

Despite the initial set back, technology in cryopreservation continued to improve throughout the 1980s, leading to an increase in embryo survival rate and pregnancy rates. During the initial years of experimentation, at best approximately 50% of embryos survived the freeze/thaw process and resulted in a pregnancy rate of 13.4% per embryo transfer procedure, as only 4.6% of the individual thawed embryos implanted. By 2003, frozen embryo transfers

accounted for 21,981 of the 112,872 IVF cycles (17.8%) performed in the US, with an overall live birth rate of 27.0% per embryo transfer procedure. **(Wang and Sauer, Dec 2006)**

CASE STUDY: A 45-year-old woman received embryos from IVF by intracytoplasmic sperm injection (ICSI) with her own oocytes that were cryopreserved (slow freezing in a low-sodium medium) 11 years and 7 and a half months before, when she was 33 years old. From seven metaphase-II oocytes thawed, five survived, four were fertilized after ICSI and two cleaving embryos were transferred on day 3. Pregnancy was achieved, ending with the delivery of two healthy girls. As far as is known, this case represents, to date, the longest storage period of cryopreserved human oocytes resulting in a live birth. **(Carlos J. Quintans, Sept 2012)**

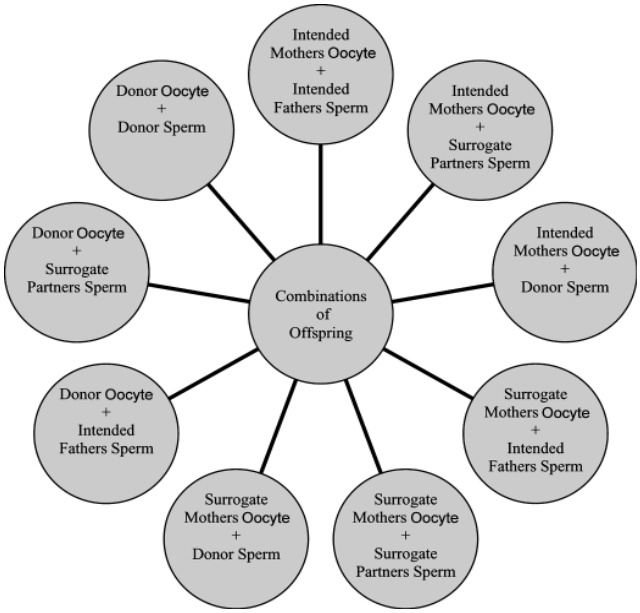
- ## Pregnancy in women above 40

It is crucial for women to understand thatas age increases, the chances of pregnancy decreases. After the age of 32, the fertility in women starts decreasing gradually. Thus it becomes very difficult for women to become pregnant in the later stages of life. However with the help of recent techniques in IVF like oocyte donation and embryo cryopreservation, the chances are slightly increased. **(Seng *et. Al. 2005*)**

- ## Surrogacy

Many reviews have been studied which addresses the psychosocial research carried out on surrogacy triads (surrogate mothers, commissioning mothers and offspring) and shows that research has focused on a number of specific issues: attachment and disclosure to surrogate offspring; experiences, characteristics and motivations of surrogate mothers; and changes in profiles of the commissioning/intended mothers. Virtually all studies have used highly selected samples making generalizations difficult. There has been a notable lack of theory, no interventions and only a handful of longitudinal studies or studies comparing different populations. Few studies have specifically questioned the meaning of and need for a family or the influence and impact that professionals, treatment availability and financial factors have on the choices made for surrogate and intended mothers. Societal attitudes have changed somewhat; however, according to public opinion, women giving up babies still fall outside the acceptable remit. Surrogate and intended mothers appear to reconcile their unusual choice

through a process of cognitive restructuring, and the success or failure of this cognitive appraisal affects people's willingness to be open and honest about their choices. Normal population surveys, on the contrary, are less accepting of third party reproduction; they have no personal need to reconsider and hence maintain their original normative cognitively consonant state. **(Olga B.A.van den Akker, 2006)**



The nine (theoretically) possible combinations of offspring resulting from surrogate arrangements, where the gestation in all instances is with the surrogate mother. (Olga B.A.van den Akker, 2006)

