RESEARCH REVIEW JOURNALS
Volume-04
Issue-05
May-2019

ISSN: 2455-3085 (Online)
RESEARCH REVIEW International Journal of Multidisciplinary
www.rrjournals.com[UGC Listed Journal]

Overview of Human In Vitro Fertilization (IVF) and Implantation

[1]Shiwani Tripathi, [2]Dr. Apurva Pathak & [3]Dr. Neelam Tripathi

[1]Research Scholar, Department of Biotechnology, Sri Satya Sai University of Technology & Medical Sciences, Sehore, M.P.
[2]Research Guide, Department of Biotechnology, Sri Satya Sai University of Technology & Medical Sciences, Sehore, M.P
[3]Research Guide, Department of Biotechnology, Sri Satya Sai University of Technology & Medical Sciences, Sehore, M.P

ARTICLE DETAILS

Article History
Published Online: 15 May 2019

Keywords
Fertilization, Infertility, Human in Vitro Fertilization (IVF), Implantation, assisted reproductive technology (ART).

ABSTRACT

In vitro fertilization (IVF) is a method of fertilization that involves combining an egg with sperm outside of the body ("in glass"). The procedure entails measuring and inducing a woman's ovulatory cycle, as well as extracting an ovum or ova (egg or eggs) from her ovaries and allowing sperm to fertilize them in a laboratory liquid. Human embryo implantation is a three-stage process (apposition, adhesion and invasion) involving synchronized crosstalk between a receptive endometrium and a functional blastocyst. The purpose of this paper is to understand and describe the IVF process and different players of the implantation process.

1. Introduction

The beginning of a new life begins with fertilization, which is characterised as the union of two germ cells (egg and sperm) that restores the somatic chromosome number and initiates the development of a new individual with parental species characteristics. In vitro fertilization (IVF) is a form of assisted reproductive technology (ART) for manually mixing an egg and sperm to create a viable preimplantation embryo in a laboratory environment, as opposed to normal or in vivo fertilisation. The three main processes of in vitro embryo development (IVP) are in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC). The proper expression of several genes at different developmental stages is necessary for mammalian germ cell maturation and early embryo development. The use of gene expression analysis to classify genes involved in in vitro embryo development (IVEP) is becoming increasingly successful, allowing for a much more accurate prediction of embryo survival and normality.

In vitro fertilization (IVF) is an excellent way to learn more about the processes involved in in vivo fertilisation. IVF may also be used to examine issues such as oocyte maturation, spermatozoa capacitation, egg activation, regulation of the embryo's genome, cell division mechanisms, maternal and paternal genome effect, nucleus-cytoplasm interaction, and so on. IVF also allows researchers to investigate the mechanism of nuclear differentiation using nuclear graft, which is an important step in the development of embryo cloning techniques. IVF has many benefits in farm animals, including the ability to produce a large number of embryos in a short period of time, the ability to use embryos from dead cows or those with fertility issues, the ability to produce embryos from a cow during the first third of pregnancy, and the ability to produce embryos in pre-pubertal heifers.

2. Fertilization

Sperm will enter the uterus and travel to the opposite end of the fallopian tubes to find the released egg. Fertilization is the result of the interaction of sperm and egg. Fertilization is a rare occurrence in which a male gamete fuses with a female gamete only if they happen to meet during their fertile life span. The successful penetration of a single sperm through the egg coat triggers a series of sperm-egg interaction events, releasing the oocyte from meiosis II arrest. As a result, the first cell of new life, the diploid zygote, is formed.

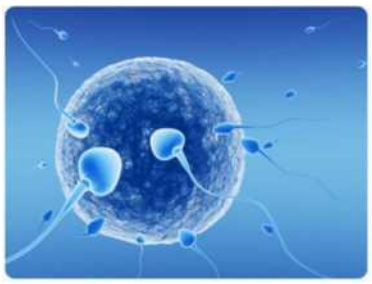


Fig 1. Fertilization

Embryos go through a series of mitotic cell divisions, also known as cleavages, following fertilisation. During the oocyte's journey through the fallopian tube to the uterus, fertilization and embryo development occur naturally. Once inside the uterus, the embryo implants into the endometrium, the uterine lining, where it continues to develop into a foetus and baby until childbirth. The uterus creates an ideal environment for this to happen. Infertility can be caused by dysfunction at any of these points.

3. Infertility

Infertility (clinical definition): a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse " (Human Reproduction, Vol.24, No.11 pp. 2683-2687, 2009, Advanced Access publication on October 4, 2009). The biological inability of a person to contribute to conception is referred to as infertility. Infertility can also refer to a woman's inability to carry a pregnancy all the way to term.

Infertility in a couple who has never had children is known as primary infertility. Failure to conceive after a previous pregnancy is referred to as secondary infertility. Both the man and the woman may be infertile or sub-fertile in some cases, and the couple's infertility is caused by the combination of these conditions. Infertility can be caused by immunological or

genetic factors; it's also possible that each partner is fertile on their own but the couple can't conceive together without help.

Infertility is described as the failure to conceive or bring a pregnancy to term after one year of unprotected sexual intercourse, or the presence of a medical condition that reduces the probability of conception or pregnancy to term. Observational studies of couples using natural methods to conceive have shown that approximately 80% of couples would conceive in the first six menstrual cycles, with an additional 10% conceiving spontaneously in the following six cycles . For the next three years, about half of the 10% of couples who are listed as infertile after one year of attempting to conceive will have a spontaneous pregnancy (Gnoth et. Al.2005).

According to the World Health Organization (WHO), infertility affects one out of every four couples in developing countries. According to WHO estimates, India's overall prevalence of primary infertility ranges from 3.9 to 16.8%. The prevalence of infertility in India varies by state, ranging from 3.7 percent in Uttar Pradesh, Himachal Pradesh, and Maharashtra to 5% in Andhra Pradesh and 15% in Kashmir. In addition, prevalence varies across tribes and castes in the same area. Though the exact prevalence of infertility in Tamil Nadu is currently unknown, Syamala states that 2.3 percent of married women aged 20 to 49 are infertile.(Syamala, 2012).

Infertility is a common problem that affects more than 10% of all couples worldwide. Most people consider it to be mentally exhausting, and it can lead to depression, social alienation, and a poorer quality of life (Johansson,2009). Historically, infertile people had no recourse to medical services, causing them to jeopardize their wellbeing and even their lives by engaging in more or less mysterious infertility-treatment procedures. Female infertility is often caused by damage to the Fallopian tubes, which prevents interaction between the egg and the sperm (Fig. 1), while male infertility is associated with decreased sperm quantity and quality.

