

Miscarriage: Biochemical and Clinical

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Nomenclature

β-hCG (beta-hCG) It is a quantitative blood test to measure the level of human chorionic gonadotropin hormone (hCG) in the body. hCG is produced from the syncytiotrophoblast cells of pregnancy.

Introduction

“... I recall nothing which in times past has caused me more anxiety and doubt, or in regard to which I have found it more difficult to get any satisfactory rules from books, than the treatment of abortion.”

—T.G. Thomas, 1908.

Assisted reproductive technology (ART) is defined as all interventions that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of reproduction. This includes, but is not limited to, in vitro fertilization (IVF) and embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, gamete and embryo cryopreservation, semen, oocyte and embryo donation, and gestational carrier cycles (Zegers-Hochschild et al., 2017).

For a woman or a couple hoping to have a child through ART, a positive pregnancy test seems like the first hurdle crossed in what will be a very long journey. Thus when after the first few days, the couple is told that the pregnancy has “failed,” there are numerous psychological repercussions that a patient goes through, along with the physical and clinical.

It is thus important to understand what miscarriage is, so as to know what can be done to prevent it.

Nomenclature

The word *abortion* is derived from the Latin word *aboriri*, which means to miscarry.

According to The International Glossary on Infertility and Fertility Care (2017), a spontaneous miscarriage/abortion is defined as spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age (Zegers-Hochschild et al., 2017).

Different nomenclature defining events associated with early pregnancy and miscarriage described in Table 1.

Incidence

In natural cycles, up to 55% of conceptions are estimated to be lost due to implantation failure or pre-clinical (biochemical) miscarriage and approximately 15% lost in clinical miscarriage (Macklon, 2002). In a recent study by Boomsma et al. (2009) it was shown that in stimulated cycles, the contribution of implantation failure for the numbers of conception losses is higher (50%) than

Table 1 Nomenclature describing early pregnancy events

Nomenclature	Definition
Biochemical pregnancy (preclinical spontaneous abortion/miscarriage)	A pregnancy diagnosed only by the detection of HCG in serum or urine and that does not develop into a clinical pregnancy (Zegers-Hochschild et al., 2009)
Clinical miscarriage	Intrauterine pregnancy demise confirmed by ultrasound or histology (ASRM Practice Committee, 2013; Stephenson and Kutteh, 2007)
i. First-trimester miscarriage	Intrauterine pregnancy demise before 12 weeks of gestational age
<i>Early miscarriage</i>	Intrauterine pregnancy loss of <10 weeks size on ultrasound (Kolte et al., 2014)
● Anembryonic miscarriage	Intrauterine pregnancy loss with a gestational sac but without a yolk sac or an embryo on ultrasound
● Yolk sac miscarriage	Intrauterine pregnancy loss with a gestational sac and yolk sac, without an embryo on ultrasound
● Embryonic miscarriage	Intrauterine pregnancy loss with an embryo without cardiac activity on ultrasound
<i>Fetal miscarriage</i>	Intrauterine pregnancy loss ≥ 10 weeks size with a fetus (≥ 33 mm) on ultrasound (Stephenson and Kutteh, 2007)
ii. Second-trimester miscarriage	Intrauterine pregnancy loss after 12 weeks of gestational age
Vanishing sac(s) or embryo(s)	Spontaneous disappearance of one or more gestational sacs or embryos in an ongoing pregnancy, documented by ultrasound (Zegers-Hochschild et al., 2017)

described for natural cycles (30%) while pre-clinical (biochemical losses) and clinical miscarriage is approximately 15% and 10%, respectively.

While reviewing other literature, biochemical pregnancy loss rate is 27% (54/200) after frozen embryo transfer (ET) and 22.1% (122/500) after fresh ET. Clinical abortion rate is 14.5% after frozen ET and 9% after fresh ET (Aflatoonian et al., 2010).