- ## Assisted Hatching

Assisted Hatching (AH) is a technique performed prior to Embryo Transfer (ET) & after In Vitro Fertilization (IVF). This technique involves the artificial lessening or gaping of the Zona Pellucida (ZP). It is performed as an attempt to increase the probability of embryo implantation. AH can be performed by mechanical, chemical, or laser-assisted means. Subgroups of patients that may benefit from AH include those with prior implantation failure, those undergoing Frozen-Thawed Embryo Transfer (FET) cycles, and women who are 38 years of age or older. IVF programs should identify subgroups of women within their patient population who may benefit clinically from AH. **(Shahryar K Kavoussi, 2014)**

IMPORTANT NOTIFICATIONS OF IVF

- **Poor response in first cycle**

Most patients with an unexpected poor response in the first cycle had a normal response in the second cycle, leading to an acceptable cumulative ongoing pregnancy rate after three cycles. Patients with an expected poor response in the first cycle should be advised to withdraw from treatment after the first cycle because of a poor prognosis. **(*Klinkert et. Al. , May 2004*)**

Poor ovarian response in IVF cycles is associated with diminished ovarian reserve and poor pregnancy outcome. Little is known about pregnancy outcome after a poor response in women with a normal ovarian reserve. The study was conducted on women undergoing IVF/intracytoplasmic sperm injection from January 2003 to December 2008 in the FertilityPLUS Clinic in Auckland, New Zealand. All women with a poor response in the first cycle were selected. Primary outcome was live birth after the second cycle. Secondary outcomes were poor response in the second cycle and the predictive values of female age and basal FSH at first cycle and IVF outcome at second cycle. Of the 2487 women starting IVF, 142 women (5.7%) with a poor response in the first cycle were selected, of which 66 (46.5%) women had a repeated poor response in the second cycle. There were 31 live births in the second cycle (21.8%). Female age was the only significant predictor for repeated poor response and clinical pregnancy, but the predictive value was low. Therefore poor response in women with a normal ovarian reserve should not be a reason to discontinue further IVF treatment. **(Lobke M Moolenaar *et. al. 2013*)**

- **IVF VS ICSI**

Traditional dogma suggests that intracytoplasmic sperm injection (ICSI) should be performed to ensure successful oocyte fertilization in an in-vitro fertilization (IVF) cycle. This study postulated that there would be no difference in the fertilization rate when ICSI was compared with IVF. This hypothesis was tested in a randomized trial of IVF versus ICSI. **CASE STUDY:** A total of 150 immature oocytes were collected in eight cycles of IVF for patients diagnosed with polycystic ovarian syndrome (PCOS). Patients were primed with minimal FSH before transvaginal oocyte aspiration. Sibling oocytes were inseminated by 50% IVF and 50% ICSI. There was no significant difference in fertilization, useable or total blastocyst development between the two insemination technique groups. Clinical pregnancy results for combined fresh and cryopreserved transfers were identical between the two

insemination techniques with a total of two fresh and five cryopreserved IVF-inseminated embryos resulting in three clinical pregnancies (42.9%) and five fresh and two cryopreserved ICSI-derived embryos resulting in three clinical pregnancies (42.9%). **(M. Walls, *et. al. 2012*)**

- **Woman's emotional adjustment to IVF**

  The research indicates that women starting IVF were only a little diverse emotionally from the standard groups. The women's levels of negative emotions were raised due to unsuccessful treatment, which persisted after consecutive unsuccessful cycles. In general, most women proved to adjust well to unsuccessful IVF, although a extensive group showed subclinical emotional problems. When IVF resulted in pregnancy, the negative emotions disappeared, indicating that treatment-induced stress is considerably related to threats of failure. **(Verhaak *et. al. 2006*)**

CONCLUSION:

IVF can be a very safe alternative to natural childbearing if used under regulation. Often times, couples with infertility problems turn to this process for help with conceiving. It is helpful for women with blocked fallopian tubes or for male with low sperm count. The IVF treatment can greatly reduces the surgery on woman's fallopian tube. Today, if used with care and consideration, IVF can take an unexpected turn. In completely understanding the process of IVF, it is important to know the history, methodology and the real life case that went right and wrong.

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