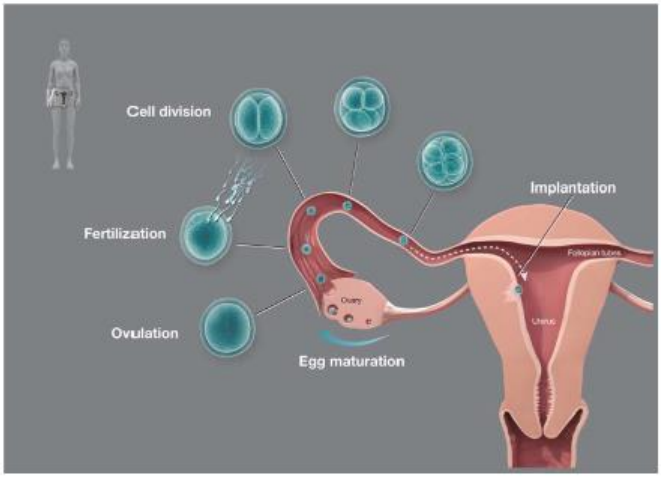


Fig. 1. The fertilization process in humans.

Female gametes are contained in the ovaries in the form of separate follicles, with each follicle containing one germ cell surrounded by one or more layers of granulosa cells. Humans (and other mammals) are born with a predetermined pool of primordial oocytes that are halted during the dictyate stage of meiosis I. FSH signaling and other factors promote the maturation of individual follicles in sexually mature women on a monthly basis, resulting in primary and secondary follicles. A cascade of events, including further oocyte growth and meiotic resumption, is triggered in response to a rapidly increased concentration of luteinizing hromone. Meiosis reactivation completes the first meiotic chromosome segregation event, resulting in one set of chromosomes being arrested at the metaphase stage of meiosis II and a second set of chromosomes being discarded (the first polar body). Following this, the mature follicle ruptures and ovulation occurs, which is the release of the egg from the ovary into the fallopian tubes (the oviduct). Sperm will penetrate the uterus and travel to the opposite end of the fallopian tubes to find the released egg. The successful penetration of a single sperm through the egg coat initiates a series of sperm-egg interaction events that alleviate the egg's meiosis II arrest. This results in the creation of two haploid sets of chromosomes, one of which will fuse with the haploid set of chromosomes provided by the sperm and the other of which will be discarded (the second polar body). The fertilization process produces an embryo, which divides multiple times as it passes through the fallopian tubes into the uterus. While within the uterus, the embryo (now at the blastula stage) will insert into the uterine lining's wall, known as the endometrium. This place will then be used for further embryo development.

4. Causes of Infertility

Infertility may be caused by male or female infertility, or by a combination of factors affecting both partners. Infertility has been due to male factors in up to 25% of couples, female factors in up to 58 percent of couples, and unexplained in up to 17 percent of couples, as shown in Figure 1.1. Braunwald(2002). When couples present after struggling to conceive after 12 months of attempting, common procedure is to conduct a comprehensive medical and sexual history as well as a medical examination of all partners. Both partners are then given the option of conducting an investigation. The aim of these initial routine investigations is to make a diagnosis, direct management, predict prognosis, or classify patients who require additional testing. Many causes of infertility are reversible and can be treated without the use of assisted reproductive technology (ART). In 5 to 15% of couples, no cause is found, and they are diagnosed with "unexplained infertility." These couples are eligible for ART therapy.

4.1 Risk Factors

Infertility is primarily dictated by a woman's age. Natural female fertility declines steadily after the age of 30, then rapidly after the age of 35, eventually ceasing at menopause. Obesity (BMI greater than 29) and low body weight (BMI less than 19 and irregular or absent menstruation) are other factors related to female infertility, as is smoking. Male infertility has also been related to obesity. Men's semen content has been linked to excessive alcohol consumption, smoking, and elevated scrotal temperature due to sedentary work positions, occupational heat exposure, and wearing tight underwear, though the effect on male fertility is unknown. Infertility has been linked to prescription and recreational medications, as well as workplace hazards such as solvent exposure in both males and females Braunwald(2002).

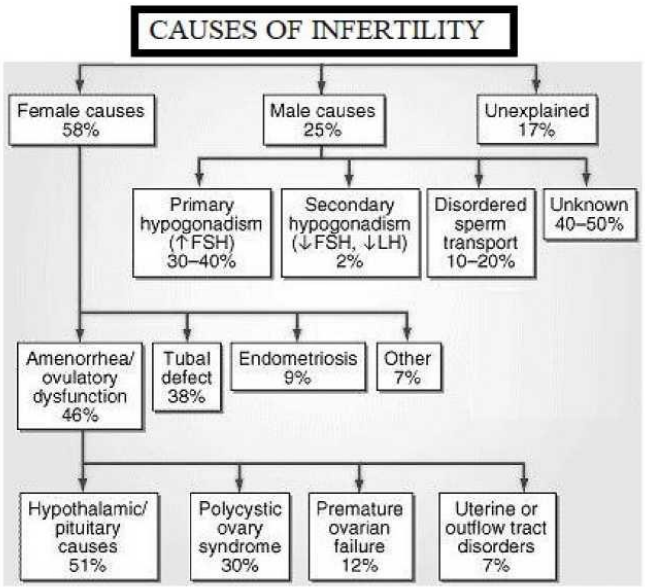


Figure 2. Causes of male and female infertility. Source(Braunwald(2002)

4.2. Male Factor Infertility

A comprehensive history, physical examination, semen analysis, and, in some cases, hormone measurement are all part of a routine investigation of male partners. The sperm analysis is key to this evaluation. Normospermia (no abnormality) means that spermatogenesis (sperm production) is natural and the reproductive tract is patent. A discovery of no sperm (azoospermia) confirms male infertility on the other hand. Low sperm count (oligospermia); low sperm motility (asthenozoospermia); irregular sperm types (teratozoospermic) and combinations of these anomalies are associated with male subfertility, but these abnormalities may be just one factor leading to a couple's failure to conceive.

Although the WHO has identified reference values for sperm count, motility, and morphology, males classified as abnormal according to these criteria can still be able to conceive naturally, restricting the predictive value of semen analysis findings other than azoospermia. A sperm density of less than 10 x 106 per mL, a motility of less than 30%, or a normal morphology of less than 4% are all considered severe male factors . Problems with sperm development, such as abnormal hormone production or testicular failure, or problems with sperm transport from the testes to ejaculation, such as obstruction of the genital tract, may cause abnormal sperm parameters.

