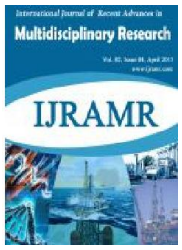
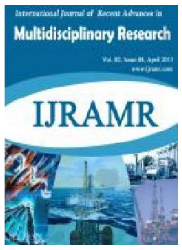


International Journal of Recent Advances in Multidisciplinary Research
Vol. 02, Issue 04, pp.0345-0356, April, 2015



Research Article

ASSISTED REPRODUCTIVE TECHNOLOGY: A BRIEF DISCUSSION

***Srijita Dutta**

*Department of Pharmacology, Nshm Coolege of Pharmaceutical Technology, Nshm Knowledge Campus, Kolkata- Group of Institutions, 124(60). B.l.Saha Road, Kolkata-700053, India

ARTICLE INFO

Article History:
Received 15th January, 2015
Received in revised form
28th February, 2015
Accepted 18th March, 2015
Published online 30th April, 2015

Keywords:
Assisted reproductive technology, Infertility, Cost effectiveness, Cloning, Stem cells.

ABSTRACT

As many as one in six couples will encounter problems with fertility, defined as failure to achieve a clinical pregnancy after regular intercourse for 12 months. Increasingly, couples are turning to assisted reproductive technology (ART) for help with conceiving and ultimately giving birth to a healthy live baby of their own. With this in mind, it is important that each step of the ART cycle is supported by good evidence from well-designed studies. Herein, the health economics research in this area are critically appraised. The cost-effectiveness of different interventions should be considered when making decisions about treatment. The rapid advancements in sciences have revolutionized modern medicine in a number of ways; genetic engineering, Assisted Reproductive Technologies (ART), human cloning, stem cells etc. has opened up the unimagined and promise unquestionable and undreamed benefits to mankind. At the same time, they raise many questions of law and ethical issues relating to public interest, social and religious sentiments and family concern. There is a certain element of risk associated with all assisted reproductive procedures. It is, therefore, necessary to ascertain the therapeutic and research value of the AR procedure in each case. This article reviews techniques of ART along with its costs, ethical & legal issues as well as implementation in today's society.

INTRODUCTION

The special programmes by WHO on human reproduction has estimated that there are 60 to 80 million infertile couples worldwide; between 6-10% of the couple are infertile, (World Health Organization, 2009) The advent of Assisted Reproductive Technologies (ART) from the late 70s has not only enhanced the possibility of pregnancy but has also made women conceive in situations which would not have been possible decades ago. However, many of these technologies require enormous technical expertise and infrastructure, are expensive and the couple's endurance physically, emotionally, socially and economically.(Barreiro *et al*., 2007) In order to ensure quality of care, it is imperative that standardised protocols and guidelines should follow for the establishment and accreditation of ART Centres. National guidelines for Accreditation, Supervision and Regulation of ART Clinics have been formulated by ICMR in 2005 (Terriou *et al*., 2005) to provide optimum benefit of these newer technologies by skilled team of experts, at affordable health and economic cost. Assisted reproductive technologies include any fertilization involving manipulation of gametes/ embryos outside the human body and transfer of gametes/embryos into the body. (Royce *et al*., 1997) The new reproductive technologies give great help and offer biomedical parenthood to various infertile couples who have exhausted all other avenues to have a child of their own. The first live birth resulting from IVF worldwide occurred in England in 1978. Nowadays IVF is a well-established and accepted treatment for infertility in most developed countries. Advances in ART, including the use of donor gametes and ICSI, have led to increased numbers of infertile couples being treated with ART in recent years. ART accounts for 1 to 3% of annual births in developed countries (Semprini *et al*., 1992). In 2002, 2.3% of babies born in Australia were conceived following the use of ART, (Peña *et al*., 2003). Over the past twenty years, there has been a steady decline in the number of embryos transferred in a single cycle due to growing concerns about the risk and very serious health consequences of multiple pregnancy. (Human immunodeficiency virus and infertility treatment, 2004) In 1993, 55.3% of treatment cycles transferred three or more embryos. In 2002 this had decreased to 5.8% of cycles. Over this same time period, the success of ART in Australia and New Zealand has steadily increased. (Nicopoullos *et al*., 2004)

****Corresponding author: Srijita Dutta***
Department of Pharmacology, Nshm Coolege of Pharmaceutical Technology, Nshm Knowledge Campus, Kolkata- Group of Institutions, 124(60). B.l.Saha Road, Kolkata-700053, India

Measurement of ART Effectiveness

Estimates of ART effectiveness may vary according to the definitions of treatment success and the treated population. Live

birth rates provide the most clinically relevant measure of ART success. Other outcomes commonly reported in the literature include the rates of fertilisation, implantation and pregnancy. Pregnancy may be defined as the occurrence of an elevated human chorionic gonadotrophin concentration; the occurrence of a clinical pregnancy; or the presence of an on-going pregnancy beyond 20 weeks. These intermediate outcomes do not, however, represent the desired outcome of a live birth, (Marina *et al*., 1998) The European Society of Human Reproduction and Embryology (ESHRE) consensus document (2003) recommends the singleton live birth as a potential measure of ART success, given the increased risks to mother and infant associated with multiple pregnancy (Stroup, 2000). In Australia, the Fertility Society of Australia (FSA) Code of Practice states 'the objective of ART must be the live birth of a single healthy child'.(Liberati *et al*., 2009) The outcome of a single term gestation, live baby has been referred to as Birth Emphasising a Successful Singleton at Term, or BESST, (Bujan *et al*., 2007) Studies that only report ART live birth rates per oocyte recovery or per embryo transfer cycle exclude women with failed or cancelled controlled ovarian hyperstimulation (COH) and thus may overestimate the true success rates of ART. Defining an IVF or Intracytoplasmic Sperm Injection (ICSI) treatment as the initiation of treatment with COH, allows inclusion of the results of the transfer of an initial fresh embryo and any subsequent frozen embryos stored after the initial oocyte retrieval within the same treatment cycle, (Garrido *et al*., 2004).

Clinical need

In developed nations throughout the world, the total fertility rate has been declining and, in many countries, is currently below the population replacement value of 2.1 births per woman. In Australia, the total fertility rate in 2002 was 1.76, which is comparable to that in the UK, USA and Canada (Veiga, 1999). It is estimated that 12–25% of couples are affected by infertility, but the number of couples seeking medical advice is not accurately known (Savasi *et al*., 2007). However, there is no national data collection on infertility in Australia. A recent population-based telephone survey of Australian men (Men in Australia, Telephone Survey, MATeS) found a self-reported failure to conceive of 7.6% , (Chu *et al*., 2006) In developed nations, as an increasing number of women delay having children until a later age, the prevalence of infertility and need for fertility assistance services is likely to increase. The median age of child-bearing in Australian women increased from 26.8 years in 1982 to 28.7 years in 1992, and to 30.2 years in 2002. (Sauer *et al*., 2002)

Infertility

Infertility is defined as a circumstance where there has been either an inability to conceive or carry a pregnancy to a live birth after one year of unprotected sexual intercourse; or there is a medical condition that will reduce the likelihood of either conception or carrying a pregnancy to a live birth, (Bujan*et al*., 2004). Observational studies conducted in couples using natural methods to conceive have indicated that approximately 80% of couples will conceive in the first six menstrual cycles, and an additional 10% will conceive spontaneously within the next six cycles.(Kowalska *et al*., 2005) Of the 10% of couples classified as infertile after one year of trying to conceive, approximately half will achieve a spontaneous conception over the next three years.

Overview:

Infertility affects approximately 13-14% of reproductive-aged couples. It is defined as the inability to conceive after 1 year of properly timed, unprotected intercourse. This definition is based on the cumulative probability of pregnancy.

