

# Infertility and Intracytoplasmic Sperm Injection (ICSI)

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## Abstract

Infertility is generally defined as a Time to pregnancy (TTP) of longer than 12 months among couples who engage in unprotected intercourse in the fertile days of the menstrual cycle, any specific threshold is arbitrary. The prevalence of infertility differs greatly from one country to another, being 15% globally, >30% in some developing countries, and 17-28% in industrialized countries. The term 'implantation failure' can be used to describe both patients who have never shown quantifiable signs of implantation such as increased levels of hCG, and those who have increased hCG production without later ultrasound evidence of a gestational sac. Intracytoplasmic sperm injection (ICSI) is a form of assisted reproductive technology in which a single sperm is injected into the cytoplasm of an egg in order to fertilize it. Complete fertilization failure following ICSI is an uncommon occurrence (1–3 percent), although it does occur even when spermatozoa appear to be normal. Furthermore, in some individuals, low to moderate fertilization (30 percent) has been reported in repeated ICSI cycles. Fertilization failure with ICSI is not the same as it is with traditional IVF technique. 60–90 percent of oocytes which showed a fertilization failure in traditional IVF are devoid of sperm nuclei, assuming that sperm ejection or penetration failure is the most common reason for the failure in fertilization process.

**Keywords:** Infertility, Intracytoplasmic sperm injection, assisted reproductive technology, implantation failure, fertilization failure.

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## Infertility

### Definition and Epidemiology

After a year of regular, unprotected intercourse, infertility is defined as the inability to become pregnant; it affects up to 15 percent of reproductive-aged couples globally and is recognized as a medical issue.<sup>[1]</sup> Additionally, it also considered as an event that has a negative impact on a person's social standing; studies have shown that it generates significant social, emotional, and psychological suffering.<sup>[2]</sup>

### Infertility – Prevalence & Burden

Infertility rates vary considerably from country to country, ranging from 15 percent worldwide to >30 percent in certain poor countries and 17-28 percent in industrialized ones.<sup>[3]</sup>

In accordance with an information obtained from the Centers for Disease Control and Prevention (CDC), around 6 percent of married women aged 15-44 in the United States are infertile, and about 12 percent of them, regardless of marital status, have experienced trouble becoming pregnant or bringing a baby

to term. It is predicted to be in the range of 15.7 percent in Denmark. However, there is no national study on infertility among Iraqi couples.<sup>[4]</sup>

### Causes of Infertility

Infertility is not a disease per se, but a symptom of a potential underlying pathology in one or both members of the couple. Polycystic ovary syndrome, tubal blockage, endometriosis, ovulatory disorders, and uterine abnormalities are among the causes of infertility that may be associated with perinatal complications, whereas causes of andrological origin can result in changes to sperm quality or quantity, leading to male factor infertility.<sup>[5]</sup> Infertility implicates both the male and female partner. More than one cause is identified in up to 30 % of couples, but infertility remains unexplained for 15%.<sup>[6]</sup>

### Primary and Secondary Infertility

Primary subfertility was defined as a delay in conceiving for a couple who has never had a child whereas secondary subfertility was described as a delay in conceiving for

a couple who has previously conceived but had a failed pregnancy, such as miscarriage or ectopic pregnancy.<sup>[7]</sup> Genetic factors, hormone problems, genetic abnormalities, congenital malformations, or reproductive system illnesses are main reasons of infertility; secondary causes include lifestyle-related variables such as obesity, food, smoking, alcohol consumption, and chemical exposures.<sup>[8]</sup>

### **Male Infertility**

Male factor infertility is diagnosed in approximately 20 % of couples seeking treatment [Figure 1], and contributes to another 30 %.<sup>[9,10]</sup> Azoospermia (lack of sperm) and oligospermia (low sperm count) can be of congenital origin, or can develop as a result of endocrine disturbances, testicular failure, ejaculatory dysfunction, and genitourinary infections.<sup>[11,12]</sup> While an underlying cause of poor sperm quality can sometimes be diagnosed, more often no known pathology is identified, and lifestyle or environmental factors are also involved.<sup>[13]</sup>

