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Research Article

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Genetic Aspects of Implantation Failure

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Abstract

Implantation failure refers to the inability of a fertilized egg, or embryo, to successfully implant itself in the endometrial lining of the uterus, leading to pregnancy loss. The repeated failure of good quality embryo implantation is referred to as recurrent implantation failure (RIF). This can occur for a variety of reasons, including chromosomal abnormalities in the embryo, problems with the endometrium, or issues with the immune system. Factors such as advanced maternal age, obesity, smoking, and certain medical conditions can also increase the risk of implantation failure. While treatment such as in vitro fertilization (IVF) can help to improve the chances of successful implantation, there is currently no definite way to prevent or treat implantation failure. Patients and healthcare professionals have substantial diagnostic and treatment hurdles as a result of many etiological factors and lack of knowledge about RIF. Numerous investigations have revealed a relationship between hormone level imbalance, perturbations of angiogenic and immunomodulatory factors, certain genetic polymorphisms, and the incidence of RIF, but still, the precise multifactorial pathophysiology of RIF is unknown. However, many studies are ongoing in this field to understand the underlying causes and to find new ways to help couples achieve pregnancy. This review article is a detailed discussion on the different molecular and genetic aspects for the improvement of diagnosis and treatment of implantation failure.

Keywords: Implantation Failure, IYF, RIF, Genetic Factors, Treatment.

1. Introduction

In mammals, implantation is a critical stage of pregnancy, implying not only the success of the pregnancy but also the health of the progeny [1]. Implantation can only take place in a receptive uterus [2]. Hoozemans et al. defined implantation as "a coordination event that involves both embryonic and maternal active participation" [3]. Makrigiannakis also described that "implantation is the stage in an embryonic development, in which the blastocyst apposes, attaches and finally invades the underlying endometrial surface of the female's uterus" [4]. Sharkey & Smith defined implantation as "the process by which the free-floating blastocyst attaches to the endometrium, invades into the stroma and establishes the placenta" [5]. Hoozemans et al. explained that, "the implantation process contains three stages, apposition, attachment and invasion into the endometrium" [3].

Ashary et al. noticed that, "implantation is the first stage of gestation, the endometrium is to implant the embryo and nourish it to ensure pregnancy" [6]. The process involves coordination between an implanted embryo and an endometrium. Santos et al. estimated that, "in humans, reproductive efficiency has been shown to be rather low, with a probability to achieve pregnancy estimated to 20–30%" [7]. Moreover, Fleming et al. added that, "apart from endogenous factors (such as genetic mutations) that could be detrimental for pregnancy development, various environmental insults (nutrition, pollution and endocrine disruptors, infections stress) have been identified as factors that may affect gamete quality and fertilization, journey of the early embryo through the oviduct, cellular interactions between endometrium and hatched blastocyst or conceptus, foeto-placental development and parturition" [8]. Since 80% of pregnancies end in miscarriage in the first trimester, it has been hypothesized that an error in embryo implantation is

the main reason for failed pregnancies [9].

Gene mutations and alterations in methylation have an ambiguous effect on RIF in the absence of chromosomal euploidy. Implantation failure in mice has been linked to certain gene abnormalities that cause the loss or lack of endometrial factors, such as cytokines and transcription factors [10,11]. The endometrium becomes receptive for a limited period of time under the influence of steroid hormones and paracrine signals from the developing embryo [5]. Murphy noted that "the endometrium is receptive to implantation during the window of implantation (WOI), a spatially and temporally restricted phase that is complex and multifactorial, during which changes occur at the molecular, cellular and tissue levels" [12].