There is no underlying cause in about a quarter of infertile males. Sperm production problems may be caused by irregular testicular development, secondary testicular failure, hypothalamic/pituitary failure, or sperm motility or function issues. The cause of irregular testicular development is normally unclear, but it is often related to conditions like cryptorchidism or sex chromosome abnormalities. Testicular failure may be caused by testicular torsion, trauma, orchitis, as well as exposure to radiotherapy or chemotherapy. Hypothalamic/pituitary disorder is a rare cause of low sperm production, but it is significant because it is the only one that can be treated.

Fertility is reduced by a variety of congenital sperm morphology defects. Furthermore, the presence of anti-sperm antibodies or infection impairs sperm motility and function. Infectious occlusion of the epididymis, vasectomy, or neuropathy - such as spinal injury or sexual problems - can all cause problems with sperm transport through the male genital tract. Varicoceles (dilation of the veins that drain the testes thought to hinder fertility due to elevated intratesticular temperature) and genital tract obstruction are the most common underlying diagnoses. Surgical intervention can be used to address any of these issues. Medical care is available for less common causes such as sperm autoimmunity, gonadotrophin deficiency, and other endocrine disorders.

4.3. Female Factor Infertility

Routine investigation of female partners includes a detailed history, physical examination and investigation with hormone testing to assess ovulatory function; chlamydia screening to indicate potential tubal damage; and hysterosalpingography to assess tubal patency.

➢ **Ovulatory dysfunction**

Ovulatory failure is diagnosed by taking a menstrual cycle history and testing progesterone levels in the blood. Following the discovery of irregular menstruation or a low progesterone level, other hormones involved in ovulation control, such as follicle stimulating hormone (FSH), luteinizing hormone (LH), and prolactin, as well as estradiol released by the ovary, are measured (NCCWCH,2004; Evers,2002). The outcomes of these tests are used to identify the causes and locations of the underlying hormone deficiency, as well as to assess the best therapeutic strategies for addressing the underlying issue. Women with low FSH levels due to hypothalamic failure, for example, may be treated with gonadotropin-releasing hormones, whereas those with high prolactin levels can be treated with dopamine antagonists and/or the underlying trigger. Women with normal hormone levels are said to have an issue with the hypothalamic-pituitary-ovarian axis, which controls hormones. The use of ovarian stimulation medications is used to treat this subgroup. In women with premature ovarian failure, there is no medication available to restore ovulation (associated with a low concentration of estradiol despite high levels of FSH). This may be a primary issue or a side effect of chemotherapy, radiotherapy, or other iatrogenic treatments. Oocyte donation may be used to help these women get pregnant.

➢ **Reproductive tract disorders**

Tubal obstruction and other pelvic anatomy distortions may prevent natural fertilization of the egg and sperm, as well as the embryo's transport to the uterus for implantation. Post-infectious tissue injury, post-surgical adhesions, and uterine developmental defects are among the causes. A history of pelvic inflammatory disorder or surgery, chronic pelvic pain, and clinical test results all point to the diagnosis. To ascertain the risk of tubal injury, a blood test is performed to detect prior exposure to the sexually transmitted disease Chlamydia trachomatis. While its value as a standard investigation has been challenged, hysterosalpingography is routinely performed to determine tubal patency (Evers, 2002). The operation is surgical and can be completed laparoscopically. However, surgery has a low success rate (only 10% for serious tubal

disease), and ART is a safer choice for patients who have a bad prognosis for surgery.

➢ **Endometriosis**

Endometriosis and infertility have a complicated relationship that seems to vary greatly from woman to woman. While endometriosis undoubtedly reduces the chances of pregnancy each month in many women, the mechanisms are unknown. Surgery enhances fertility in women with endometriosis, according to limited level II RCT evidence and meta-analysis evidence (Adamson,19994, Parazzini 1999), but the evidence for the role of IVF and ARTs in management is uncertain (11). In women with endometriosis, IVF can result in pregnancy quickly, but it's unclear if one period of IVF is equivalent to attempting to conceive spontaneously for one month, six months, two years, or longer, or if the form or severity of endometriosis has an effect on IVF efficacy. (Adamson,2005).

➢ **Uterine fibroids**

This is a common disorder in which the uterine muscle develops benign smooth muscle and fibrous tumours. While many women with fibroids tend to have normal fertility, there is some evidence that fibroids may play a role in infertility in some women (Peric & Fraser,2006). Submucosal fibroids that distort the uterine cavity are likely to have a negative impact on fertility and decrease the chances of IVF treatment success. (Pritts,2001).

➢ ***Adenomyosis***

This is another disorder that affects the anatomy and function of the uterine muscle and may make it difficult for some women to conceive. However, this disease often affects women in their later reproductive years, when fertility is less of a concern. The role of ART in management is unknown.

➢ **Defects in sperm-mucus interaction**

Defects in sperm-mucus interaction are of uncertain significance. Such 'defects' are generally based on the post-coital test where non-motile sperm are observed in the cervical mucus. The validity of the post-coital test has been questioned and this diagnosis is not universally accepted. An abnormal post-coital test may, however, indicate the presence of anti-sperm antibodies produced by either the male or female partner with the potential to interfere with sperm motility and egg fertilization.

5. Infertility Treatments

Some of the most common causes of infertility in couples may react to treatment aimed at reversing the underlying problem. This may include surgical steps to treat genital tract obstruction or endometriosis, or hormone therapy to restore ovulatory function, as defined in the aetiology section above. In cases where other therapies are ineffective, the underlying cause of infertility is not treatable, or the infertility is unexplained, ART procedures are efficient.

5.1 In Vitro Fertilization (IVF)

IVF entails a number of procedures. Regulated ovarian hyperstimulation, oocyte extraction, sperm retrieval and preparation, IVF, and embryo transfer are the five basic measures. In preparation for fertilisation, sperm are washed, spun in a centrifuge, and incubated in a specialised medium. To classify mature oocytes appropriate for fertilisation, retrieved oocytes are isolated from follicular fluid and classified. After 16 to 20 hours, the sperm and oocyte samples are mixed in a culture medium in a Petri dish and tested for fertilisation. Fertilized oocytes are destroyed, as are oocytes that have fertilised abnormally. A pronuclear zygote is a cell that includes a pronucleus from both the oocyte and the sperm after fertilisation. Cell division begins after the two pronuclei merge, and the cell reaches a 4-cell level after about 40 hours. At this stage, embryos are examined for viability.