Table 1. Cumulative Probability of Pregnancy in Couples With Normal Fertility (All Reproductive-aged Women):(Lee *et al*., 2001)

Month	Monthly Probability	Cumulative Probability
1	0.2	0.20
2	0.2	0.36
3	0.2	0.49
4	0.2	0.59
5	0.2	0.67
6	0.2	0.74
7	0.2	0.79
8	0.2	0.83
9	0.2	0.86
10	0.2	0.89
11	0.2	0.91
12	0.2	0.93

Cycle fecundability is the probability that a single menstrual cycle will result in pregnancy (see Table 1 above). Cycle fecundity is the probability that a single cycle will result in a live birth. Assuming a constant monthly probability of conceiving (fecundability) of 20%, the theoretical cumulative pregnancy rate after 12 months is 93%. However, studies show that this number is actually lower. The number and quality of a woman's oocytes declines with age. The decline in the number of oocytes begins at 20 weeks' gestation when the female fetus has approximately 6-7 million oogonia, (Manigart *et al*., 2006).

Cause of Infertility

The cause of infertility can be easily identified in some couples. In other couples the cause is much less clear, and multiple factors may contribute. The major recognized causes of infertility are listed in Table 2.

Table 2. Causes of Infertility: (Mencaglia *et al*., 2005)

Cause	Couples	Women
Male	35%	--
Ovulatory	15%	40%
Tubal	35%	40%
Other	5%	10%
Unexplained	10%	10%

Obtaining a thorough history and physical examination is essential in evaluating an infertile couple. The questions asked during the evaluation should include the following topics:

- Current and past medical/surgical problems and recent changes in health
- Employment and potential chemical/environmental exposures
- Family history of miscarriages
- Personal and family history of birth defects or inheritable disorders
- Updated medicine list (including any over-the-counter medicine or herbs)

- Tobacco, alcohol, and drug use
- Coital frequency and any sexual dysfunction
- Thorough gynecologic history, including pelvic pain, discomfort with intercourse, menstrual cramps, cycle length, and duration of flow[23]
- Prior pelvic infections
- Duration of infertility
- Prior pregnancies from either partner
- Use of prior contraception

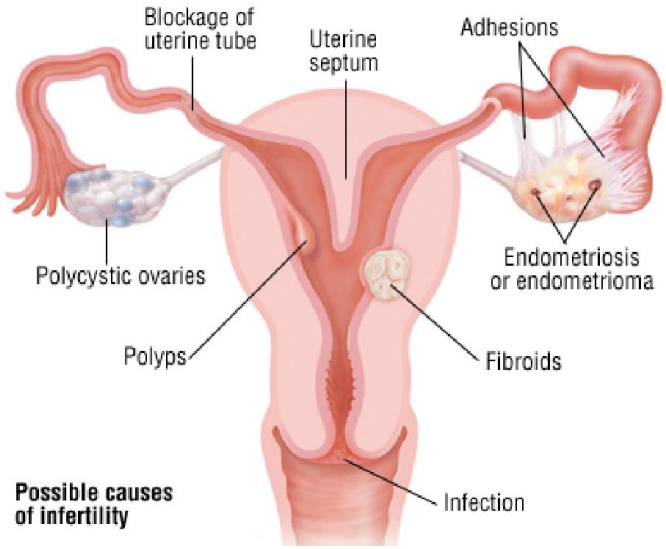


Figure 1: Female Infertility (Mencaglia *et al*., 2005)

Risk factors

The NICE review provides detailed guidelines based upon the evidence available for known risk factors for infertility. These factors are outlined briefly below.

- Female age is the major determinant of infertility. Natural female fertility falls gradually after age 30 years, with a rapid decline after age 35 years to cessation at menopause.

Other factors associated with female infertility are obesity (body mass index greater than 29) and low body weight (body mass index less than 19 and irregular or absent menstruation) and smoking, (Tay *et al.,* 2007).

- Obesity has also been associated with male infertility. Excessive alcohol intake, smoking and elevated scrotal temperature due to sedentary work position, occupational heat exposure and wearing tight underwear has been associated with reduced semen quality in men, although the impact of this on male fertility is not known, (PubMed)
- Prescription and recreational drugs and occupational hazards such as exposure to solvents have been associated with infertility in both males and females.

Diagnostic Tests

When evaluating an infertile couple, diagnostic studies should be selected as indicated. If the history is unclear, then tests that address the above-mentioned major categories of infertility should be obtained.

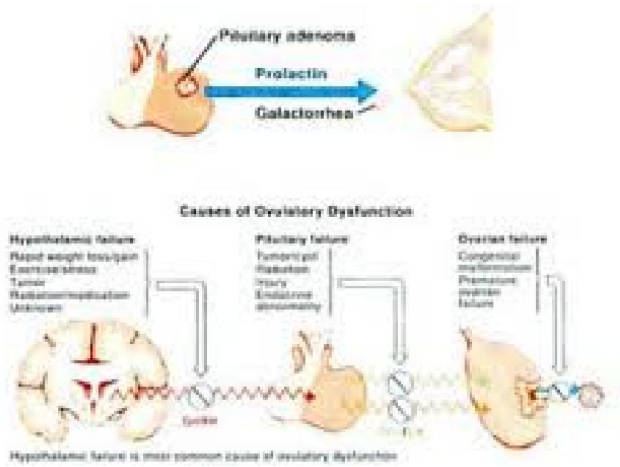


Figure 2: Diagnosis of Infertility in Women

Male factor testing-After a medical history and physical examination, semen analysis is the single best test for evaluating for male factor infertility

For optimum and consistent results, abstinence is required 3-5 days prior to semen collection. The World Health Organization (WHO) has established methods for semen analysis, but methods may vary among facilities. Additionally, the WHO has established normal reference values. Another commonly used method for evaluating morphology is the strict Kruger method, (Farhi *et al.,* 2007). Sperm concentration - 20 million/mL or more Motility - At least 50% or more with forward progression Morphology - At least 30% or more normal forms (14% strict Kruger criteria) White blood cells - Fewer than 1 million/mL Round cells - Fewer than 5 million/mL More recently, other tests have been devised to evaluate sperm. The Halosperm test and the Sperm Chromatin Structure Assay (SCSA) have been devised to evaluate the DNA fragmentation of sperm.

Ovulatory function testing- Women of reproductive age who have regular menstrual cycles lasting from 21-35 days are likely ovulatory. However, for patients to become more accustomed to predicting ovulation so that they can appropriately time intercourse, they may wish to initiate basal body temperature monitoring or use luteinizing hormone (LH) detection kits.(Dovey *et al*., 2008)

Basal body temperature (BBT) monitoring is largely a historical method for determining the correct timing of intercourse. A 0.5–1.0ºF rise in temperature is noted 2 days after the luteinizing hormone (LH) peak, which occurs after the day of ovulation. This results from progesterone production from the corpus luteum. Since most studies show that the best day to introduce sperm into the female reproductive tract is either the day of ovulation or the day before ovulation, BBT monitoring -is not useful for coital timing in a current cycle but best serves as a method to confirm the time of ovulation and helps the patient to predict future cycles based on data she has gathered over prior cycles, (Zadehmodarres *et al*., 2009). A deficiency in progesterone production by the corpus luteum (CL) has historically been attributed to infertility and recurrent pregnancy loss in many women with otherwise unexplained miscarriages. The significance and presence of an inadequate luteal phase (also referred to as luteal phase defect (LPD)) has been questioned throughout the literature.