From the clinical point of view, RIF refers to the repeated failure of good quality embryo implantation [13]. According to Garneau & Young, "RIF is the unsuccessful implantation after repeated transfers of morphologically good quality embryos into a normal uterus" [14]. Cimadomo et al. refers to RIF as the failure of the embryo to reach a stage when an intrauterine gestational sac is recognized by ultrasonography [15]. In a study done by Maesawa et al., "biochemical pregnancy is actually not uncommon, and its reported incidence varies from 8 to 33% in the general population, including those who spontaneously conceived" [16]. Hoozemans et al. stated that, "for successful implantation, embryo maturation and uterine receptivity must occur in concert such that a window of implantation is open for 48 hours, 7–10 days after ovulation" [3]. In a study done by Coughlan et al, the term "implantation failure" states in two different types of cases, those in whom there has no evidence of implantation and those who have evidence of implantation, and in fact both the cases depend on the presence of Human chorionic gonadotropin (hCG) [17]. RIF is usually determined by considering two criteria: the number of good quality embryos transferred and the number of embryo transfer (ET) procedures performed with good quality embryos [18].

Implantation failure is due to several factors including maternal factors as well as embryonic causes. Simon & Laufer mentioned that, "maternal factors include uterine anatomical abnormalities, thrombophilia, non-receptive endometrium and immunological factors" [19]. Franasiak et al. mentioned that, "embryonic causes include either genetic abnormalities or other factors essential to the embryo that impair its ability to develop in the uterus, to hatch and to implant" [20]. Margalioth et al denoted that "chromosomal abnormalities in embryos are one of the possible causes of implantation failure" [21]. Franasiak et al. added that, "chromosomal abnormalities, such as aneuploidy or chromosome rearrangements affect the implantation [20]. In the year 1999, Stern also noted that "an increased prevalence of chromosomal structural abnormalities has been documented in RIF patients". The most common fetal chromosomal abnormalities are caused by meiotic nondisjunction like trisomy and monosomy, and structural chromosomal abnormalities (balanced translocation or inversions). According to Brosens, "maternal age is the main risk factor for embryonic aneuploidy" [22].

Hoozemans et al. observed that, "the immunological action against the embryo is the maternal restraint, it may cause implantation failure or failure of adequate placentation [3]. Hence immunomodulation is necessary to prevent the maternal immune system rejecting the embryonic transplant". Maternal age plays a crucial role in the quality of the embryos that are used for IVF [17]. Salumets et al. found that "the major predictive factor contributing to pregnancy outcome in frozen embryo transfer, specifically with Intracytoplasmic Sperm Injection (ICSI) technique, was maternal age" [23]. Increased body mass index (BMI) (> 25 kg/m2) has also been shown to impact implantation rate [24].

When compared to non-smoking individuals receiving artificial reproductive technology (ART), smoking has been demonstrated to dramatically increase the probability of miscarriage (time undefined) for each pregnancy [25]. Cigarette toxins might play a role in disrupting corpus luteum formation and implantation of the embryo [26]. Maternal smoking was shown to be more frequently associated with spontaneous miscarriage with normal foetal karyotype than with defective foetal karyotype, indicating that the toxic effects of carbon monoxide and nicotine may be the primary causes of harm [27]. Cortisol synthesis in the body increases in response to psychological, immunological, and other stresses, implying that it functions as a marker warning to the female body that it is not in optimal reproductive condition [28].

Healthy embryos and a functional endometrium is essential for successful implantation. The cross-talk between the embryo and the endometrium, which is essential for successful implantation, can be negatively impacted by issues arising from the host environment, such as aberrant uterine anatomy, non-receptive endometrium, the mother's health, and other genetic variables. Repeated implantation failure is a challenge for any IVF clinic since the infertile couples who have unsuccessful IVF/ET treatments are put through a great deal of psychological, emotional, and financial stress, and the medical professionals who are trying to assist them are frustrated. In this study, an effort was made to categorize the many different causes of RIF provided with the following RIF kinds with the hope that it would enable couples who have implantation failure after embryo transfer to receive the appropriate care.

2. The Embryo in Implantation Failure

Global gene analysis of the dormant versus active blastocysts demonstrates that heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) encoded by Hbegf gene is significantly up-regulated during blastocyst activation [29]. One of the most important factors is the embryo's quality. Following the transfer of 2, 3, 4, 5, 6, and 7 embryos, the odds of all embryos failing to implant are 0.81, 0.73, 0.66, 0.59, 0.53, and 0.48, respectively, assuming that the likelihood of successful implantation is decreased to 0.10. In other words, all seven embryos have a 48% probability of failing to implant.