### **Female Infertility**

#### **Ovulation Dysfunction**

Ovulatory dysfunction is the predominant cause of infertility among women, contributing to approximately 20-25 % of cases. Hyperandrogenism and persistent anovulation are characteristics of polycystic ovarian syndrome (PCOS) which is considered as the most frequent diagnosed cause of ovulatory dysfunction and is implicated in up to 70 % of cases involving anovulation. Low and high BMI are associated with amenorrhea and oligo-ovulation, respectively.<sup>[14]</sup>

#### **Tubal Factors and Endometriosis**

Fallopian tube disease is involved in approximately 15 % of infertility cases. Asymptomatic Chlamydia infection, tubal abnormalities -such as blockage, adhesions and inflammation- impair fertility through the inability of the oocyte to be fertilized or, after fertilization, to travel through the fallopian tube and implant. While the etiology of endometriosis is not fully understood, it is detected in approximately 5-10 % of women with a history of infertility. Ectopic endometrial tissue in the pelvic cavity, commonly on the ovaries and fallopian tubes, can cause adhesions that result in reduced fertility.<sup>[15]</sup>

#### **Uterine Abnormalities**

Congenital malformations of the uterus, while rare, can result in reduced fertility from either the inability of the embryo to implant or due to the failure of the uterus to sustain a pregnancy. Other, more common, uterine abnormalities that may be associated with infertility include fibroids, polyps, and uterine adhesions.<sup>[16]</sup>

### **Combined Male and Female Infertility**

Both the male and the woman may be infertile or subfertile in some circumstances, and couples infertility results from the combination of these factors. In some situations, the cause is thought to be immunological or genetic; it's possible that each person is fertile on their own but the pair is unable to conceive without help.<sup>[17]</sup>

### **Unexplained Infertility**

In approximately 20-30 % of cases, diagnostic and screening tests provide no evidence of underlying pathology, and a diagnosis of unexplained infertility is given. Previously reported that women with no identifiable pathology, except for advanced age, are more likely to be given a diagnosis of unexplained infertility. Despite of age association, overall evidence suggests that undetectable or undiagnosed reproductive disturbances are likely to represent the majority of unexplained cases.<sup>[18]</sup>

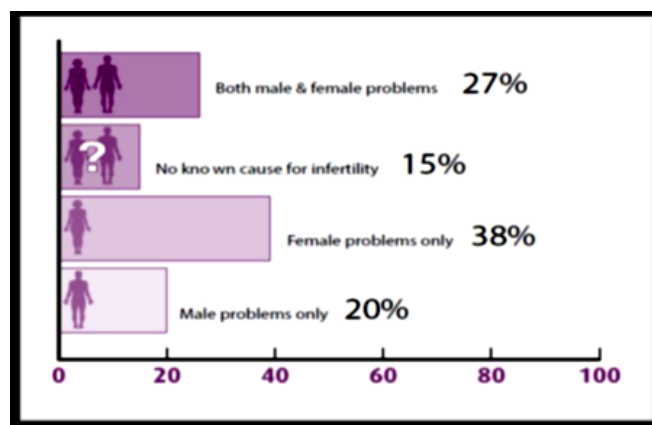


Figure 1: Proportion of couples with infertility due to male or female problems Source (6)

### **Assisted Reproduction Technology (art)**

This term (ART) defines all procedures whereby human oocytes, sperm, and/or embryos are handled in-vitro for the purpose of initiating a pregnancy. There are several well-established ART treatments, including IVF, gamete intrafallopian transfer (GIFT), intracytoplasmic sperm injection (ICSI), embryo transfer, and gamete and embryo cryopreservation.<sup>[19]</sup>

## **In Vitro Fertilization (ivf)**

This technique involves a series of interventions which include controlled ovarian hyper stimulation, oocyte retrieval, sperm retrieval and preparation, in vitro fertilization, and embryo transfer.