Ovarian reserve testing- Several simple tests for ovarian reserve exist. Initial testing usually includes cycle day 3 laboratories including follicle stimulating hormone (FSH), estradiol (E2), and leuteinizing hormone (LH). Typically, if the FSH level is greater than 15 mIU/mL or the estradiol level is greater than 75 pg/mL, the prognosis is poor. Day 3 antral follicle scans and ovarian volume may also be used to evaluate ovarian reserve and are simple and accurate, (Nyboe Andersen *et al*., 2005) In patients older than 40 years or for whom poor ovarian reserve is suspected, a clomiphene citrate challenge test may be performed. Clomiphene citrate (100 mg PO qd) is administered on cycle days 5-9. FSH and estradiol levels are drawn on days 3 and 10.

Cervical disease testing- Women who have had cervical cone biopsies or trauma to the cervix are at risk for cervical abnormalities and cervical stenosis. If a cervical abnormality is found, the most logical approach is to recommend bypassing the cervix with intrauterine inseminations (IUI), (Kovacic *et al*., 2010) especially if the rest of the findings from the infertility evaluation are normal.

- Hirsutism or hair loss
- Abnormal weight gain
- Oily skin or acne
- Purple striae on the abdomen
- Polyuria/polydipsia
- Irregular menses
- Heat or cold intolerance

Headaches, visual problems or lactation without prior pregnancy, (Melo *et al*., 2008; Prisant *et al*., 2010).

Infertility treatments

In some couples presenting with infertility, treatment can be directed at reversing the underlying cause. As described in the aetiology section above, this may involve surgical measures to treat genital tract obstruction or endometriosis; or hormone treatment to restore ovulatory function. ART procedures are effective in cases where alternative treatments are of doubtful value; the underlying cause is not amenable to other treatments; or the infertility is unexplained. (Queiroz *et al*., 2008)

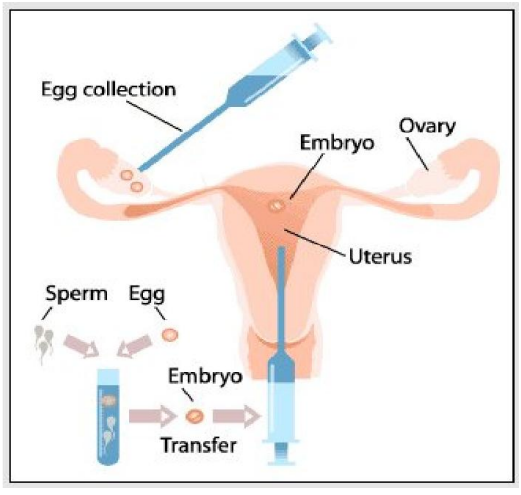


Figure 3: Infertility Treatments (Queiroz *et al*., 2008)

Indication for ART: (Egger *et al*., 1993**)**

- When husband is impotent.
- When husband is infertile.
- When husband is unable to depose semen in female genital i.e. hypospadesis, epispadesis etc.
- When Rh incompatibility between husband and wife.
- When husband is suffering from hereditary diseases.

Oxidative stress in the ART setting

Despite the advancement of ART techniques, gametes and embryos when handled, prepared and manipulated for ART procedures, are exposed to various potential ROS-inducing factors. In vitro, the risk of oxidative stress development is greater than in vivo(Egger *et al*., 2001) and its negative impact may be amplified due to the lack of physiological defense mechanisms, absence of natural antioxidants and the presence of multiple potential sources of ROS (van Leeuwen *et al*., 2005).

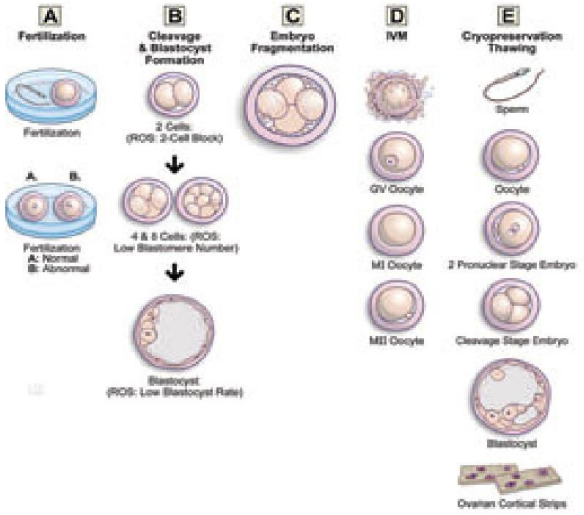


Figure 4: Oxidative Stress In ART Setting (Chu *et al*., 2005)

These sources of ROS during ART procedures could either be endogenously from gametes or via exogenous environmental factors (Chu *et al*., 2005). However, unless measures are taken to curb ROS production, both the endogenous and exogenous sources of ROS will ultimately lead to the development of oxidative stress, which would then negatively impact on fertilization rates and pregnancy outcome.

ART: Step-by-Step Guide

Every cycle of ART involves multiple steps, and each occurs at a specific time during a four to six-week period. The following is an overview of the IVF procedure. The procedure begins in the month proceeding the actual ART cycle. An IVF cycle typically includes the following steps or procedures: (Guttmacher *et al*., 1956)

- Medications to grow multiple eggs
- Retrieval of eggs from the ovary or ovaries
- Insemination of eggs with sperm
- Culture of any resulting fertilized eggs (embryos)
- Placement ("transfer") of one or more embryo(s) into the uterus

- Support of the uterine lining with hormones to permit and sustain pregnancy
- In certain cases, these additional procedures can be employed:
- Intracytoplasmic sperm injection (ICSI) to increase the chance for fertilization
- Assisted hatching of embryos to potentially increase the chance of embryo attachment ("implantation")(Dain *et al*., 2011 and Rova *et al*., 2012)
- Embryo cryopreservation (freezing)
- The success of IVF largely depends on growing multiple eggs at once:
- Injections of the natural hormones FSH and/or LH (gonadotropins) are used for this purpose
- Additional medications are used to prevent premature ovulation
- An overly vigorous ovarian response can occur, or conversely an inadequate response.

Cycle Preceding ART Cycle: (Picaud *et al.,* 2012**)**

- Sometimes oral contraceptives are begun.
- Sometimes a GnRH agonist (e.g. Lupron®) is initiated.
- Sometimes a mock transfer is performed to identify potential problems in embryo transfer.
- Occasionally, an IVF cycle starts with a cycle day of the menstrual period without other medications.

ART Cycle

- Prestimulation treatment
- Ovarian stimulation with gonadotropins (e.g. Bravelle®, Menopur®, Follistim®, Repronex®, Gonal-F®, Follistim AQ pen, and/or Gonal-F RFF Pen)
- Monitoring follicle development with ultrasound and serum hormone levels
- Final oocytes maturation and hCG administration (Profasi®, Pregnyl®, Novarel® or Ovidrel®)
- Transvaginal oocyte retrieval
- Insemination
- Embryo transfer
- Progesterone supplementation(Hamdan *et al*., 2015)
- Pregnancy test Early pregnancy follow-up

ART Medications

Several medications are used in a typical IVF cycle. These medicines belong to several categories and each are an important part of a stimulated cycle. The types and amount of medications used vary according to the medication protocol prescribed by physician.

GnRH Agonists

Gonadotropin releasing hormone (GnRH) is a hormone produced in the brain that indirectly stimulates ovarian function. Agonists of GnRH are synthetic forms of this hormone which do not directly induce follicle development or ovulation but which have become very important in ART therapy, (Perkins *et al*., 2015)

GNRH Antagonists

Antagonists of GnRH are also available (Cetrotide® and Antagon®). These are started later in the cycle than Lupron® and directly and immediately inhibit FSH and LH production. Protocols that use these medications may require fewer injections. (McLernon *et al.,* 2010) Ultrasound measurements of follicular growth are used to determine when to start these medicines.