As a result, in order to arrive at a therapeutically meaningful definition, several researchers specified that good-quality embryos had been transplanted [20]. Poor embryo quality is considered to

be the major cause of implantation failure [30]. Coughlan noted that, "enhancing the quality of the transferred embryo and the endometrium's receptivity is the main treatment plan for couples who experience implantation failure" [31]. Proteomic studies indicated that the embryonic secretome may differ between those that implant and those that fail, although prospective validation studies are as yet lacking [32].

2.1 Quality of the Embryo

Successful implantation is a complex process that involves multiple factors, including genetic factors, oocyte quality, and uterine receptivity. Any defects or abnormalities in any of these factors can lead to implantation failure and recurrent pregnancy loss. It is essential to understand these mechanisms and their interactions to develop effective treatments for couples experiencing infertility or recurrent implantation failure. Assisted reproduction techniques require careful evaluation of gametes and embryos. According to the embryo quality, the implantation rate was highest for "top quality embryos", which were 8-cell embryos on day 3 with symmetrical blastomeres, less than or equal to 10% fragmentation, and no multinucleated blastomeres [33]. Embryo quality depends on gamete quality and culture conditions. Various grading systems evaluate embryos at different stages. Selecting the embryo with the highest potential reduces the number transferred without compromising success. Invasive and non-invasive methods, such as preimplantation genetic testing and morphokinetics, aid in selection. This review compares effective evaluation and selection methods.

Oocyte quality is also a crucial factor in successful implantation. Identifying oocyte maturity is crucial for optimal fertilization timing. Morphology assessment predicts future development and implantation potential. Oocyte quality can be influenced by various factors, including maternal age, lifestyle, and environmental factors. When there is a poor response to ovarian stimulation with fewer oocytes retrieved, a large proportion of immature oocytes, a lowered fertilisation rate, and a low embryo utilisation rate, compromised oocyte quality is frequently suggested as a cause of RIF [34]. Age-related decline in oocyte quality is associated with increased chromosomal nondisjunction resulting in aneuploid embryos, decrease in mitochondrial membrane potential and increase of mitochondrial DNA damage.

The two primary local growth factor systems, namely the bone morphogenetic system and the insulin-like growth factors (IGF) system, are affected by both gonadotropins (luteinizing hormone [LH] and follicle stimulating hormone [FSH]), which in turn impact oocyte competence [35]. Hernandez-Gonzalez et al. recognized that "not only the oocyte but the cumulus cells (CCs) play an important role in the implantation process [36]. The cumulus oophorus is a mass of granulosa cells (GCs) associated with the oocyte from the antral follicle stage to fertilization and until early embryo development". The nurturing of oocyte growth, development, and acquisition of developmental competence is primarily facilitated by the ovarian follicular microenvironment and maternal signals, which are transmitted through GCs and CCs [37].

Sperm quality can play a crucial role in successful implantation, as it affects the ability of the sperm to fertilize the egg and support early embryonic development. Sperm count, motility, and morphology are some of the key factors that can influence sperm quality. The ability of sperm to fertilize decreases when there are abnormalities in their genomic material. Poor-quality spermatozoa may also result in the generation of poor-quality embryos. It is commonly acknowledged that standard sperm analysis criteria do not adequately indicate sperm quality. Cigarette smoking, genital tract infection, and past chemotherapy or radiation are all factors that lead to sperm DNA damage.