5.2 Ovarian Hyperstimulation Under Strict Supervision (COH)

The ovary produces approximately ten follicles, each containing an oocyte, in a typical 28-day menstrual cycle, of which one follicle matures under the influence of the pituitary hormones FSH and LH, with ovulation occurring around day 14 of the cycle. COH is a surgical procedure that involves the induction of several ovarian follicles. COH is used in most ART cycles to allow for multiple oocyte harvesting. The availability of multiple oocytes increases the probability that IVF will produce a variety of embryos for transfer, allowing for the selection of embryos with a greater chance of implanting in the uterus (based on growth rates and morphology). The pharmaceuticals used to treat COH work by allowing the growth of several mature follicles rather than a single dominant follicle by raising and maintaining high FSH levels. Clomiphene citrate, an anti-estrogen that increases pituitary FSH release, can be used as part of COH protocols prior to artificial insemination, although it is seldom used prior to IVF nowadays. Exogenous follicle stimulating hormone is widely used to promote follicle growth prior to IVF. The LH concentration is usually inhibited with gonadotrophin releasing hormone (GnRH) analogues, either agonists or antagonists, to prevent premature release of LH triggering premature ovulation of the oocytes. Serum estradiol levels and transvaginal ultrasound are used to monitor follicular growth. Human chorionic gonadotropin (HCG) is provided once the follicles have grown to an appropriate size, normally after 9 to 11 days, to induce oocyte maturation in preparation for oocyte selection. However, the cycle may be terminated prior to oocyte collection if either an inadequate number of follicles develops or an excessive number of follicles develops. Donor oocytes may be used to support women who are unable to grow enough follicles on their own. Oocyte retrieval occurs between 32 and 36 hours after HCG administration and before spontaneous ovulation. This can be done trans-vaginally with sedation by inserting a needle into the ovary under ultrasound control, or laparoscopically with general anaesthetic.

5.3 Extraction of sperm

On the same day as the oocyte extraction, fresh sperm are extracted from the male partner's ejaculate, while frozen-thawed sperm can also be used. If the male partner is azoospermic, sperm may be extracted from the testes or epididymis using percutaneous or open biopsy techniques. The needle aspiration of sperm from the testis, epididymus, or vas is used in percutaneous methods. It takes about 30 minutes and can be done in an outpatient setting under local or regional anaesthesia. An open biopsy of the testis is a more invasive

procedure that is done under general anaesthesia in an operating room. It comes with a higher risk of surgical and anaesthetic complications, but it also offers a larger volume of tissue, which may be required in men with serious sperm defects or preferred for later ART attempts.

5.4 Embryo Transplantation (ET)

ET is performed as an outpatient procedure either 2 to 3 days after fertilisation, when the embryo is at the cleavage (4 to 8 cell) level, or when the embryo is at the cleavage (4 to 8 cell) stage (or after 4 to 6 days if transferred at the blastocyst stage). Various grading systems are widely used to pick the healthiest embryos for trans-vaginal injection into the uterus. Following ET, the female is treated with progesterone or alternative regimens on a regular basis until week 10 of pregnancy to help with implantation and pregnancy maintenance. The number of embryos transferred is determined by the provider's experience as well as the age and interests of the couple being treated. The number of cycles involving the transfer of three embryos in India decreased from 47.3 percent in 2007 to 43.09 percent in 2009. However, between 2007 and 2009, the rate of double embryo transfer rose from 21.4 percent to 24.78 percent, whereas single embryo transfer was performed in 10% of all cycles and remained constant over the three years (Malhotra at. al.2013). Any healthy embryos that remain can be frozen and transferred at a later date if necessary.

5.5 Frozen Embryo Transfer and Cryopreservation (FET)

The ability to freeze embryos for later frozen-thawed embryo transfer in unstimulated treatment cycles has the potential to increase the number of embryo replacement cycles without the need for additional regulated ovarian hyperstimulation and oocyte retrieval. While the live birth rate per ET is lower for frozen than fresh cycles (26.8% per transfer in 2007 to 29.92 percent per transfer in 2009 (Malhotra et. al. 2013), this increases the overall pregnancy rate per treatment cycle while also lowering the risk of ovarian hyperstimulation syndrome (OHSS). Any remaining healthy embryos may be frozen for storage and transfer at a later date if required.

5.6. Sperm Injection into The Cytoplasm (ICSI)

ICSI is an ART procedure that involves injecting a single sperm into the cytoplasm of an oocyte in order to fertilise it. Although some have suggested that it be used as a first-line ART treatment for couples with male factor infertility and those who have had poor fertilization with traditional IVF, it is indicated for the treatment of couples with male factor infertility and those who have had poor fertilization with conventional IVF (Abu-Hassan & Al-Hasani,2003). For couples with extreme male factor infertility, ICSI is the only treatment choice. It can be achieved with sperm that has been surgically extracted or ejaculated. Oocytes are tested for fertilization after 16 hours, and viable embryos are transferred 1 to 3 days later, as mentioned above for IVF. Concerns have been raised that ICSI could be linked to an increased risk of congenital malformations and long-term genetic effects because it allows for the production of embryos from defective sperm that would not otherwise be able to fertilise, such as sperm from males with genetic defects (Boyle t. al, 2004).

5.7. Intrafallopian Gamete Shift (GIFT)

GIFT is a laparoscopic procedure that involves aspirating follicles and transferring oocytes and sperm into the fallopian tubes at the same time. GIFT was created as a more physiological alternative to IVF in women with a high chance of success, but its use has decreased in the last decade as IVF strategies have increased in effectiveness.

5.8 Artificial Insemination (AI) Procedures

AI applies to procedures in which sperm from the male partner is implanted into the female partner's vaginal vault, cervix (intracervical insemination), or uterus (intrauterine insemination). It can be used as an initial treatment for moderate male factor infertility or unexplained infertility, with or without ovarian stimulation, as a less invasive alternative to IVF or ICSI (7). Couples with azoospermia, male genetic defects or infectious diseases, other co-morbidities, or sexual factors that prevent natural insemination and reproduction may have no other choice than to use donor sperm in donor insemination.

5.9 Intrauterine Insemination (IUI)

Under ultrasound guidance, washed sperm are inserted into the uterus, bypassing the normal cervical mucus barrier. It's an outpatient procedure that can be conducted with or without COH. It's made to carry a large number of sperm into close proximity to one oocyte after normal ovulation or several oocytes after COH. IUI with COH is used to treat unexplained infertility in cases where an obstructive origin (at least one open fallopian tube) has been ruled out, as well as extreme male factor infertility. The treatment has the advantage of being less invasive and well tolerated than IVF. The procedure has been linked to a lower success rate and a higher rate of multiple pregnancy than IVF, which are also disadvantages. Low-dose COH regimens combined with the cessation of insemination when more than three dominant follicles form, on the other hand, can be expected to minimise the latter.