When reviewing the incidence based on type of oocyte and embryos, the incidence of clinical miscarriage in patients after transferring embryos using self-oocytes, donor-oocytes and vitrified-warmed embryos are 15.9%, 21.8%, and 25.2%, respectively (Banker et al., 2016).

If we look at the incidence of miscarriage following ART in different registries: according to Australian & New Zealand Assisted Reproduction Database (ANZARD) (2015) out of the 17,659 clinical pregnancies, 79.9% resulted in a delivery and 19.0% resulted in clinical miscarriage. The outcomes of 193 (1.1%) clinical pregnancies were not known because women could not be followed up or contacted by fertility centers.

According to FIVNAT registry (2015), out of 2149 pregnancies, 525 (24%) had spontaneous miscarriage.

Miscarriage Types

Broadly, miscarriages are of two types: biochemical and clinical miscarriages.

Biochemical Miscarriage

Spontaneous pregnancy demise based on decreasing serum or urinary b-hCG levels, without an ultrasound evaluation.

In the general population, most cases of biochemical pregnancy remain undetected. Thus the incidence is different for these cases as compared to patients undergoing assisted reproductive treatment. In the latter group, more stringent monitoring and early testing allows us to detect transient β -hCG rises which would otherwise have been missed (Annan et al., 2013).

Numerous studies have advocated that ovarian stimulation alters the endometrial receptivity, hence increasing biochemical pregnancy rates (Kasius et al., 2014).

But a study by Zeadna et al. (2015) attempts to dispel this theory. Their study showed fertile controls who conceived spontaneously has a higher biochemical pregnancy rate (18%) as compared to women undergoing fresh or frozen embryo transfers (13.8%).

A study by Yang et al. (2015) reviewed over 10,000 cycles to show that biochemical pregnancy in the first IVF cycle is a negative predictor for future positive results.

Etiology (Annan et al., 2013)

The exact etiology of biochemical miscarriage after ART is unknown.

Numerous factors have been implicated:

1. Genetically abnormal embryos
2. Poor endometrial receptivity
3. Genetically abnormal oocyte
4. Sperm DNA damage
5. Stress

Pathogenesis

Soon after fertilization, or embryo transfer, the embryo begins to secrete β -hCG. The amount of β -hCG continues to rise as the trophoblastic reaction and embryo invasion continues. Hence a patient tests positive for pregnancy at this early stage. However, in case of a biochemical miscarriage, further growth of the embryo stops, thus preventing any clinical evidence of the pregnancy to be visible on ultrasound (Annan et al., 2013).

Clinical Miscarriage

A pregnancy which can be visualised on ultrasound is known as a clinical pregnancy. A spontaneous loss of this type of intra-uterine pregnancy prior to 22 completed weeks of gestational age is known as spontaneous miscarriage/abortion (Zegers-Hochschild et al., 2017).

Broadly, there are two types of clinical miscarriage (Fig. 1):

- i. First trimester miscarriage
- ii. Mid-trimester miscarriage

Types

- i. First trimester miscarriage (Kolte et al., 2014):

Spontaneous pregnancy demise before 12 weeks of gestational age is called first trimester miscarriage.

This can be divided into two parts: early miscarriage and fetal miscarriage

- Early miscarriage:

Early miscarriage is defined as intrauterine pregnancy loss of <10 weeks size on ultrasound and it is divided in to three categories:

- (a) *Anembryonic miscarriage*:

It is an intrauterine pregnancy loss with a gestational sac but without a yolk sac or an embryo on ultrasound. In this case, the trophoblast has invaded the decidua but the embryonic disc has not developed.

- (b) *Yolk sac miscarriage*:

Intrauterine pregnancy loss with a gestational sac and yolk sac, without an embryo on ultrasound.

- (c) *Embryonic miscarriage*:

It is an intrauterine pregnancy loss with an embryo without cardiac activity on ultrasound.

- Fetal miscarriage:

It is defined as pregnancy loss ≥ 10 weeks size with a fetus (≥ 33 mm) on ultrasound.