Bashiri et al. found that, "damaged DNA of sperm has been correlated with poor fertilization, reduced implantation and pregnancy rates, and increased production of aneuploid embryos" [38]. Over the past decades, there have been many reports of inverse correlations between genetic abnormalities in sperm and male infertility, as well as the success of assisted reproductive treatments (ART). Shamsi et al. showed that, "birth of offspring with use of sperm with DNA damage results in increased chances of morbidity and childhood cancer" [39]. According to a 2004 study done by Bungum et al., 30% of men choosing ART have a significant percentage of sperm with DNA breaks. Studies done by Shamsi et al. observed that 40.06 percent of sperm in infertile males with severe sperm pathologies had DNA damage, compared to 47.7% of sperm with high DNA damage in male partners of couples who had miscarriages [40].

3. The Mother in Implantation Failure

Maternal age plays a crucial role in pregnancy rates as well as the quality of embryos used for IVF. Many difficulties that emerge clinically in the first trimester, such as miscarriage, or in the second half of pregnancy, such as preeclampsia, preterm birth (PTB), foetal growth restriction (FGR), and gestational diabetes (GDM), have their origins in implantation and placentation disorders [41]. Gellersen et al. stated that the endometrium is a multi-layered, dynamic mucosa that overlays the myometrium of the uterus [42]. It comprises a variety of cells, including luminal and glandular epithelial cells, stromal fibroblasts, and vascular and immune cells. During a menstrual cycle, dramatic changes occur in both the phenotype and abundance of many of these cells, especially in the superficial endometrial layer. Takano et al. observed that, "endometrial growth is dependent on estrogen stimulation whereas the postovulatory rise in progesterone levels triggers a coordinated programme of differentiation, characterized by proliferative arrest and secretory transformation of the epithelial cells, transient oedema, in- flux of uterine natural cells (uNK), vascular remodeling, and differentiation of stromal fibroblasts into specialized decidual cells" [43].

3.1 Receptivity of the Endometrium

The primary role of the endometrium is to collaborate with the myometrium in accepting the embryo during implantation, supporting its development, and guaranteeing a punctual delivery of the fully-formed fetus. Being able to identify a uterus that is receptive can play a crucial role in avoiding reproductive failures

and can also be a determining factor in the success of ART. Endometrial receptivity is a result of effects provided by ovarian steroid hormones, and is synchronized with fertilization and embryo development [44]. Teh et al. also added that, "after being exposed to estrogen and progesterone in sequence, the human endometrium attains its receptive state" [45]. Paulson mentioned that, "estrogen and progesterone are the two key hormones required for preparation of the human endometrium for implantation" [46].

Studies have shown new functions for the decidualized endometrium as a biosensor of embryo quality, with the embryo itself accounting for just around 30% of implantation failures and insufficient uterine receptivity accounting for roughly 70% [47]. The receptivity of the endometrium may be adversely affected by ovarian hyperstimulation, leading to a discordant maturation between the embryo and the endometrium which may result in failed implantation [33]. Roque et al. explained that, "reduced endometrial receptivity brought on by supraphysiological hormone levels during the follicular phase of controlled ovarian stimulation may have a negative impact on the rate of implantation and pregnancy" [48]. The occurrence of an early increase in progesterone levels during the follicular phase in ovarian stimulation is linked to decreased rates of implantation and pregnancy. This is thought to be a result of an unresponsive endometrium and possibly lower quality oocytes embryos.

3.2 Implantation Failure and Genetics

Genetic factors are vital in successful implantation to occur. The presence of abnormal genetic material in the embryo or/and endometrium will cause the implantation to fail. There is also growing evidence that genetic factors regulating invasion and endometrial angiogenesis is essential for embryo implantation [49]. Maruyama & Yoshimura suggested that, "there are overlaps between the genetic variables that cause recurrent spontaneous abortion and infertility and those that cause implantation failure" [50]. Chromosomal abnormalities, such as aneuploidy or chromosome rearrangements, are well known to cause early pregnancy failure and recurrent pregnancy loss (RPL) [20]. For successful implantation, embryo maturation and uterine receptivity must occur in concert such that a "WOI" is open for 48 hours, 7–10 days after ovulation [3].