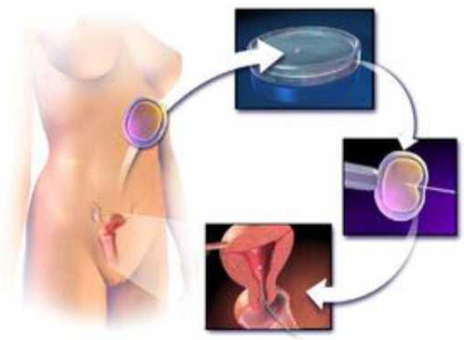
6. Brief of In -Vitro Fertilization (IVF)

Early biological studies involving the cultivation of tissues outside of the living organism were conducted in glass containers such as beakers, test tubes, or Petri dishes, thus the Latin word in vitro, which means "in glass." To differentiate it from an in-situ process (such as in vivo fertilization), where the tissue resides within the living body in which it is usually located, the scientific expression "in vitro" is also used.

"Test tube babies" is a common word for babies born by IVF. Test tubes are tube-shaped glass or acrylic resin tanks used in chemistry and biology laboratories. IVF, on the other hand, is typically done in Petri dishes, which are larger and shallower than regular dishes and are commonly used to grow cultures.

IVF is a form of assisted reproductive technology (ART) in a wider context.

IVF is a method of fertilization in which an egg is mixed with sperm outside of the womb ("in glass"). The procedure entails controlling and activating a woman's ovulatory phase, as well as extracting an ovum or ova (egg or eggs) from her ovaries and allowing sperm to fertilize them in a laboratory liquid. The fertilized egg (zygote) is inserted in the same or another woman's uterus after undergoing embryo culture for 2-6 days in the intention of developing a successful pregnancy.



IVF stands for in vitro fertilization, which is a form of assisted reproductive technology used to treat infertility and gestational surrogacy.

A fertilized egg will be inserted into the uterus of a woman, and the infant born as a result is genetically unrelated to the surrogate. Fertility tourism has risen as a result of certain countries banning or restricting the provision of IVF care. Costs and age are two factors that limit the supply of IVF in order for a woman to bear a stable pregnancy to term. IVF is usually used only after less intrusive or costly alternatives have declined or have been ruled out.

In vitro fertilization (IVF) is a method of fertilization that involves combining an egg with sperm outside of the body ("in glass"). The procedure entails measuring and inducing a woman's ovulatory cycle, as well as extracting an ovum or ova (egg or eggs) from her ovaries and allowing sperm to fertilize them in a laboratory liquid. Since undergoing embryo culture for 2-6 days, the fertilized egg (zygote) is inserted in the same or another woman's uterus in the hopes of ensuring a healthy pregnancy.

IVF stands for in vitro fertilization, which is a method of assisted reproductive technology used to treat infertility and gestational surrogacy. A fertilized egg will be inserted into the uterus of a woman, and the infant born as a result is genetically unrelated to the surrogate. Fertility tourism has risen as a result of certain countries banning or regulating the provision of IVF treatment. Costs and age are two factors that limit the supply of IVF in order for a woman to carry a healthy pregnancy to term. IVF is usually used only after less intrusive or costly alternatives have failed or have been ruled out.

7. Human In Vitro Fertilization - A Challenge

Researchers researching reproduction started to explore the possibility of identifying conditions that would allow human oocytes to be fertilised in vitro in the early twentieth century. The tremendous complexity of the fertilization process posed an obstacle, and in the early 1960s, despite advances in animal reproductive science, little progress had been made in IVF of human oocytes. Before successful human IVF could be achieved, many technological advances and discoveries would be needed, including the ability to control the oocyte maturation process, the ability to retrieve oocytes at a developmental stage suitable for IVF, the ability to activate sperm in vitro, the ability to define conditions that would promote fertilization as well as early embryo development in vitro, and finally, the ability to control the oocyte maturation process.

In the late 1950s, Robert G. Edwards, a researcher at the National Institute for Medical Research in London, was dedicated to developing a way to cure human infertility. Edwards had a broad understanding of the fertilization process thanks to years of basic research on animal reproductive physiology, and he was well prepared for this challenge (Edwards, 1954; Edwards,1955 ; Edwards and Sirlin, 1956 ; Sirlin and Edwards,1957 ; Fowler, and Edwards (1957) ; Edwards and Fowler, 1958 ; Edwards and Gates,1959; Edwards and Sirlin 1959) .The first issue he had to address was locating a method for obtaining mature oocytes suitable for IVF. His first idea was to try to find conditions that would facilitate human oocyte maturation in vitro. He knew from Pincus' work that mammalian oocytes only needed a few hours of in vitro cultivation before they assumed meiotic maturation2. Edwards spent years trying to identify in vitro conditions that would trigger dormant human oocytes, starting with immature human oocytes released from ovarian tissues. Edwards' work was rewarded in 1965 when he discovered that, contrary to popular belief, human oocytes needed 24 hours of in vitro incubation before they began their maturation process (Edwards 1965). Importantly, the in vitro maturation method produced oocytes at a late developmental stage (meiosis II metaphase) that were appropriate for IVF. The next step in Edwards' research was to find conditions that would encourage oocyte fertilization in vitro. A graduate student of Edwards' at Cambridge University, Barry D. Bavister, had recently described buffer conditions to help in vitro activation of hamster sperm (Bavister, 1969). Edwards demonstrated in 1969 that activated human spermatozoa could promote fertilization of in vitro matured oocytes using these buffer conditions (Edwards et.al. 1969). This finding was a significant breakthrough in reproductive science, as it paved the way for the development of a treatment for infertility.

- **The Breakthrough**

Edwards' research on in vitro maturation of human oocytes, however, had given him a crucial insight. He had found that while human oocytes that had matured in vitro could be fertilized, they failed to progress beyond the 2-cell stage. This failure could be attributed to the lengthy periods that in vitro matured oocytes had to spend outside the body. Edwards now instead decided to try to use oocytes that had completed their maturation process in vivo. Edwards postulated that if mature human oocytes could be retrieved from the ovary prior to the onset of ovulation, these oocytes would be more competent to undergo IVF and early embryo development (Fig. 2).