## **Intracytoplasmic Sperm Injection (icsi)**

It is a form of assisted reproductive technology in which a single sperm is injected into the cytoplasm of an egg in order to fertilize it.<sup>[20]</sup>

## **Intracytoplasmic Sperm Injection (icsi)**

Intracytoplasmic sperm injection was among the most important technological developments in ART that first appeared in the early 1990s. Immediately after the introduction of ART, the technique was quickly recognized as routine clinical practice in fertility clinics globally. Depending on the statistics that obtained from national and regional registers throughout the world, this method was utilized in 39.6 percent of ART cycles in 1997 and 58.9 percent in 2004. Thousands of babies have been born as a consequence of ICSI across the world. ICSI has been proven to be increasingly utilized in individuals that don't have a significant male factor diagnosis, indicating that the method is now employed as a medical adaptation and development to help couples who suffered from infertility. Indeed, while there isn't much evidence to back up these claims, the common thought in favor of the success of ICSI has led to a boost to utilize it even for moderate and borderline male factor infertility, unexplained infertility, and women in their forties and fifties.<sup>[21]</sup>

## **Fertilization Failure In Icsi**

Complete fertilization failure following ICSI is an uncommon occurrence (1–3 percent), although it does occur even when spermatozoa appear to be normal. Furthermore, in some individuals, low to moderate fertilization (30 percent) has been reported in repeated ICSI cycles. Fertilization failure with ICSI is not the same as it is with traditional IVF technique. 60–90 percent of oocytes which showed a fertilization failure in traditional IVF are devoid of sperm nuclei, assuming that sperm ejection or penetration failure is the most common reason for the failure in fertilization process.<sup>[22]</sup>

In contrast, a large sperm head is found in 60–70 percent of unfertilized oocytes following ICSI, suggesting that the oocyte was successfully injected and that the primary reason of unsuccessful fertilization was the deficit stimulation of the oocyte. Unfertilized oocytes subjected to ICSI have been reported to fail to activate in 40–70 percent of cases. Failure in

the decondensation of sperm head, early condensation of the chromatin of sperm, abnormalities in spindle or sperm aster, or simply improper injection of sperm are some of the other causes.<sup>[23]</sup>

ICSI's effectiveness demonstrates that sperm contact with the plasma membranes of oocyte is not required for the stimulation of oocytes. The stimulation of oocytes and later development after ICSI, on the other hand, are specific to sperm which cannot be replaced by artificially introducing Ca<sup>2+</sup> within the oocyte from the surrounding medium, nor can insertion of a glass pipettes with or without culture medium injection sustain calcium releasing intracellularly that stimulate injected egg.<sup>[24]</sup>

## **Recurrent Implantation Failure (rif)**

It is recognized as clinical pregnancy failure following four high quality attempts to transfer the embryo and at least 3 fresh or even frozen cycles of IVF/ICSI in women under forty years old.<sup>[25]</sup> Both individuals who have never demonstrated measurable implantation symptoms, such as higher levels of Human Chorionic Gonadotropin (hCG), and those who produced high hCG levels but no subsequent ultrasound confirmation of a gestational sac, are referred to as implantation failure.<sup>[26]</sup> Implantation failure can affect both individuals who are undergoing ART and those who are attempting to conceive naturally. Only patients receiving ART are at risk of recurrent implantation failure (RIF). Despite that there is no universally acknowledged criteria for RIF, researchers believed that it occurs after 3 unsuccessful IVF/ICSI embryo transfer rounds with excellent quality embryos.<sup>[27]</sup> Previous work also provides a more comprehensive working criteria that considers mother age, the number of embryos transplanted, and the total number of completed cycles.<sup>[26]</sup>

## **Incidence**

There is a scarcity of data that correctly depicts the incidence or frequency of repeated implantation failure and biochemical pregnancy due to differences in terminology. Biochemical pregnancy is not rare, with rates ranging from 8–33 percent in the general population, including those who conceived spontaneously. However, since majority patients who knew they had a biochemical pregnancy were receiving ART, it's unclear how accurate these numbers are. As a result, it's probable that these patients monitored the levels of their hCG sooner than individuals who conceived naturally and waiting to test for pregnancy until they miss a period. It is reported that 30 percent of pregnancies are lost before implantation in spontaneous conception, with 10 percent being clinical pregnancy losses. It's also worth noting that spontaneous

conception occurs in only around 30 percent of typical fertile couples on the first try, with many more succeeding on later attempts. Furthermore, it's worth investigating if biochemical pregnancy is a pathological condition. [16,28]