The crucial stage of embryo implantation is influenced by a range of genetic factors, and a number of single-nucleotide polymorphisms (SNPs) have been linked to RIF [51]. Chen et al. noted that, "microRNAs are known to regulate various functions and have the ability to influence the expression of multiple genes that are crucial for fetal and placental development during the peri-implantation period [52]. However, these same factors may also be closely linked to the development of recurrent implantation failure (IVF) and recurrent pregnancy loss (RPL)".

Multiple studies have demonstrated that having an inherited predisposition to thrombophilia may increase the likelihood of experiencing repeated failures in achieving a successful pregnancy after recurrent implantation failure. During pregnancy, the haemostatic system undergoes changes that lead to a state of increased blood clotting, which becomes more pronounced as the pregnancy progresses and reaches its peak towards the end. The most significant change is observed in the coagulation process, which shows higher levels of activity in factors VII, VIII, X, and von Willebrand factor, as well as a marked increase in fibrinogen [53]. Kamel et al. mentioned that, "alterations in the blood clotting system serve as a natural protective mechanism during the peripartum phase, but may increase the likelihood of complications for both the mother and fetus throughout the gestational period [54]. The mother is at risk of such complications from the moment of conception until after delivery".

Arachchillage & Makris defined thrombophilia as "a predisposition to form clots inappropriately [55]. This condition increases the development of venous thromboembolism (VTE) and thromboembolic disease, which can be acquired or inherited". Stevens et al. denoted that, "inherited or hereditary thrombophilia commonly implies the conditions in which a genetic mutation affects the amount or function of a protein in the coagulation system" [56]. Activated immune conditions including elevated proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8), aberrant alloimmunity and presence of autoantibodies may contribute to thrombosis as well [57]. It is clear that vascular thrombosis can be attributed to inherited thrombophilia, however, the impact of inherited thrombophilia on women experiencing RIF remains a topic of debate.

Thrombophilia type	Description	Gene location	Association with RIF	References
Factor V Leiden (Homozygous & Heterozygous)	Genetic mutation that affects blood clotting; common cause of abnormal clotting; homozygous Factor V Leiden carriers have higher clotting risk than heterozygous carriers.	F5 gene on Chromosome 1	High risk factor for infertility and RIF	Kujovich, 2011
Prothrombin Gene Variant G20210A (Heterozygous)	Genetic mutation that affects the prothrombin; dominant autosomal trait.	F2 gene on Chromosome 11	3–8 times higher risk than homozygous type	Kozma et al., 2015

MTHFR C677T/ MTHFR gene mutations	Genetic mutation in the MTHFR (methylenetetrahydrofolate reductase) gene; inherited in an autosomal recessive pattern.	Chromosome 1, specifically on the long (q) arm at position 36.3	Higher fetal loss at early stages of pregnancy	Altomare et al., 2007
Antithrombin Deficiency	Genetic disorder affecting blood clotting regulation.	Chromosome 1 q25.1-q25.2	High risk of maternal venous thromboembolism	Bravo-Pérez et al., 2019
Protein C Deficiency	Rare genetic disorder that affects the body's ability to regulate blood clotting; autosomal dominant trait.	Chromosome 2 q13-q14	Pregnancy increases risk of venous thromboembolism by 7.8%	Croles et al., 2017
Protein S Deficiency	Autosomal incomplete dominant genetic disease; loss-of-function mutations in the PS coding gene PROS1; More common than protein C deficiency	PROS1 gene near the centromere on chromosome 3 at 3q11.2	Increases the risk of recurrent pregnancy loss by 15-fold.	Lalan et al., 2012; Zhang et al., 2022

Table 1: Inherited Thrombophilia and Its Association with Pregnancy Related Issues

Inherited thrombophilia is believed to play a role in recurrent implantation failure after IVF treatments, and has been the subject of research efforts. Factors are recruited to promote haemostasis, which involves increased expression of tissue factor, the main initiator of haemostasis through thrombin generation, and plasminogen activator inhibitor type 1 (PAI1, SERPINE 1), which deactivates tissue-type plasminogen activator (t-PA, PLAT), the primary agent in fibrinolysis. Nelson & Greer has been hypothesised that invasion of maternal vessels by syncytiotrophoblast can be affected by localised thrombosis at the implantation site, leading to IVF failure [58]. Simcox et al. also hypothesised that thrombophilia may cause placental insufficiency due to placental vascular thrombosis [59].