Gonadotropins

To increase likelihood of pregnancy through ART, multiple oocytes must be produced. This is accomplished through the administration of gonadotropins, hormonal medications that directly stimulate the ovaries. Stimulation can be achieved with a variety of drug regimens. Gonadotropin medications come in several forms; Repronex® and Menopur® are a combination of FSH and LH. They replace a woman's own LH and FSH which are normally produced by the pituitary gland. Bravelle®, Follistim® AQ Cartridge for use with Follistim Pen®, Follistim® AQ Vial, Gonal-F®, and Gonal-F® RFF Pen are preparations that contain only FSH. (Kresowik *et al*., 2011) This process ensures uniform purity and potency. Because the dose of hormones that are used in ART is greater than what the body normally produces, the ovaries typically develop more than one oocyte as occurs in a natural cycle.

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) is an injectable medication that is administered to complete oocyte maturation. The brand names for hCG are Profasi®, Ovidrel®, Novarel®, and Pregnyl®. Human chorionic gonadotropin is structurally similar to the LH that is produced by a woman's pituitary gland. It acts on the ovary in a manner similar to a woman's own LH. (Shah *et al*., 2011) Human chorionic gonadotropin, like LH, stimulates the final maturation of the oocytes in the follicle. It also stimulates progesterone production from the ovary after egg retrieval. This progesterone is important to prepare the uterus for implantation of the embryo.

ART procedures

In Vitro Fertilization (IVF)

This type of treatment, the first to be introduced in 1978 still remains as the mainstay of ART today. The object of IVF is to add sperm to eggs in the laboratory (instead of the Fallopian tube) in situations where the normal pathway via the Fallopian tubes is damaged or absent, or sperm is not functioning normally. The process of IVF requires a significant time contribution and is emotionally stressful. When performed for the correct reasons, it offers a pregnancy (success) rate of approximately 30-50% percent per single attempt, (Kalra *et al*., 2011). Units specializing in ART undertake this type of treatment. A treatment cycle requires the stimulation of multiple eggs in the ovary using drugs such as clomiphene citrate, HMG, FSH GnRH agonists/antagonists and/or HCG. At an appropriate time, eggs are collected either by ultrasound as an outpatient procedure, or rarely by laparoscopy. The sperm is collected, prepared and added to the eggs in the laboratory, (Abma *et al*., 1997 and Barbieri *et al*., 1999). Once fertilization and division of the eggs has occurred, usually after forty-four hours, normally one or two embryos are replaced into the mother's uterus by means of a thin plastic tube (transfer catheter) inserted via the vagina and cervix.

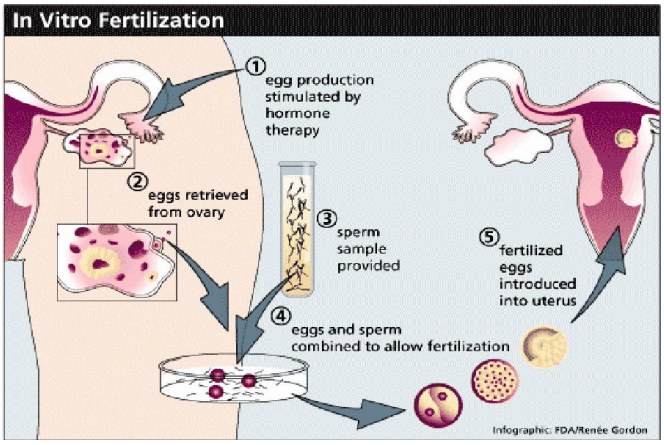


Figure 5. In- Vitro Fertilization (Kalra *et al*., 2011)

GIFT- GIFT is short for Gamete Intrafallopian Transfer, and it was once a very common and popular form of ART. However, it is not used that much anymore. GIFT is where the woman's eggs and man's sperm are combined in a lab. The eggs are then inserted into the fallopian tubes. In GIFT treatments, the fertilization takes place inside a woman's body and not inside a lab, (Biljan *et al*., 1999) GIFT is a good option for couples who want a more natural ART treatment, that allows the fertilization to take place inside the woman's body naturally.

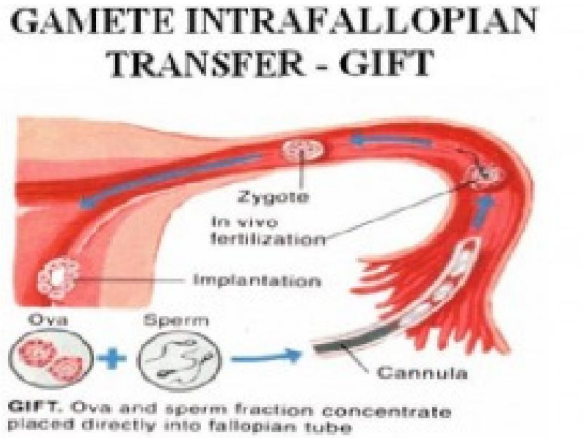


Figure 6: Gamete Intrafallopian Transfer (GIFT), developed in 1984, (Biljan *et al*., 1999)

ZIFT- ZIFT is short for Zygote Intrafallopian Transfer, and it is much like GIFT. The eggs are mixed with the sperm in a lab, and then placed back inside the woman's fallopian tubes. The difference with ZIFT is that the eggs are not inserted back into the woman's body until the eggs have already been fertilized. As with GIFT, this process was once very popular, (Centers for Disease Control and Prevention, 2002 and Confino *et al*., 1990) but has now become less common with the increased success of IVF.

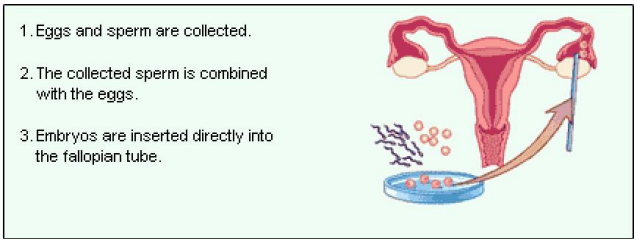


Figure 7: Zygote Intrafallopian Transfer (ZIFT), (Confino *et al*., 1990)

ICSI- ICSI is short for **Intracytoplasmic Sperm Injection**, and it is most commonly used for couples with serious problems with the man's sperm. ICSI represents one of the major advances in human reproduction and ART that occurred in the 90's. It differs from IVF by what happens once the eggs and sperm reaches the laboratory. Essentially a single moving sperm is injected into a single mature egg. Thus the couple requires very few sperm for this type of therapy.[54] ICSI has opened up treatment to a whole new group of patients in whom classical IVF was unsuccessful. This includes males with very low sperm counts (< 5 million / ml), males with antisperm antibody problems, couples who have had failed fertilisation at IVF. Once embryos are formed, embryo transfer is identical to IVF (de La Rochebrochard *et al*., 2006).

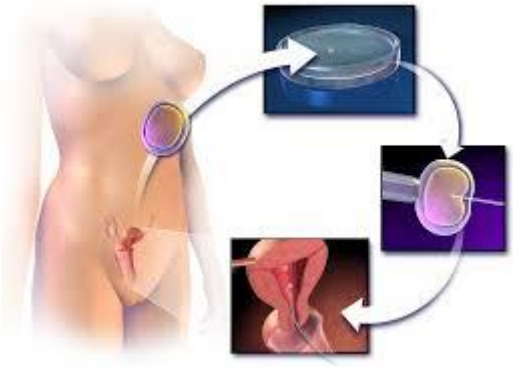


Figure 8. Illustration depicting intracytoplasmic sperm injection (ICSI), an example of assisted reproductive technology.[55]

Concerns have been raised that ICSI may be associated with an increased risk of congenital malformations and long-term genetic consequences due to the ability to produce embryos using abnormal sperm that would not otherwise be able to achieve fertilisation, including sperm from males with genetic defects (Evenson and Wixon, 2005).