## Risk Factors of Infertility

### Maternal Age

It has long been recognized that the frequency of aneuploidy rises with maternal age. As a result, several writers set age limits in their research. Pregnancy rates have also been observed to decline as the mother's age rises as illustrated in table 1. [29] Maternal age was revealed to be the most important predictor of pregnancy outcome in frozen embryo transfer, especially using the Intracytoplasmic Sperm Injection (ICSI) method. There was a substantial increase in the frequency of biochemical pregnancy starting at the age of 39. Both mother age and embryo quality influence the rate of delivery. Previous research has shown that as the mother's age rises, so does the rate of embryo-endometrial asynchrony. [16]

### Body Mass Index (bmi)

Increased BMI (> 25 kg/m<sup>2</sup>) has also been linked to a lower rate of implantation as illustrated in [Figure 2]. [30] When compared to individuals of normal weight (BMI 18.5–24.99 kg/m<sup>2</sup>), obese patients who classified as class I, II, and III (BMI > 30 kg/m<sup>2</sup>) showed a highest implantation failure risk. Obese women needed longer cycles of stimulation with gonadotropin, but statistically had less number of oocytes which are available for collection (8 vs. 10 in non-obese women, P=.03) which implies that obesity may have an impact on oocyte quality and follicular development. [31]

### Smoking

In compared to non-smoking individuals receiving ART, smoking has been found to increase the chance of miscarriage (time undefined) for each pregnancy. Smoking patients had decreased estradiol levels during ovarian stimulation in women undergoing IVF. Toxins from cigarettes may interfere with corpus luteum development and embryo implantation. [32] According to Kunzle et al, it's also important to think about how smoking affects male fertility. Male smokers had a lower sperm count, a larger proportion of abnormal morphology, reduced motility, and a higher pH level assessed by citrate concentration. [16,33]

### Stress

Cortisol hormone that released during stress has been linked to about 2.7-fold increased miscarriage risk in the first three

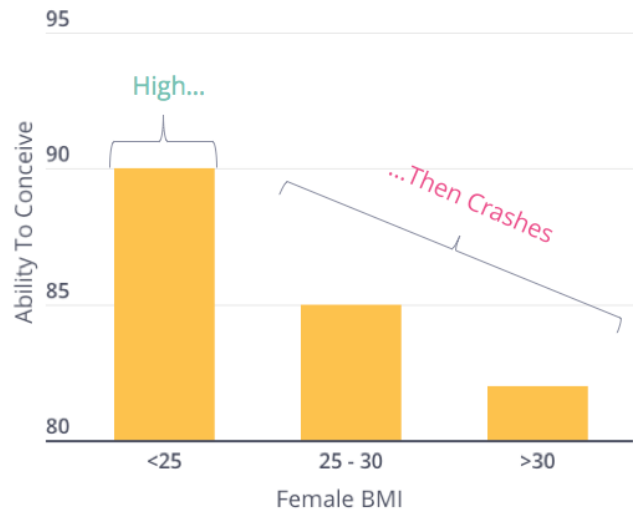


Figure 2: Ability to conceive naturally and female BMI.

weeks following conception when compared to women who showed low levels of cortisol. The production of cortisol in the body increases in response to immunological, psychological, and other stresses, implying that it can be considered as a marker alerting the woman that her body is not in the optimum reproduction condition. [34]

## Pathophysiological Mechanisms of RIF

### Immunological

#### Natural killer (NK) cells– Peripheral and uterine

Numbers of peripheral NK cells was shown to be approximately equal in women whose subsequent pregnancy ended in either biochemical pregnancy, loss or both, indicating that these immune cells may play a role in pregnancy beyond implantation. [35]

#### T-helper-1/T-helper-2 ratio and Tumor necrosis factor (TNF-α levels)

Generally there is an agreement that high Th1 cells levels are linked to the rejection of embryo, whereas high Th2 levels cells are linked to pregnancy. [36,37]

#### Auto-antibodies

Anti- cardiolipin antibodies, anti- nuclear antibodies, and anti-phospholipid antibodies are among the autoimmune antibodies associated in biochemical pregnancy loss. It was reported that patients with these autoantibodies showed a higher possibility of biochemical pregnancy in addition to morphological abnormalities in the embryos themselves, particularly in women who have anti-cardiolipin antibodies. [38,39]