Furthermore, the thrombomodulin-protein C mechanism plays a crucial role in inhibiting coagulation and fibrinolysis to avoid excessive production of tissue factors. This, in turn, prevents the formation of thrombin and the generation of fibrin degradation products that can be harmful to trophoblast cells [60]. The significant function of the haemostatic system in the implantation process is highlighted by the possibility of heparin's favorable impact [61]. Overall, understanding the genetic factors and haemostatic system's role in implantation can aid in identifying and treating factors contributing to implantation failure and improving outcomes for couples seeking to conceive.

3.3 Molecular Aspects of Implantation Failure

Dey & colleagues reported that, “molecular and genetic evidence indicates that ovarian hormones together with locally produced signaling molecules, including cytokines, growth factors, homeobox transcription factors, lipid mediators and morphogen genes, function through autocrine, paracrine and juxtacrine interactions to specify the complex process of implantation” [62]. However, more studies were done by on the hierarchical structure of the molecular signaling pathways that control interactions between the uterus and the embryo in the first trimester of pregnancy [63]. Canfield et al. explained that, “implantation is considered to occur when a blastocyst breaches the luminal endometrial epithelium [64]. However, determining precisely when this occurs in the human being is complicated. The only established clinical marker of implantation is hCG”.

Progesterone is widely acknowledged to be necessary for embryo implantation in almost all of the species investigated, but the significance of the two estrogen surges that occur during the pro-estrous and luteal phases prior to embryo implantation is still controversial [62,65,2]. IL-6 is minimally expressed in human endometrium throughout the proliferative phase but has significant immunoreactivity during the mid-secretory phase, primarily in glandular and luminal epithelial cells [66,67]. Therefore, a role in human implantation could also be postulated for this cytokine, as for leukemia inhibitory factor (LIF) and interleukin (IL)-11, since IL-6 has some functional redundancy with IL-11 and LIF. There is growing proof that IL-11 plays a significant role in human implantation. Recent studies have shown that the human endometrium contains IL-11 and its receptor (IL-11R) [68,69]. Koler et al. showed that, “RIF patients show deregulated gene expression during the receptive phase compared to controls” [70].

Bashiri et al. identified that, “implantation failure is diagnosed as a lack of ultrasound signs of pregnancy in the uterine cavity [26]. In several studies, a biochemical pregnancy was included (an increase in β-hCG without any ultrasound sign of pregnancy) to the definition of RIF [26]. Moreover, Coughlan et al. pointed out that, “implantation process is complex, the assessment of causes of RIF should be performed on several levels [17]. The most common analyses are chromosomal testing of both parents, the estimation of ovarian function (FSH, LH, anti-mullerian hormone (AMH) measurement) in women, and sperm DNA fragmentation in men, as well as assessment of uterine pathologies and fallopian tube

permeability (hysterosalpingogram, laparoscopy)".

RIF patients show deregulated gene expression during the receptive phase compared to controls [70]. Studies focusing on p53 tumour suppressor gene, which regulates cell apoptosis, angiogenesis and is a potential mediator of pregnancy show significantly more homozygous genotypes in RIF patients [71]. The human LIF gene, which is required for implantation, has been discovered as a p53 target gene. Through direct sequence specific DNA binding and transcriptional activation, p53 controls both basal and inducible LIF transcription [72]. Hu et al. studied LIF as a gene target for p53, which increases its expression [72]. The molecule p53 binds to the p53-binding element in the first intron and alters the expression of LIF in different tissues, including endometrial tissue. The absence of p53 leads to the reduction in LIF and thereby impairing the implantation process.