- ii. Mid-trimester miscarriage:

Miscarriage/abortion resulting after 12 weeks of gestation is defined as mid-trimester miscarriage.

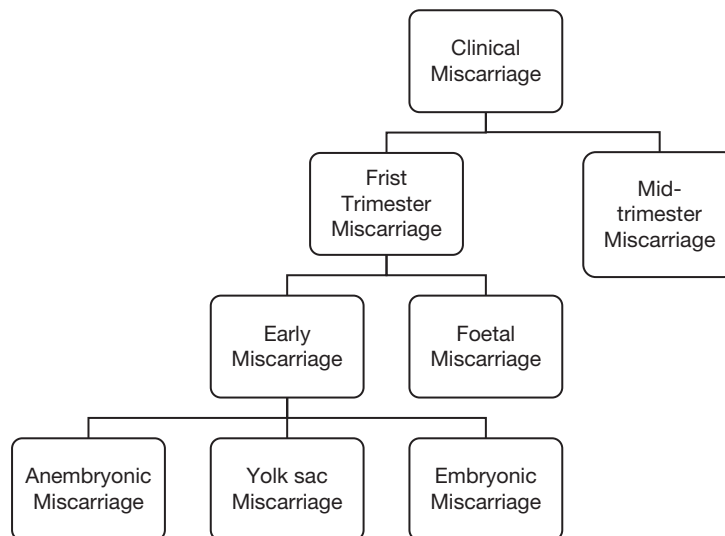


Fig. 1 Types of clinical miscarriage.

Etiopathogenesis of clinical miscarriage following ART

Causes/factors related to ART

1. *Ovarian stimulation:*

The use of exogenous gonadotrophins for controlled ovarian hyperstimulation (COH) as part of IVF has become a well-accepted practice; however, this can have an impact on oocyte and embryo quality and endometrial receptivity.

Early research suggested that the exposure of the developing oocyte to supraphysiological concentrations of gonadotrophins may disturb oocyte maturation and the completion of meiosis leading to chromosomal aneuploid oocytes and/or embryos (Hodges et al., 2002). Whereas animal studies seem to support an association between FSH exposure and embryonic aneuploidy, human studies have been more conflicting.

Possible mechanisms suggested either FSH-induced chromosome dysfunction in oocytes or recruitment of poorer-quality oocytes when the process of naturally selecting a dominant follicle is overridden with ovarian stimulation. If exogenous FSH increases aneuploidy, it could lead to an increased miscarriage rate in patients undergoing IVF, because most miscarriages are the result of chromosomal abnormalities (Andersen et al., 2000; Lathi et al., 2007). But, Massie et al. (2011) concluded that the incidence of embryonic aneuploidy was not higher in pregnancies conceived with FSH stimulation compared with spontaneous conceptions in infertility patients. This suggests that exogenous gonadotrophins exposure does not increase the risk of aneuploidy.

Multiple ovarian follicle maturation by COH will produce supraphysiological serum estradiol (E2) levels that may induce morphologic (Kolb et al., 1997) and biochemical (Simón et al., 1996) endometrial alterations related to uterine receptivity without affecting embryo quality. Thus, with high E2 concentration, implantation and LBR (live birth rate) decreases without affecting miscarriage rate (Ji et al., 2013).

2. *Number of oocytes retrieved:*

There was a strong association between the number of oocytes retrieved and the clinical miscarriage rate. The miscarriage rate fell from 20% to 13% with increasing numbers of oocytes. Stepwise logistic regression ($P < 0.001$) identified three cut-points (4, 10, and 15 oocytes) at or beyond which the probability of clinical miscarriage fell. It failed to find any oocyte number above which the miscarriage rate rose again. The miscarriage rates were 20.0% (1–3 oocytes), 15.5% (4–9 oocytes), 13.8% (10–14 oocytes) and 13.1% (≥ 15 oocytes). After adjusting for female age, the effect of oocyte numbers on the clinical miscarriage rate was reduced, but not eliminated.