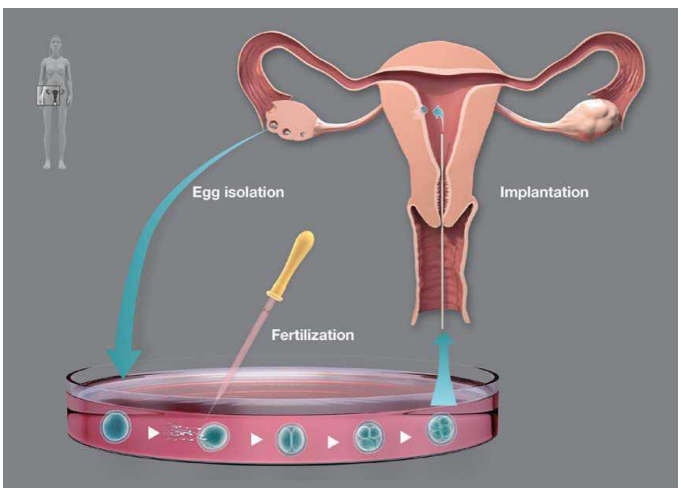


Fig. 2. The principle for IVF as developed by Edwards

Oocytes arrested at the metaphase stage of meiosis II are retrieved prior to ovulation from the ovary by laparoscopy. The oocytes are placed in a culture dish with medium and mixed with sperm. The medium condition promotes sperm activation in vitro, a necessary requirement for the fertilization process. The egg-sperm interactions relieve the meiosis II arrest of the egg. This results in the formation of two haploid sets of chromosomes, one set that will fuse with the haploid set of chromosomes contributed by the sperm, and a second that is discarded (the second polar body). The fertilization process results in the formation of an embryo that undergoes a number of cell divisions in vitro. The embryo is transferred back to the uterus at the eight-cell stage (2.5 days after onset of fertilization) using a thin needle. The embryo will divide further in the uterus until it reaches the blastula stage and thereafter implant into the wall of the uterine lining, the endometrium. Further embryo development will take place at this location.

Edwards' latest approach was influenced by his previous studies on mouse reproductive biology. Externally given gonadotrophins, i.e. hormones that mimicked the function of the intrinsically acting hormone (luteneising hormone), were shown to regulate the initiation of meiotic maturation of oocytes (Edwards and Fowler 1958; Edwards, R. G. and Gates, 1959). He also knew that maturing mouse oocytes to the metaphase II stage of meiosis took an equal amount of time in vitro and in vivo, and that the timing of the meiotic maturation process in humans was understood from his in vitro studies of human oocytes. Edwards' (now at Cambridge University) new technique, however, posed a significant technological challenge: no procedure he knew of could extract a sufficient number of human oocytes at the correct stage of development from the ovary. Access to human oocytes in the late 1960s involved surgical removal of a small portion of the ovary from infertile women, a method that was not appropriate for IVF. Edwards learned about laparoscopy after reading a research article written by Dr. Patrick C. Steptoe(Steptoe (1968)) . A fiber-optic endoscope implanted through an incision near the navel was used to imagine the human female reproductive tract during laparoscopy. Steptoe was a professional surgeon and obstetrician who pioneered the use of laparoscopy in England and showed that oocytes could be aspirated from the ovary. He operated at the Oldham and District General Hospital. Edwards realised right away that this procedure could be used to extract oocytes from the ovary during the metaphase stage of meiosis II at an appropriate time during the menstrual cycle. After priming ovaries with gonadotrophins, Edwards contacted Steptoe, and the two of them demonstrated in 1970 that mature preovulatory oocytes at the metaphase II stage of meiosis could indeed be retrieved from infertile women (Steptoe and Edwards (1970).

Edwards went on to say that in vitro fertilization of preovulatory oocytes with in vitro activated sperm could result in 8-cell stage human embryos (Edwards et.al. (1970). This was a ground-breaking discovery in two ways: it was the first time in a mammalian system that in vitro activated sperm was shown to contribute to embryo growth beyond the 2-cell level, and it was the first time human embryos were shown to undergo cell divisions in vitro (Fig. 3). He then demonstrated in 1971 that human oocytes fertilised in vitro could be further cleaved in vitro, resulting in 16-cell stage embryos and blastocysts (Steptoe, et. Al. (1971). Edwards' findings from 1969 to 1971 were significant turning points in IVF science, laying the groundwork for the next step.

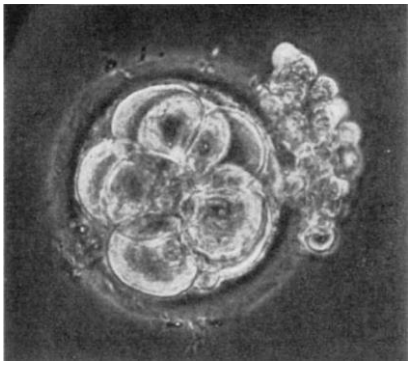


Fig. 3. Picture of 8-cell stage human embryo resulting from IVF (Fig. 2., in Edwards et a (1970) Nature, vol 227:1307-1309).

Edwards and Steptoe began transferring early embryos developed by IVF back into women in the early 1970s. They discovered that hormone treatments provided to women to promote oocyte maturation disrupted implantation of the embryo in the uterus, resulting in spontaneous abortions, after more than a hundred attempts that all resulted in short-lived pregnancies. Finally, in 1976, the first successful pregnancy was achieved after a reform in the hormone therapy regimen (Steptoe and Edwards, 1976). The pregnancy had to be terminated because the embryo had inserted ectopically in the Fallopian tube. Edwards and Steptoe then opted to forego the ovarian stimulation protocol entirely and instead focus on the patients' normal menstrual cycles, despite the fact that this meant they would only have access to one egg each cycle. They were able to predict when the maturing oocyte will enter the metaphase stage of meiosis II in vivo based on the concentration of luteinizing hormones in the women's urine. They hoped that by performing a laparoscopy before ovulation, they would be able to extract the egg. Steptoe and Edwards were successful in their efforts, and in 1978 they announced the birth of Louise Joy Brown, a normal, fit, and healthy infant, through successful IVF of human oocytes (Steptoe and Edwards, 1978). Edwards' long-term vision and perseverance had eventually paid off, ushering in a new age in infertility care.

8. Implantation Window: A Phenomenon

The embryo adheres to the uterine wall during implantation, which happens early in pregnancy. The embryo is a blastocyst at this point of embryonic growth. The fetus receives oxygen and nutrients from the mother via this adhesion, allowing it to expand.

In several animals, embryo implantation is the most crucial stage in the reproductive phase. The blastocyst becomes closely bound to the maternal endometrial surface to shape the placenta, which will establish an interface between the developing fetus and the maternal circulation. (Denker,1993) A receptive endometrium, a normal and functional embryo at the blastocyst developmental level, and a coordinated dialogue between maternal and embryonic tissues are all needed for successful implantation. Implantation takes approximately 9 days after ovulation, although it can take anything from 6 to 12 days. [Wilcox et. al.1999]

8.1 The Implantation Window

There are a number of requirements that must be met in order for an implantation to be successful. Implantation is only available for a certain amount of time; this is known as the "implantation window." Preparations in the uterine endometrium, both structurally and in the composition of its secretions, begin the implantation window.

• Uterus adaptation

The uterus undergoes modifications in order to accept the embryo to allow for implantation.

• Predecidualization

The endometrium thickens, becoming more vascularized, and the glands in the endometrium become tortuous and secrete more. About 7 days after ovulation, these shifts are at their peak.