Embryo Freezing and Replacement (Transfer)

Advances in freezing technology allow unused embryos, provided they are structurally normal, to be frozen and stored. Embryos can be stored for years without any apparent defects occurring. These can be thawed and replaced in normal cycles greatly simplifying the patient's treatment cycle and costs. At this time it would appear that the pregnancy rate from frozen embryos is lower than that from fresh embryos.[57] Unfortunately at the time of writing while some eggs have been successfully frozen the technology has not reached the same level as that for embryos and is not a routine clinical procedure. In Australia, a grading system based on embryo morphology is commonly used to select the healthiest embryos for injection into the uterus trans vaginally. After embryo transfer, the female is treated with progesterone daily for up to the 10th week of pregnancy (or alternative regimens) to assist implantation and maintenance of pregnancy.[58] The number of embryos transferred depends on the practice of the provider and the age and preferences of the treated couple. Any remaining healthy embryos may be frozen for storage (cryopreservation) for thawing and transfer at a later date if needed.

Blastocyst Culture

The embryo is usually replaced at the 2-8 cell stage of development. The blastocyst (multicellular stage with fluid

compartment) stage may be reached by prolonging the culture of the embryo in the laboratory for a longer period and if it reaches the blastocyst stage can be transferred to the uterus at that time. This scientific approach helps in selecting the embryo(s) with a greater chance of succeeding. To achieve this degree of development special laboratory conditions are required. (Guzick *et al*., 2001)

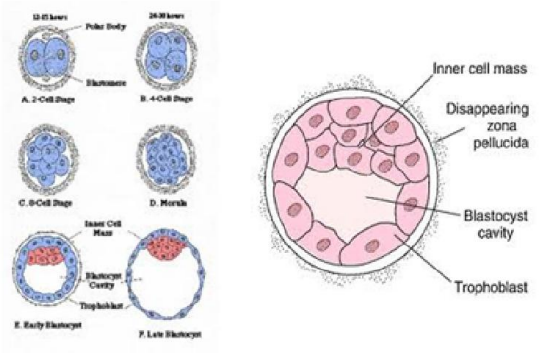


Figure 9. The blastocyst stage embryo is a ball of cells approximately 100-150 cells in total, (Guzick *et al*., 2001)

Assisted Hatching

The term hatching applies to the embryo (which has now reached the blastocyst stage), which develops in the original egg covering called the Zona Pellucida or clear zone. Once the embryo reaches the uterus and before it can implant into the uterine wall this outer clear zone must open and the embryo comes out (hatches). Failure of hatching will prevent conception and currently it is thought that, it may apply to a small number of patients, (Hassan *et al*., 2003) especially those on ART programmes who are 38 years and older, who have had many embryos replaced without a pregnancy and embryos that have been frozen prior to replacement. Assisted hatching means procedures which artificially thin out the zona pellucida. Different techniques to assist hatching exist and one of these involves the use of a non-contact laser.

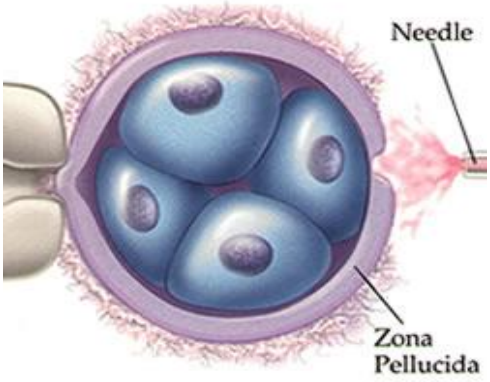


Figure 10. IVF: Assisted Hatching (Isaacs *et al*., 1997**)**

GENETIC TESTING

Genetic testing represent one of the most exciting fields of medicine in the new century and its impact is and will be enormous. Genetics involves the study of heredity, by testing the information carried in each and every human cell that makes each one of us unique. In the field of fertility certain genetic tests are already available. These genetic tests can already be performed to diagnose special causes of infertility. In addition the embryo can undergo certain genetic tests before it is returned to the mother's uterus during IVF/ICSI cycles to exclude certain genetic problems and this is referred to as Pre-implantation Genetic Diagnosis (PGD).(Johnson *et al*., 2004)

Intrauterine insemination- Intrauterine insemination (IUI) involves the placement of washed sperm into the uterus under ultrasound guidance to bypass the natural cervical mucus barrier. It is performed under sedation as an outpatient procedure with or without controlled ovarian hyperstimulation (COH). It is designed to bring a high concentration of sperm into close contact with one oocyte (after natural ovulation) or with multiple oocytes (after COH).(Kidd *et al*., 2001; Klonoff-Cohen *et al*., 2004 and Kovacs *et al*., 1978) The main indications for IUI with COH are in the treatment of unexplained infertility where investigations have excluded an obstructive cause (at least one open fallopian tube) and severe male factor infertility.

Figure 11. Intrauterine Insemination (IUI), (Kidd *et al*., 2001)

Advantages are that the procedure is less invasive and better tolerated than IVF. Disadvantages are that the procedure has been associated with a lower success rate and higher multiple pregnancy rate than IVF. However, the use of low-dose COH regimens with abandonment of insemination when more than three dominant follicles develop may be expected to reduce the latter. In Australia, best practice involves ovarian stimulation with low-dose clomiphene citrate (usually 50– 100mg daily) or FSH alone (usually 50–75 IU daily),[(65)] with IUI treatment only when one or two dominant follicles are present on the day of HCG administration.

Surrogacy/Gestational Carrier

A pregnancy may be carried by the egg donor (traditional surrogate) or by another woman who has no genetic relationship to the baby (gestational carrier). If the embryo is to be carried by a surrogate, pregnancy may be achieved through insemination alone or through ART. The surrogate will be biologically related to the child.[(66)] If the embryo is to be carried by a gestational carrier, the eggs are removed from the infertile woman, fertilized with her partner's sperm, and transferred into the gestational carrier's uterus. The gestational carrier will not be genetically related to the child. All parties benefit from psychological and legal counseling before pursuing surrogacy or a gestational carrier.

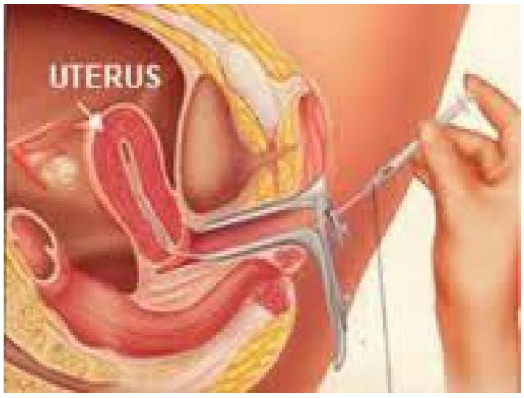


Figure 12. Gestational Surrogacy, (Legro *et al*., 2007)

Donation

Artificial Insemination (AI)- Any procedure in which human sperm are introduced into the reproductive tract of a woman by a non-coital method other than as part of an IVF or GIFT procedure, (Misell *et al*., 2006)

Donor Insemination (DI)- Introduction of sperm which has been donated, from a man other than the woman's partner, into the reproductive tract of a woman by a non-coital method, (Misell *et al*., 2006)

Donation- A process by which a person who has the responsibility to make decisions about the keeping or use of any gametes or embryo gives consent for their use by another person or persons.

In ART there are three types of donation - egg, sperm or embryo.

- **Donor embryo:** A fertilised egg where the sperm and oocyte used do not belong to the couple attempting to conceive. A donor embryo may be donated from a couple, or may be made up from a donated oocyte and donated sperm.
- **Donor sperm:** Sperm not belonging to the male partner of the couple attempting to conceive. The donor may or may not be known to the couple, (Mitwally et al., 2005)
- **Donor oocyte:** An unfertilised egg not belonging to the female member of the couple attempting to conceive. The donor may or may not be known to the couple.