4. Polymorphism of Genes and Implantation Failure

Genetic factors play an important role in the success of implantation. Recent research has demonstrated that genetic variables, including polymorphisms in certain genes, might affect the implantation process and cause RIF. Polymorphisms are differences in a gene's DNA sequence that happen in a population at a frequency of at least 1%. They have been linked to a number of diseases and conditions, including infertility and RIF, and can affect the gene's expression or function. The success of implantation and pregnancy may depend on a number of genes, including those that control metabolism, immunological function, coagulation, and hormone signalling. Implantation failure can result from changed gene function or expression caused by polymorphisms in these genes, which can disturb the delicate balance of the implantation process.

The abnormal genetic material in the endometrium can lead to implantation failure [73]. Numerous findings from recent studies suggest that genetic variables controlling angiogenesis and invasion processes play a significant role in embryo implantation. Studies in the literature demonstrate that implantation failure can result from genetic flaws, including genetic polymorphisms of the genes involved in these processes [74]. The genetic variables that cause implantation failure coincide with those that cause recurrent spontaneous abortion and infertility [75].

4.1 LIF Gene

Steck et al. states that leukaemia inhibitory factor (LIF) is a glycoprotein that plays an important role in reproduction, with particular relevance in the regulation of implantation, but also has a variety of functions in different organ systems" [76]. Cullinan et al. studied that "the expression of LIF, related members of this group of cytokines, oncostatin M and ciliary neurotrophic factor, and the LIF receptor j3 and glycoprotein gpl30 in normal human tissues and in the endometrium of fertile women" [77]. Fenwick et al. explained that "LIF protein and mRNA are detectable in the human endometrial system only during the secretory phase of the menstrual cycle" [78]. Le´de´e-Bataille et al. reported that low concentrations of LIF in uterine flushings at day 26 were highly predictive of subsequent implantation [79]. Hambartsoumian demonstrated that low uterine concentrations of LIF protein in the secretory menstrual phase has been reported to be associated with a high risk of implantation failure after embryo transfer and in unexplained infertility [80]. He also states that LIF secretion found in the proliferative phase of the menstrual cycle and that LIF secretion in endometrial explant cultures was different between fertile and infertile women. In fertile women, the endometrial LIF secretion was 2.2-fold higher in the secretory than in the proliferative phase, whereas infertile women did not exhibit such an elevation of LIF production in the luteal phase.

LIF concentration in uterine flushings of fertile women on days 18–21 of the menstrual cycle was 3.5-fold higher than in infertile women with recurrent IVF failure, and 2.2 times higher than in infertile women without multiple failure of implantation. Mikolajczyk et al. also states that "LIF overexpression in uterine secretions may be used as a potential indicator of uterine receptivity in fertile women" [81]. Chen et al. noted that "the majority of unexplained infertile women show significant decrease in LIF expression level, signifying the importance of LIF in implantation" [82]. Recently Hu et al. identified that "p53 has a specific binding site on LIF promoter and regulates both basal and inducible transcription of LIF" [72].

4.2 p53

Phylogenetic research on p53 revealed that it is an evolutionarily conserved gene and that p53-like transcriptional factors exist in invertebrates that do not have adult malignancies. These findings imply that p53 might be involved earlier in these species [83]. A genetic polymorphism known as polymorphism of p53 codon 72 is being explored extensively for its significance in reproductive medicine. However, Razieh et al. noted that the results on the correlation between polymorphism and abnormalities, recurrent pregnancy loss and RIF, are still inconclusive [84]. The p53 gene (17q13) has 11 exons with a single nucleotide polymorphism (SNP) at codon 72, which results in a proline instead of an arginine substitution by changing G to C. One of the p53 protein's gene targets is leukemia inhibitory factor (LIF), which regulates lymphocyte differentiation and proliferation by secreting cytokines [85]. The p53 protein, containing an arginine at codon 72, induces apoptosis, LIF expression, and cellular transformation considerably more efficiently [86].