• Decidualization

If conception happens, decidualization follows predecidualization. This is an extension of it, with the uterine glands, zona compacta, and decidual cell epithelium growing more. The decidual cells fill up with lipids and glycogen and carry on the polyhedral form that decidual cells are known for. It's possible that the blastocyst is mostly responsible for the decidua's additional growth and maintenance. This is shown by the fact that decidualization happens to a greater extent during pregnancy periods than during non-conception cycles. Moreover, as stimuli mimicking the embryo's normal invasion are used, identical modifications are detected (Boron & Walter, 2004).

• Decidua throughout pregnancy

The decidua survives after implantation for at least the first trimester. However, during the early months of labour, during implantation, it is more noticeable. The conclusive placenta takes up its function as a surrounding tissue. However, certain aspects of the decision-making process persist during breastfeeding (Boron & Walter,2004).

• Pinopodes (Uterodomes)

Pinopods are bleb-like protrusions on the endometrial epithelium's apical base. (Usadi et. al, 2003), they occur between the 19th and 21st day of pregnancy. (Boron & Walter,2004) this leads to a fertilization age of around 5 to 7 days, which is very close to the implantation duration. They just last two or three days. (Boron & Walter,2004) progesterone aids in their growth. These constructs are many micrometers deep and extend past the microvilli stage into the uterine lumen. They were initially discovered in the endometrium of mice (Nilsson,1958) and then in human endometrium. (Johannisson & Nilsson,1972; Martel et. al. 1987). The word 'pinopod' comes from the Greek word for 'drinking foot.' The most popular technique for observing these structures is electron microscopy. (Johannisson & Nilsson,1972; Martel et. al. 1987) Pinopod speech is restricted to a two-day timeframe during the menstrual cycle, which corresponds to the putative implantation window. (Nikas,1999, Aghajanova et. al. 2003) others also discovered that pinopods are present from the mid-secretory period to the late secretory phase, with cycle-dependent morphological shifts. This indicates that anatomy, rather than the existence or absence of pinopods, is extremely important. Since pinopod expression is controlled during the menstrual cycle, they may be used as implantation markers (Usadi et. al, 2003).

8.2 Adaptation of Secretions

• Nourishment

Until implanting, the embryoblast spends about 72 hours in the uterine cavity. It can't get nutrition from the mother's blood during this period, so it has to depend on nutrients secreted into the uterine cavity, such as iron and fat-soluble vitamins (Boron & Walter,2004).

• Growth and implantation

The endometrium secretes several steroid-dependent proteins that are essential for growth and implantation, in addition to providing nourishment. In addition, cholesterol and steroids are secreted (Boron & Walter,2004). The synthesis of matrix substances, adhesion molecules, and surface receptors for matrix substances all help with implanting.

8.3 Mechanism

When the blastocyst makes contact with the uterine wall, the process of implantation begins. Apposition, adhesion, and invasion are the three steps of the implantation phase. (Enders,1967) trophoblast cells bind to the sensitive endometrial epithelium during blastocyst apposition. The blastocyst would then attach to the basal lamina of the endometrium and the stromal extracellular matrix (ECM). Uterine flushing will no longer dislocate the established embryo–endometrial linkage at this stage. An intrusive blastocyst invasion into the luminal epithelium follows (Enders,1967).

Despite the fact that the blastocyst can implant in a variety of human tissues, including the endometrium, this condition can only occur between days 20 and 24 of a normal menstrual cycle (day LH + 7 to LH + 11). The human endometrium is prepared for blastocyst attachment during this time, known as the implantation window (Psychoyos,1973), since it has acquired an accurate morphological and functional condition triggered by ovarian steroid hormones. (Finn & Martin,1974)

• Zona hatching

The blastocyst must first lose its zona pellucida in order to perform implantation. This is referred to as "hatching." Plasmin is a drug that is likely to be involved. The plasmin precursor, plasminogen, is present in the uterine cavity, and blastocyst factors help convert it to active plasmin. The lytic results of plasmin in vitro support this theory (Boron & Walter,2004).

• Apposition

The apposition is the first, although tenuous, relation between the blastocyst and the endometrium

• location

The apposition is generally rendered on the endometrium where there is a slight crypt, perhaps because it raises the region of interaction with the very spherical blastocyst. On the other side, the blastocyst develops when the zona pellucida has been sufficiently lysed to produce a rupture, allowing clear

interaction with the underlying trophoblast and the endometrium's decidua (Boron & Walter, 2004).

• Adhesion

Adhesion to the endometrium is far more efficient than loose apposition. The trophoblasts stick together by invading the endometrium with trophoblast cell protrusions.

• Communication

At this point, there is a lot of contact between the blastocyst and the endometrium. The blastocyst sends messages to the endometrium, such as modifications in the cytoskeleton of decidual cells, to help it respond to its presence. This frees the decidual cells from their attachment to the underlying basal lamina, allowing the blastocyst to carry out the subsequent invasion.

Receptor-ligand interactions, both integrin-matrix and proteoglycan interactions, carry out this coordination.

• Integrin-matrix

Integrins are cell-membrane-spanning receptors that bind extracellular matrix proteins such as collagen, laminin, fibronectin, and vitronectin.

Integrins are present on the surface of the blastocyst's trophoblast cells as well as the decidual cells on the uterine wall in this instance. The trophoblast's integrins react with the collagen, laminin, and fibronectin that envelope the decidual cells. The blastocyst is most likely directed between the decidual cells and down to the basal lamina by fibronectin.

Integrins, on the other hand, are present on decidual cells and react with matrix proteins surrounding them, such as fibronectin in this situation. When tiny peptides with sequences identical to fibronectin are found in the decidua, they inhabit the integrins of the decidua, preventing them from attaching to blastocyst fibronectins, preventing implantation.

The integrins, on the other hand, are only present on the decidua for a short time, precisely between days 20 and 24, due to the implantation window phenomenon.

• Proteoglycan receptors

Proteoglycan receptors, which are located on the surface of the uterus' decidua, are another ligand-receptor channel active in adhesion. Proteoglycans, their counterparts, are found around the blastocyst's trophoblast cells. Just at the implantation window is this ligand-receptor mechanism present.

• Invasion

Invasion is the establishment of the blastocyst in the endometrium to a higher level.

• Syncytiotrophoblasts

The trophoblast protrusions that bind to the endometrium begin to proliferate and infiltrate the endometrium. This penetrating cell divide into syncytiotrophoblasts, a new kind of cell. The prefix syn- denotes that the borders between these cells vanish, resulting in a single mass containing several cell nuclei (a syncytium). The trophoblasts that form the inner cell mass are referred to as cytotrophoblasts.