How often is assisted reproductive technology (ART) successful

Success rates vary and depend on many factors. Some things that affect the success rate of ART include:

- Age of the partners
- Reason for infertility
- Clinic
- Type of ART
- If the egg is fresh or frozen
- If the embryo is fresh or frozen

The U.S. Centers for Disease Prevention (CDC) collects success rates on ART for some fertility clinics. According to the 2006 CDC report on ART, the average percentage of ART cycles that led to a live birth were:(70)

- 39% in women under the age of 35
- 30% in women aged 35-37
- 21% in women aged 37-40
- 11% in women aged 41-42

ART can be expensive and time-consuming. But it has allowed many couples to have children that otherwise would not have been conceived. The most common complication of ART is multiple fetuses. But this is a problem that can be prevented or minimized in several different ways.(Paulson *et al*., 2001)

Success Rates

Success varies with many factors. The age of the woman is the most important factor, when women are using their own eggs. Success rates decline as women age, specifically after the mid-30's. Part of this decline is due to a lower chance of getting pregnant from ART, and part is due to a higher risk of miscarriage with increasing age, especially over age 40.

Success rates vary with the number of embryos transferred. However, transferring more and more embryos at one time does not increase the chance of live birth significantly, but may only increase the risk of a multiple pregnancy, and its associated risks, (Peters *et al*., 1992). The impact of the number of embryos that are transferred also varies with the age of the woman. SART, in conjunction with, The American Society for Reproductive Medicine (ASRM), has published guidelines for the recommended number of embryos to transfer. Patients may require several cycles of treatment to have a baby, (Petrozza *et al*., 1997) Success rates remain fairly constant over several cycles, but may vary greatly between individuals. It is important to note that patient characteristics vary among programs; therefore, success rates should not be used to compare treatment centers.

Risks of Art

The medical risks of ART depend upon each specific step of the procedure. The following are some of the primary risks of ART procedures:

Ovarian stimulation carries a risk of hyperstimulation, where the ovaries become swollen and painful. Fluid may accumulate in the abdominal cavity and chest, and the woman may feel bloated, nauseated, and experience vomiting or lack of appetite. Up to 30% of woman undergoing ovarian stimulation have a mild case of ovarian hyperstimulation syndrome (OHSS) that can be managed with overthe-counter painkillers and a reduction in activity.(Pittaway *et al*., 1983) In moderate OHSS, women develop or accumulate fluid within the abdominal cavity, and gastrointestinal symptoms may occur. These women are monitored closely, but generally do very well with simple outpatient management. The condition tends to resolve without intervention unless pregnancy occurs, in which case recovery may be delayed for several weeks. Up to 2% of women develop severe OHSS characterized by excessive weight gain, fluid accumulation in the abdomen and chest, electrolyte abnormalities, over-concentration of the blood, and, in rare

cases, the development of blood clots, kidney failure, or death. (Schlaff *et al*., 1990)

Safety of ART

Research about the safety of ART compared to spontaneous pregnancies is based on observational studies that compare outcomes for couples achieving pregnancy with ART with those achieving pregnancy spontaneously. As outlined in the following, these studies show that ART procedures are associated with greater health risks for the mother and child than spontaneous pregnancies. These risks are largely associated with the use of controlled ovarian hyperstimulation regimens and multiple embryo transfers in ART, for example, OHSS, and complications due to multiple pregnancies. Other differences observed in antenatal and perinatal outcomes may reflect differences between fertile and subfertile couples, (Stumpf *et al*., 1980; Styer *et al*., 2008) such as maternal age and paternal sperm abnormalities, rather than the independent effects of ART. However, research is ongoing to confirm or exclude an association between ART, in particular ICSI, and the risk of congenital malformations.

Ethics

Some couples find it difficult to stop treatment despite very bad prognosis, resulting in futile therapies. This may give ART providers a difficult decision of whether to continue or refuse treatment Tietze *et al*, 1957. Some assisted reproductive technologies can in fact be harmful to both the mother and child. Posing a psychological and a physical health risk, which may impact the ongoing use of these treatments. The adverse affects may cause for alarm, and they should be tightly regulated to ensure candidates are not only mentally, but physically prepared, (Tiitinen *et al*., 2003)

The role of the ART ethical guidelines in the regulation of ART

The Ethical guidelines on the use of assisted reproductive technology in clinical practice and research 2007 underpin the regulation of ART practice within Australia. Accreditation of ART treatment centres by the Reproductive Technology Accreditation Committee (RTAC) is the basis of a nationally consistent approach for overseeing ART clinical practice.(Watson *et al*., 1994) RTAC accreditation requires ART treatment centres to comply with government laws and guidelines concerning the practice of ART. The ART Guidelines are included in this requirement. RTAC was established by the Fertility Society of Australia.

Conclusion

This review has attempted to address the current state of several assisted reproductive technology interventions, with a particular focus on the clinical benefit provided to patients in addition to their safety. Not all established or developing techniques have been discussed as they are beyond the scope of this review, but the considerations raised will none the less be relevant for other technologies such as in vitro maturation, artificial oocyte activation, sperm selection by hyaluronic acid-binding assays and pronuclear transfer for overcoming mitochondrial disease.
Although the same could be said for all areas of medicine, assisted reproductive technology in particular has developed a very strong commercial backing. It is therefore particularly important to ensure that all new technologies are adequately and rigorously tested for both safety and efficiency, ideally before being used clinically. While not all of the techniques discussed here are routinely offered, many are reserved for use in patients with repeated IVF failure. The use of largely experimental techniques particularly in a vulnerable cohort of patients further highlights the need for the risks and benefits to be fully investigated.

REFERENCES

Abma JC, Chandra A, Mosher WD, *et al*. 1997. Fertility, family planning, and women's health: new data from the 1995 National Survey of Family Growth. *Vital Health Stat 23*. May;1-114.

Barbieri RL. Infertility. In: Yen SSC, Jaffe RB, Barbieri RL, 1999. eds. *Reproductive Endocrinology - Physiology, Pathophysiology, and Clinical Management*. 4th ed. Philadelphia, Pa: WB Saunders; 588.

Barreiro, P., Castilla, J.A., Labarga, P., Soriano, V. 2007. Is natural conception a valid option for HIV-serodiscordant couples?. *Hum Reprod*.;22:2353–2358.

Biljan MM, Mahutte NG, Tulandi T, *et al*. 1999. Prospective randomized double-blind trial of the correlation between time of administration and antiestrogenic effects of clomiphene citrate on reproductive end organs.*Fertil Steril*. Apr ;71(4):633-8.

Bujan, L., Pasquier, C., Labeyrie, E., Lanusse-Crousse, P., Morucci, M., Daudin, M. 2004. Insemination with isolated and virologically tested spermatozoa is a safe way for human immunodeficiency type 1 virus-serodiscordant couples with an infected male partner to have a child. *Fertil Steril*. 82:857–862.

Bujan, L., Sergerie, M., Kiffer, N., Moinard, N., Seguela, G., Mercadier, B. *et al*, 2007. Good efficiency of intrauterine insemination programme for serodiscordant couples with HIV-1 infected male partner: a retrospectivecomparative study. *Eur J Obstet Gynecol Reprod Biol*. 135:76–82.

Centers for Disease Control and Prevention. 2002 Assisted Reproductive Technology Success Rates. In:*National Summary and Fertility Clinic Reports*. Atlanta, Ga: Centers for Disease Control and Prevention; 2002.

Chu, M.C., Pena, J.E., Nakhuda, G.S., Thornton, M.H. 2nd, Sauer, M.V. 2006. Assessing the reproductive performance of men co-infected with HIV-1 and hepatitis C undergoing assisted reproduction. *Arch Gynecol Obstet*.274:155–159.