These findings conclude that poor responders have a higher clinical miscarriage indicating that poor ovarian response is associated with a parallel decline in both oocyte quantity and quality (Sunkara et al., 2014).

Though decline in the LBR (live birth rate) observed with higher number of eggs could be due to the deleterious effect of the raised serum E2 levels affecting at the level of embryo implantation (Valbuena et al., 2001; Mitwally et al., 2006; Joo et al., 2010) but not beyond that.

Ji et al. (2013) concluded that the fresh embryo LBR per started cycle increased with the number of retrieved oocytes up to Groups 2 and 3 (6–10 and 11–15 oocytes) and then (>15 oocytes) decreased, because of the high number of cycles with all embryos being cryopreserved, in order to avoid moderate—severe OHSS in group 4 (>15 oocytes). However, the cumulative LBR per started cycle continued to increase with oocyte number, as did the incidence of moderate—severe OHSS. There was no significant difference in the miscarriage rates among the patient groups.

3. *Frozen embryo transfer (FET):*

There is no difference of miscarriage rates between fresh and FET, though FET resulted in an increase in the clinical pregnancy rate (RR 1.31; 95% CI 1.10–1.56) and the ongoing pregnancy rate (RR 1.32; 95% CI 1.10–1.59) (Evans et al., 2014). But, on comparing different FET protocols, a higher miscarriage rate was observed in the HRT (hormone replacement therapy) group when compared to hCG or LH surge (21.2% vs. 12.9% vs. 11.1%, $P < 0.01$) (Cerrillo et al., 2017).

4. *Cryopreservation technique:*

Earlier, slow freezing technique was used to cryopreserve gametes/embryos. Recently vitrification technique has replaced this due to better results. According to Kaartinen et al. (2016), the clinical pregnancy and delivery rates were similar for both freezing methods. The clinical miscarriage rate in the slow-freezing group was higher (29% vs. 15.7%). Slow freezing and thawing has been detected to induce numerical chromosomal changes in human embryos, which could be explained by increased ice crystal formation. The embryo DNA integrity index was higher in vitrified than in slow-frozen blastocysts which could explain the lower miscarriage rate.

5. *Multiple pregnancy:*

More than one embryo is very often transferred in an IVF—ICSI cycle to improve the pregnancy rate. This leads to a higher incidence of twins, triplets and sometimes even high order multiple pregnancies.

According to Sullivan et al. (2013), the number of embryos transferred per ART cycle varied among countries. The average number of embryos transferred overall has decreased from 2.47 in 2002 to 2.35 in 2004 reflecting increase in single and double embryo transfers from 67.6% in 2002 to 73.2% in 2004. Positively, triple and quadruple embryo transfers decreased between 2002 and 2004 from 28.6% to 25.1% and 13.7% to 11.6%, respectively, and this has contributed to the decline in the trend of multiple deliveries following ART.

The lowest proportion of ≥ 4 fresh embryo transfers were reported from Australia/New Zealand at 0.4% then Europe at 3.3% and the highest from Asia at 42.5% with the Nordic countries reporting no embryo transfer cycles of ≥ 4 . The proportion of single embryo transfers (SETs) increased from with the highest reported by Sweden (67.4%), Belgium (48.9%) and then Finland (47.0%).

The proportion of twin deliveries marginally increased from 24.8% in 2003 to 25.1%, while the proportion of triplet deliveries continued to decrease, from 2.0% to 1.8%. However, these rates differed largely among countries; the percentage of twin births ranged from 5.6% in Sweden to 48.2% in Peru, and births of triplet and higher order multiples from 1% in several countries (including Sweden, Belgium, Australia, New Zealand, Norway, Denmark, and France) to 10% in Lithuania and six Latin American countries (Argentina, Brazil, Ecuador, Guatemala, Mexico, and Venezuela).