**Table 1: Conception success rates (live births per 100 women of each age group) without assisted reproductive technologies.**

|  | Woman's age when starting pregnancy attempt |          |          |
|--|---|----------|----------|
|  | 30 years                                    | 35 years | 40 years |
| Success: Conception (LB) within 12 months    | 75.4  | 66.0     | 44.3     |
| Delay: Conception (LB) within 12-23 months   | 10.9  | 12.3     | 12.7     |
| Conception (LB) within 24-25 months          | 3.0   | 3.9      | 4.7      |
| Conception (LB) within 36-47 months          | 1.4   | 1.7      | 2.0      |
| Total conceptions (LB) within 4 years        | 90.7  | 83.9     | 63.7     |
| Total conceptions (LB) ever                  | 93.9  | 85.9     | 65.1     |
| At least one miscarriage before LB           | 14.4  | 15.7     | 16.3     |
| Age Y when starting ART (in case of failure) | 34 years                                    | 38 years | 42 years |
| No conception at age Y                       | 9.3   | 17.8     | 43.0     |

### Hereditary thrombophilia

Some evidence suggests that women subgroup who experienced RIF may have inherited thrombophilias. Inheritable thrombophilias such as the deficiency in antithrombin III, factor V leiden, prothrombin and methylene tetrahydrofolate reductase (MTHFR) were shown to be more prevalent in women with RIF in comparison with controls.<sup>[16,40,41]</sup>

### Infection

RIF has been linked to chronic endometritis (CE) in a significant number of women as a result of bacterial colonization, frequently with little or no infection symptoms. According to Kushnir et al., 2016, 45 percent of infertile individuals, particularly those with RIF, developed CE. The majority of pathogens discovered are common bacteria such as Escheria Coli, Group B Streptococcus, and Enterococcus Faecalis in addition to Mycoplasma. STIs (sexually transmitted infections) such as Chlamydia, can cause RIF in some circumstances.<sup>[42]</sup> Bacteria in the endometrium cause aberrant lymphocyte numbers, resulting in an environment that disrupts normal endometrial receptivity.<sup>[43]</sup>

### Altered Expression of Associated Molecules

#### Hyperglycosylated hCG

Because it closely mimics transforming growth factor beta (TGF- $\beta$ ) and due to its ability to bind to TGF- $\beta$  receptor, hyperglycosylated hCG (hhCG) is considered to aid the invasion of the embryo in decidua during implantation. TGF-stimulates cell proliferation and prevents apoptosis. Though overall hCG levels were equal in women who had an early miscarriage, a biochemical pregnancy, or a live delivery. It was shown that the mean concentrations of hyperglycosylated hCG in the urine were lower significantly in women with a

biochemical pregnancy compared to those who had a term pregnancy.<sup>[16,44]</sup>

#### Other molecules

Endometrial receptivity and implantation have been linked to prostaglandins (PGs) and cellular adhesion molecules (CAMs). Though the mechanism is unknown, PGs have been hypothesized to participate in reproductive processes which include ovulation and implantation.<sup>[45]</sup>

### Anatomical Abnormalities and Endometrial Thickness

Polyps, myomas, and adhesions are just a few of the uterine diseases that might affect implantation rates in individuals undergoing IVF/ICSI. Myomas can deform the endometrial cavity, and adhesions that form after surgery or infection can hinder the embryo from attaching to the luminal surface. Hydrosalpinges have been found to have a detrimental influence on implantation in women undergoing IVF/ICSI. The hypothesized mechanism is that the absence of nutrients and energy in the fluid prevents normal embryo growth. Furthermore, the fluid might physically wash the embryo out, preventing it from implanting. Septate uterus may have a role in RIF, since individuals with untreated septa had poorer IVF/ICSI results than those who have undergone hysteroscopic metroplasty.<sup>[46,47]</sup>

Implantation failure can also be caused by the endometrium itself. A thin endometrium can be caused by previous endometrial damage, long-term use of birth control tablets, or poor uterine blood flow. When blood flow is restricted, epithelial cell development and vascular endothelial growth factor (VEGF) production are diminished, depriving the endometrium of the required angiogenesis and growth factors to attain the correct thickness for successful implantation.<sup>[48,49]</sup>

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