Chu, M.C., Pena, J.E., Thornton, M.H. 2005. 2nd, Sauer, M.V. Assessing the treatment efficacy of IVF with intracytoplasmic sperm injection in human immunodeficiency virus-1 (HIV-1) serodiscordant couples. *Reprod Biomed Online*. 10:130–134.

Confino E, Tur-Kaspa I, DeCherney A, *et al*. 1990. Transcervical balloon tuboplasty. A multicenter study. *JAMA*. Oct 24-31 ; 264(16):2079-82.

Crosignani PG, Collins J, Cooke ID, et at. 1992. Recommendations of the ESHRE workshop on 'Unexplained Infertility'. Anacapri, August 28-9, *Hum Reprod*. Jun 1993; 8(6):977-80.

Dain L, Auslander R, Dirnfeld M. 2011. The effect of paternal age on assisted reproduction outcome. *Fertil Steril*. Jan ;95(1):1-8.

Danforth DR, Arbogast LK, Mroueh J, *et al*. 1998. Dimeric inhibin: a direct marker of ovarian aging. *Fertil Steril*. Jul ;70(1):119-23.

de La Rochebrochard E, de Mouzon J, Thepot F, *et al*. 2006. Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. *Fertil Steril*. May ;85(5):1420-4.

Dovey, S., Sneeringer, R.M., Penzias, A.S. 2008. Clomiphene citrate and intrauterine insemination: analysis of more than 4100 cycles. *Fertil Steril*. 90:2281–2286.

Egger, M., Schneider, M., Davey Smith, G. 1998. Spurious precision? Meta-analysis of observational studies. *BMJ*. 316:140–144.

Egger, M., Smith, G.D., Schneider, M. 2001. Systematic reviews of observational studies. in: M. Egger, G.D. Smith, D.G. Altman (Eds.) Systematic reviews in health care: meta-analysis in context. BMJ Publishing Group, London; 211–227.

Ethics Committee of the American Society for Reproductive Medicine. 2004. Human immunodeficiency virus and infertility treatment. *Fertil Steril*.82:S228–S231.

Evenson DP, Wixon R. 2005. Comparison of the Halosperm test kit with the sperm chromatin structure assay (SCSA) infertility test in relation to patient diagnosis and prognosis. *Fertil Steril*. Oct ;84(4):846-9.

Faddy MJ, Gosden RG, Gougeon A, *et al*. 1992. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod*. Nov ;7(10):1342-6.

Farhi, J., Ben-Haroush, A., Lande, Y., Fisch, B. 2007. Role of treatment with ovarian stimulation and intrauterine insemination in women with unilateral tubal occlusion diagnosed by hysterosalpingography. *Fertil Steril*. 88:396–400.

Forsey JP, Caul EO, Paul ID, *et al*. 1990. Chlamydia trachomatis, tubal disease and the incidence of symptomatic and asymptomatic infection following hysterosalpingography. *Hum Reprod*. May ;5(4):444-7.

Garrido, N., Meseguer, M., Bellver, J., Remohi, J., Simon, C., Pellicer, A. 2004. Report of the results of a 2 year programme of sperm wash and ICSI treatment for human immunodeficiency virus and hepatitis C virus serodiscordant couples. *Hum Reprod*. 19:2581–2586.

Guttmacher AF. 1956. Factors affecting normal expectancy of conception. *J Am Med Assoc*. Jun 30 ;161(9):855-60.

Guzick DS, Overstreet JW, Factor-Litvak P, *et al*. 2001. Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med*. Nov 8 ;345(19):1388-93.

Hamdan M, Omar SZ, Dunselman G, Cheong Y. 2015. Influence of Endometriosis on Assisted Reproductive Technology Outcomes: A Systematic Review and Meta-analysis. *Obstet Gynecol*. Jan ;125(1):79-88.

Hassan MA, Killick SR. 2003. Effect of male age on fertility: evidence for the decline in male fertility with increasing age. *Fertil Steril*. Jun 79 Suppl 3:1520-7.

Isaacs JD Jr, Lincoln SR, Cowan BD. 1997. Extended clomiphene citrate (CC) and prednisone for the treatment of chronic anovulation resistant to CC alone. *Fertil Steril*. Apr ;67(4):641-3.

Johnson NP, Mak W, Sowter MC. 2004. Surgical treatment for tubal disease in women due to undergo in vitro fertilisation. *Cochrane Database Syst Rev*. CD002125.

Joint United Nations Programme on HIV/AIDS (UNAIDS). 2009. World Health Organization. AIDS epidemic update. December. WHO Library, Geneva; 2009.

Kalra SK, Ratcliffe SJ, Coutifaris C, Molinaro T, Barnhart KT. 2011. Ovarian stimulation and low birth weight in newborns conceived through in vitro fertilization. *Obstet Gynecol*. Oct ;118(4):863-71.

Kidd SA, Eskenazi B, Wyrobek AJ. 2001. Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril*. Feb ;75(2):237-48.

Klonoff-Cohen HS, Natarajan L. 2004. The effect of advancing paternal age on pregnancy and live birth rates in couples undergoing in vitro fertilization or gamete intrafallopian transfer. *Am J Obstet Gynecol*. Aug ;191(2):507-14.

Kovacic, B., Sajko, M.C., Vlaisavljevic, V. 2010. A prospective, randomized trial on the effect of atmospheric versus reduced oxygen concentration on the outcome of intracytoplasmic sperm injection cycles. *Fertil Steril*. 94:511–519.

Kovacs GT, Newman GB, Henson GL. 1978. The postcoital test: what is normal?. *Br Med J*. Apr 1 ;1(6116):818.

Kowalska, A., Maciejewski, J., Niemiec, T., Scholz, A., Lipniacki, A., Piasek, A. 2005. Efficacy and safety of assisted reproduction techniques in HIV-discordant couples. *HIV AIDS Rev*. 4:7–10.

Kresowik JD, Stegmann BJ, Sparks AE, Ryan GL, van Voorhis BJ. 2011. Five-years of a mandatory single-embryo transfer (mSET) policy dramatically reduces twinning rate without lowering pregnancy rates. *Fertil Steril*. Dec ;96(6):1367-9.

Kwee J, Elting ME, Schats R, *et al*. 2007. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. *Reprod Biol Endocrinol*. Mar 15 ;5:9.

Lee, S., Revas-Toro, H., Rozis, G. 2001. Tratamento com IIU e ICSI para homens HIV positivo com parceiras HIV negativo: método de redução de riscos. *J Brasil Reprod Assist*. 5:64–65.

Legro RS, Barnhart HX, Schlaff WD, *et al*. 2007. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med*. Feb 8 ;356(6):551-66.

Liberati, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C., Ioannidis, J.P. *et al*, 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Ann Intern Med*. 151:W65–W94.

Manigart, Y., Rozenberg, S., Barlow, P., Gerard, M., Bertrand, E., Delvigne, A. 2006. ART outcome in HIV-infected patients.*Hum Reprod*. 21:2935–2940.

Marina, S., Marina, F., Alcolea, R., Exposito, R., Huguet, J., Nadal, J. *et al*, 1998. Human immunodeficiency virus type 1–serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertil Steril*.70:35–39.

Marina, S., Marina, F., Expósito, R., Alcolea, R., Pérez, N., Bermejo, B. *et al*, 2002. HIV y reproducción asistida. Reproducción Asistida en parejas serodiscordantes (hombre seropositivo) al VIH-1: experiencia de 118 niños nacidos sanos. *Ginecol Obstet Clín*. 3:146–150.

McLernon DJ, Harrild K, Bergh C, Davies MJ, de Neubourg D, Dumoulin JC, *et al*. 2010. Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials. *BMJ*. Dec 21 ;341:c6945.

Melo, M.A., Meseguer, M., Bellver, J., Remohi, J., Pellicer, A., Garrido, N. 2008. Human immunodeficiency type-1 virus (HIV-1) infection in serodiscordant couples (SDCs) does

not have an impact on embryo quality or intracytoplasmic sperm injection (ICSI) outcome. *Fertil Steril*. 89:141–150.