Similar rates of multiple deliveries were seen for FET in 2003 and 2004, with twins at 17.0% and triplets or higher at 1.0%. The percentage of transfers with four or more embryos in fresh cycles increased slightly, to 11.6% from 10.8% in 2003.

According to [Tummers et al. \(2003\)](#), the overall incidence of spontaneous abortion—in twin pregnancies was 17.1% (12.1% vanishing twins and 5.0% complete miscarriages) and in singleton pregnancies was 21.7%. The incidence of miscarriage in the twin pregnancies, expressed per gestational sac, was 11.1%. Once fetal heart activity was present, the risk of abortion (per gestational sac) was 7.3%, which is significantly lower than that in singleton pregnancies. Twin pregnancies after IVF have a better potential for survival than singleton pregnancies.

According to [La Sala et al. \(2016\)](#), overall clinical miscarriages (odds ratio [OR] 4.9, 95% confidence interval [CI] 3.4, 6.9) and live births (OR 0.2, 95% CI 0.1, 0.3) were, respectively, higher and lower when compared to patients who had one clinical twin pregnancy. The overall risk of perinatal complications was significantly higher in patients who had one twin delivery rather than patients who had two consecutive singleton deliveries. Thus, he concluded that compared with two consecutive singleton pregnancies, twin pregnancies are characterized by higher success rates but worse perinatal outcomes irrespectively of vanishing twin syndrome. Thus, reducing the number of embryos transferred should be encouraged for better perinatal outcome.

6. ART laboratory parameters:

- *Air quality:*

Filtration units have been shown to reduce airborne concentrations of toxic volatile organic compounds (VOC), chemical contaminants and particles in IVF laboratories. Control of air pollution in ART laboratory, operating and transfer rooms through the construction of cleanrooms equipped with carbon-impregnated filters [like Class 100 (ISO 5; $n = 248$) clean-room] for air and incubators results in better good quality embryo formation, cleavage and pregnancy rates, and in lower spontaneous abortion rates ([Esteves et al., 2004](#)).

- *Culture media:*

The pregnancy rate and implantation rate in the group without HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] were statistically significantly higher than in the control group where media buffered with HEPES used. But no differences were observed for the number of embryos transferred, top-quality embryo rate transferred, or abortion rate between the two groups ([Morgia et al., 2006](#)).

- *ICSI (intra-cytoplasmic sperm injection):*

ICSI is suspected to increase the chromosomal aberrations as it bypasses natural selection mechanisms and may increase first trimester aneuploidy rates ([Kim et al., 2010](#)). Explanations for this include (i) physical or biochemical disturbances of the ooplasm or meiotic spindle, (ii) injection of biochemical contaminants, (iii) injection of sperm associated exogenous DNA, (iv) injection of sperm carrying a chromosomal anomaly, (v) transmission of genetic defects that may be related to the underlying male-factor infertility, (vi) male gametes with structural defects, (vii) anomalies of sperm activating factors, (viii) potential for incorporating sperm mitochondrial DNA, and (ix) female gamete anomalies.

[Kim et al. \(2010\)](#) concluded that out of total miscarriages, 52.62% were found to be cytogenetically abnormal among all patients, but no statistical difference was found between ICSI and conventional IVF group (54.3% v/s 55.3%, $P 0.503$).

General causes miscarriage

Many causes of abortion not specifically related to ART but can cause abortion in any pregnancy conceived naturally or through IUI or ART.

1. Chromosomal abnormalities ([Agenor and Bhattacharya, 2015](#)):

Almost 50% of early pregnancy losses take place due to chromosomal abnormalities, mostly aneuploidy. These maybe genetic, but more often than not, they arise de novo, due to aberrations in chromosomal structure during development.

(a) *Trisomies:* Seen most often in cases of advanced maternal age (≥ 35 years), trisomy 21, 16, and 18 are known to be a cause of early pregnancy loss.