The syncytiotrophoblasts continue their invasion by entering the basal membrane under the decidual cells, penetrating it, and attacking the uterine stroma. Finally, the egg is fully encased in the endometrium. The chorionic villi are formed when the syncytiotrophoblasts come into contact with maternal blood. This is when the placenta begins to grow.

• Secretions

During invasion, the blastocyst secretes a variety of factors for a variety of purposes. It secretes many autocrine factors that target itself and encourage it to penetrate the endometrium further. In addition, secretions separate decidual cells, preventing the embryo from being rejected by the uterus, triggering final decidualization, and preventing menstruation.

• Autocrine

The blastocyst's autocrine growth factor is human chorionic gonadotropin. Insulin-like growth factor type 2 stimulates the invasiveness of it, on the other side.

• Dislodging

The syncytiotrophoblasts dislodge decidual cells along their path by degrading both the cell adhesion molecules that connect the decidual cells and the extracellular matrix that connects them.

Tumor necrosis factor-alpha secreted by syncytiotrophoblasts degrades cell adhesion molecules. The release of cadherins and beta-catenin is inhibited as a result of this. Cadherins are a kind of cell adhesion molecule, and beta-catenin aids in their attachment to the cell membrane. Since the expression of these molecules is inhibited, the bond between decidual cells is loosened, allowing syncytotrophoblasts and the whole embryo to enter the endometrium.

Serine endopeptidases and metalloproteinases destroy the extracellular matrix. Collagenases, gelatinases, and stromelysins are examples of metalloproteinases. Type-I collagen, Type-II collagen, Type-III collagen, Type-VII collagen, and Type-X collagen are also digested by these collagenases. There are two types of gelatinases: one that digests Type-IV collagen and the other that digests gelatin.

• Immunosuppressive

The fetus is different from the mother's cells, and if the mother's immune system didn't secrete immunosuppressive agents, it would be discarded as a parasite. Platelet-activating factor, human chorionic gonadotropin, early pregnancy factor, immunosuppressive factor, Prostaglandin E2, Interleukin 1-alpha, Interleukin 6-alpha, interferon-alpha, leukemia inhibitory factor, and Colony-Stimulating Factor are examples of such agents.

• Decidualization

The blastocyst also plays a role in the final development of decidual cells into their proper shape. Any decidual cells in the vicinity of the blastocyst, on the other hand, degenerate, supplying nutrients for it.

• Prevention of menstruation

Human chorionic gonadotropin (hCG) is a hormone that not only suppresses the immune system but also "notifies" the mother's body that she is pregnant, stopping menstruation by maintaining the corpus luteum's role.

OTHER FACTORS

Other factors secreted by the blastocyst are;

- ovum factor
- Embryo-derived histamine-releasing factor
- Tissue plasminogen activator as well as its inhibitors
- Estradiol
- β1-integrins
- Fibroblast growth factor
- Transforming growth factor alpha
- inhibin

9. Implantation Failure

Despite advances in ART, the major limiting factor in IVF is still the implantation of transferred embryos. The embryo and the endometrium interact in a complex way during implantation. Despite substantial improvement in patient care, follicular recruitment, oocyte quality and aspiration, embryo quality, culture, and cryopreservation, our understanding of the implantation process has remained limited, and the resources to interfere within this process are limited.

The failure to achieve a pregnancy after several IVF cycles is known as repeated implantation failure (RIF). RIF is classified as 2 to 6 IVF cycles in which at least 10 high-grade embryos (in total) were transferred to the uterus, despite the lack of a standardised description (Tan,2005). 1 Three failed ART cycles in which 1 to 2 relatively good embryos were transferred would, however, draw special investigative attention in most currently running IVF systems (Margalioth et. al)

This situation's aetiology has yet to be determined, and there are likely a variety of explanations for this (Figure 1.2). As a result, evaluating RIF patients is particularly challenging and complex. The study of genetic factors in IVF applications is crucial for the effectiveness of the process and the determination of couple-specific management.

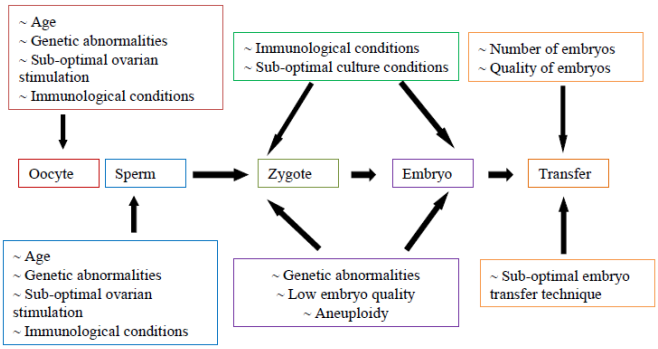


Figure 1.2. Overview on the factors affecting implantation after ART.

10. Conclusion

The current study conducts a systematic literature search for publications on the role of the first mitotic division in embryo selection for effective IVF care. Human gametes can now be manipulated for in-vitro fertilization (IVF) treatments due to advances in biotechnology. IVF protocols have improved, allowing for a greater number of high-quality embryos to be available for implantation. It resulted in an increase in the rate of implantation and pregnancy in the following years. Embryo implantation is the product of a well-coordinated series of events that include cellular adhesion, invasion, and immune regulating mechanisms, some of which are influenced by ovarian hormones via genetic processes.

References

1. Boron, Walter . Oxford: Elsevier; 2004. Emile Boulpaep. Medical Physiology: A Cellular And Molecular Approaoch. ISBN. 1-4160-2328-3.
2. Enders A. A morphological analysis of the early implantation stages in the rat. *Am J Anat.* 1967;125:1–29.
3. Psychoyos A. Hormonal control of ovo-implantation. *Vitam Horm.* 1973;31:205–25.
4. Finn CA, Martin L. The control of implantation. *J Reprod Fertil.* 1974;39:195–206
5. Usadi RS, Murray MJ, Bagnell RC, Fritz MA, Kowalik AI, Meyer WR, et al. Temporal and morphologic characteristics of pinopod expression across the secretory phase of the endometrial cycle in normally cycling women with proven fertility. *Fertil Steril.* 2003;79:970–4.
6. Johannisson E, Nilsson L. Scanning electron microscopic study of the human endometrium. *Fertil Steril.* 1972;23:613–25.
7. Denker HW. Implantation: A cell biological paradox. *J Exp Zool.* 1993;266:541–58.
8. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the Conceptus and loss of pregnancy. *N Engl J Med.* 1999;340:1796–9.
9. Nilsson O. Ultrastructure of mouse uterine surface epithelium under different estrogenic influences. 3. Late effect of estrogen administered to spayed animals. *J Ultrastruct Res.* 1958;2:185–99.