Mencaglia, L., Falcone, P., Lentini, G.M., Consigli, S., Pisoni, M., Lofiego, V. *et al*, 2005. ICSI for treatment of human immunodeficiency virus and hepatitis C virus-serodiscordant couples with infected male partner. *Hum Reprod*. 20:2242–2246.

Misell LM, Holochwost D, Boban D, *et al*. 2006. A stable isotope-mass spectrometric method for measuring human spermatogenesis kinetics in vivo. *J Urol*. Jan ;175(1):242-6; discussion 246.

Mitwally MF, Biljan MM, Casper RF. 2005. Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. *Am J Obstet Gynecol*. Feb ;192(2):381-6.

Navot D, Rosenwaks Z, Margalioth EJ. 1987. Prognostic assessment of female fecundity. *Lancet*. Sep 19 ;2(8560):645-7.

Nicopoullos, J.D., Frodsham, L.C., Ramsay, J.W., Almeida, P.A., Rozis, G., Gilling-Smith, C. 2004. Synchronous sperm retrieval and sperm washing in an intracytoplasmic sperm injection cycle in an azoospermic man who was positive for human immunodeficiency virus. *Fertil Steril*.81:670–674.

Nyboe Andersen, A., Goossens, V., Bhattacharya, S., Ferraretti, A.P., Kupka, M.S., de Mouzon, J. *et al*, 2009. Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE: ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod*. 24:1267–1287.

Paulson RJ, Milligan RC, Sokol RZ. 2001. The lack of influence of age on male fertility. *Am J Obstet Gynecol*. Apr ;184(5):818-22; discussion 822-4.

Peña, J.E., Thornton, M.H., Sauer, M.V. 2003. Assessing the clinical utility of in vitro fertilization with intracytoplasmic sperm injection in human immunodeficiency virus type 1 serodiscordant couples: report of 113 consecutive cycles. *Fertil Steril*. 80:356–362.

Perkins KM, Boulet SL, Kissin DM, Jamieson DJ. 2015. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001-2011. *Obstet Gynecol*. Jan ;125(1):70-8.

Peters A, J, Lloyd R, P, Coulam C, B. 1992. Prevalence of out-of-phase endometrial biopsy specimens. *Am J Obstet Gynecol*. Jun ;166(6 Pt 1):1738-45; discussion 1745-6.

Petrozza JC. 1997. The Role of Diagnostic Laparoscopy in Evaluation of Infertility. *Infertil Reprod Med Clin North Am*.

Picaud JC, Chalies S, Combes C, Mercier G, Dechaud H, Cambonie G. 2012. Neonatal mortality and morbidity in preterm infants born from assisted reproductive technologies. *Acta Paediatr*. Aug ;101(8):846-51.

Pittaway DE, Winfield AC, Maxson W, *et al*. 1983. Prevention of acute pelvic inflammatory disease after hysterosalpingography: efficacy of doxycycline prophylaxis. *Am J Obstet Gynecol*. Nov 15 ; 147(6):623-6.

Prisant, N., Tubiana, R., Lefebvre, G., Lebray, P., Marcelin, A.G., Thibault, V. *et al*, 2010. HIV-1 or hepatitis C chronic infection in serodiscordant infertile couples has no impact on infertility treatment outcome. *Fertil Steril*. 93:1020–1023.

Queiroz, P., Tanil, C.T., Madaschi, C., Lopes, D.R., Iaconelli Junior, A., Pasqualotto, F.F. *et al*, 2008. Safe gametes acquisition through association of seminal processing techniques with HIV serodiscordant couples (article in Portuguese). *Rev Bras Ginecol Obstet*. 30:171–176.

Rova K, Passmark H, Lindqvist PG. 2012. Venous thromboembolism in relation to in vitro fertilization: an approach to determining the incidence and increase in risk in successful cycles. *Fertil Steril*. Jan ;97(1):95-100.

Royce, R.A., Sena, A., Cates, W. Jr., Cohen, M.S. Sexual1997. transmission of HIV. *N Engl J Med*.336:1072–1078.

Sauer, M.V., Chang, P.L. 2002. Establishing a clinical program for human immunodeficiency virus 1-seropositive men to father seronegative children by means of in vitro fertilization with intracytoplasmic sperm injection. *Am J Obstet Gynecol*. 186:627–633.

Savasi, V., Ferrazzi, E., Lanzani, C., Oneta, M., Parrilla, B., Persico, T. 2007. Safety of sperm washing and ART outcome in 741 HIV-1-serodiscordant couples. *Hum Reprod* ;22:772–777.

Schlaff WD, Hassiakos DK, Damewood MD, *et al*. 1990. Neosalpingostomy for distal tubal obstruction: prognostic factors and impact of surgical technique. *Fertil Steril*. Dec ;54(6):984-90.

Semprini, A.E., Levi-Setti, P., Bozzo, M., Ravizza, M., Taglioretti, A., Sulpizio, P. *et al*, 1992. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet*;340:1317–1319.

Shah DK, Missmer SA, Berry KF, Racowsky C, Ginsburg ES. 2011. Effect of obesity on oocyte and embryo quality in women undergoing in vitro fertilization. *Obstet Gynecol*. Jul;118(1):63-70.

Stroup, D.F., Berlin, J.A., Morton, S.C., Olkin, I., Williamson, G.D., Rennie, D. *et al*, 2000. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA*.283:2008–2012.

Stumpf PG, March CM. 1980. Febrile morbidity following hysterosalpingography: identification of risk factors and recommendations for prophylaxis. *Fertil Steril*. May ;33(5):487-92.

Styer AK, Wright DL, Wolkovich AM, *et al*. 2008. Single-blastocyst transfer decreases twin gestation without affecting pregnancy outcome. *Fertil Steril*. Jun ;89(6):1702-8.

Tay, P.Y., Raj, V.R., Kulenthran, A., Sitizawiah, O. 2007. Prognostic factors influencing pregnancy rate after stimulated intrauterine insemination. *Med J Malaysia*. 62:286–289. PubMed

Terriou, P., Auquier, P., Chabert-Orsini, V., Chinchole, J.M., Cravello, L., Giorgetti, C. *et al*, Outcome of ICSI in HIV-1-infected women. *Hum Reprod*.20:2838–2843.

Tietze C. 1957. Reproductive span and rate of reproduction among Hutterite women. *Fertil Steril*. Jan-Feb ;8(1):89-97.

Tiitinen A, Unkila-Kallio L, Halttunen M, *et al*. 2003. Impact of elective single embryo transfer on the twin pregnancy rate. *Hum Reprod*. Jul ;18(7):1449-53.

van Leeuwen, E., de Vries, J.W., Jurriaans, S., Verhoeve, H.R., Prins, J.M., Repping, S., van der Veen, F. 2005. Intra uterine insemination with processed sperm for HIV serodiscordant couples in whom the man is HIV positive (article in Dutch). *Ned Tijdschr Geneeskd*. 149:423–424.

Veiga, A., Coll, O., Tur, R., Busquets, A., Barri, P.N. 1999. Assisted reproductive technologies and HIV-1

serodiscordant couples. *Prenat Neonat Med*. 4:357–361.

Watson A, Vandekerckhove P, Lilford R, *et al*. 1994. A meta-analysis of the therapeutic role of oil soluble contrast media at hysterosalpingography: a surprising result?. *Fertil Steril*. Mar ;61(3):470-7.

Zadehmodarres, S., Oladi, B., Saeedi, S., Jahed, F., Ashraf, H. 2009. Intrauterine insemination with husband semen: an evaluation of pregnancy rate and factors affecting outcome. *J Assist Reprod Genet*. 26:7–11.

****