(b) *Y-chromosome abnormalities:* Translocation in the Y-chromosome is commonly detected, leading to defective spermatogenesis.

(c) *Factor V Leiden:* This mutation is genetically inherited, predisposing the affected individual to thrombosis.

Pre-implantation genetic screening (PGS) can be offered to rule out chromosomal aneuploidies in embryos before transfer.

2. *Antiphospholipid antibodies:* especially anticardiolipin antibodies and lupus anticoagulant have been linked with pregnancy loss, right from the implantation stage, through to late preterm labor. These antibodies are said to impair trophoblastic invasion of spiral arterioles, reducing the blood supply to the fetus, causing improper placental development, PIH and changes in fetal blood flow patterns. Aspirin and low molecular weight heparin are commonly used treatment option.

3. *Anatomical defects in the uterus:* These maybe both congenital and acquired. Defects like uterine septum, unicornuate uterus, distorted anatomy due to endometriosis, uterine fibroids, polyps and intrauterine synechiae all can cause pregnancy losses, especially 2nd trimester loss. Surgical correction, when appropriate helps to reduce the risk.

4. *Cervical incompetence*: Cervical incompetence, often undiagnosed until it is too late, is a common cause of recurrent and preventable mid-trimester pregnancy loss. Cervical cerclage is performed in second trimester to prevent such losses.
5. *Endocrine cause*: The main causes include poorly controlled diabetes, overt hyperthyroidism and hypothyroidism, hyperprolactinemia and luteal phase defect.
6. *Infections*: Infections can cause sporadic losses, never recurrent. The organisms responsible are Chlamydia, ureaplasma, mycoplasma, and toxoplasma.
7. *Environmental cause* (Gardella and Hill, 2000): Heavy metals such as mercury and lead, alcohol, organic solvents, and ionizing irradiation are confirmed teratogens which on exposure can lead to miscarriage. The suspected teratogens are caffeine (>300 mg/day), and cigarette smoking, whereas the teratogenic potential of pesticides remains unknown.
8. *Maternal obesity* (Lashen et al., 2004): Obesity is associated with increased risk of first trimester and recurrent miscarriage. Weight loss should be encouraged for obese patients before ART (Sim et al., 2014).
9. *Immunological factors* (Agenor and Bhattacharya, 2015):
 - (a) *HLA antigens*: HLA expression influences various stages of gestation. Its expression is associated with increased chances of an embryo implanting successfully as well as elevated embryo cleavage rates.
 - (b) *Antisperm antibodies*: although these are mainly said to cause infertility, their role in miscarriages has also been studied, leading to lower pregnancy rates.
 - (c) *Integrins*: these are adhesion molecules that facilitate cell to cell interactions, and are considered essential for the binding of a sperm to an oocyte. They are also essential for the process of implantation and later on for placental development. Lack of integrin expression can lead to miscarriages.
 - (d) *LIF*: leukemia inhibitory factor is required in the endometrium to facilitate the process of implantation. Its absence or reduction has been linked to human reproductive failure.
 - (e) *Cytokines*: Cytokines are involved in gamete development, trophoblast invasion, implantation, placental development, decidualization and immune tolerance to pregnancy.
Th1 cytokines are said to be cytotoxic, causing tissue injury, whereas Th2 cytokines promote antibody production and cell healing. Thus when the Th1:Th2 balance is disturbed in favor of the cytotoxic cytokines, pregnancy is affected.
 - (f) *Natural killer cells*: increased NK cell activity, especially the uterine NK cells, as compared to the peripheral NK cells, has been implicated in impaired placental development leading to reproductive failure.
 - (g) *Haemostatic pathways*: Proteins C, protein S, factor V Leiden, homocysteine, are some of the factors that have been considered responsible for pregnancy loss.

Management strategies are vast and detailed. In a nutshell, they involve detecting the underlying cause first, and then solving it to be able to take a pregnancy to term.

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