Kang et al. denoted that, "the p53 allele encoding proline at codon 72 (P72) was significantly enriched over the allele encoding arginine (R72) among patients undergoing in vitro fertilization (IVF)" [71]. The P72 allele is a risk factor for unsuccessful implantation. LIF levels are considerably lower in cells with the P72 allele than in cells with the R72 allele, which would explain why the P72 variant is related to poor implantation and fertility. Zhang et al. stated that, "the p53 plays an important role in controlling female reproduction and blastocyst implantation owing to true-life" [63].

Gene	Location	Polymorphism	Encoding protein function	Polymorphism results in	References
LIF	22q12.2	rs929271, rs7832768	Leukemia inhibitory factor; promotes embryo implantation by signaling to the endometrium	Polymorphisms in LIF have been associated with RIF and recurrent miscarriage	Vagnini et al., 2019; Salleh & Giribabu, 2014
Tp53	17p13.1	rs1042522 (Arg72Pro)	Tumor protein 53; role in regulating the cell cycle, DNA repair, and apoptosis	Associated with infertility, recurrent miscarriage	Mohammadzadeh et al., 2019
ESR1	6q25.1	rs2234693 (*PvuII*)	Estrogen receptor alpha; plays a role in uterine receptivity and embryo implantation	Associated with infertility and failure of IVF	Vagnini et al., 2019; Paskulin et al., 2013
MTHFR	1p36.3	rs1801133 (C677T), rs1801131 (A1298C)	Methylenetetrahydrofolate reductase; key enzyme for folic acid metabolism	Associated with RIF, causing hyperhomocysteinemia	Zeng et al., 2021
KIR	19q13.4	KIR2DS1, KIR2DS5, KIR3DL2, KIR3DL3, KIR3DS1	Killer cell immunoglobulin-like receptors; important for maternal-fetal immune tolerance and implantation success.	Associated with recurrent miscarriage and implantation failure	Piekarska et al., 2022
IL-10	1q31-32	rs1800896, rs1800871, rs1800872	Interleukin 10; for successful implantation and maintenance of embryo during pregnancy	Associated with spontaneous abortion	Vidyadhari et al., 2017
HLA-G	6p21.3	rs1632947	Human leukocyte antigen G; plays a role in immune tolerance and maternal-fetal interactions.	Associated with recurrent implantation failure and recurrent miscarriage.	Fan et al., 2017

Table 2: Gene, its polymorphism and association with RIF

4.3 MUC-1

MUC-1 (Mucin-1) is a glycoprotein expressed on the epithelial surface of different types of tissues, including the endometrium [87]. One proposal is that in mice MUC-1 mucin forms an anti-adhesive barrier, and its downregulation after ovulation is necessary for embryo attachment. Conversely, in man, rabbits, and baboons, MUC-1 mucin concentrations increase after ovulation and persist during implantation [87]. Women with recurrent pregnancy loss (RPL) were shown to express reduced endometrial MUC-1, as compared with a normal group of patients [88]. Wu et al. demonstrated that MUC-1, a highly glycosylated polymorphic mucin-like protein secreted by the endometrial luminal epithelium is considered a "barrier to implantation" [89]. In humans, MUC-1 is expressed in the luteal and pre-implantation phases in a progesterone-dependent manner.

Alterations in the internal structure of MUC-1 Variable Number of Tandem Repeats (VNTR) have the potential to impact the quantity of core protein O-glycosylation sites, thereby affecting the immunogenicity of the molecule and potentially contributing to pregnancy loss [90]. However, the studies done by Dentillo et al. suggested that the number of VNTR repeats in MUC-1 is not linked to implantation failure in women experiencing recurrent abortion [9].

4.4 MTHFR Gene

The human Methylenetetrahydrofolate Reductase (MTHFR) gene, which consists of 11 exons, is found on the short arm of chromosome 1 (1p36.22). The MTHFR enzyme is crucial for cell division, embryo development and early pregnancy. It also plays a crucial function in the metabolism of folate. The MTHFR gene's two most prevalent variants are MTHFR A1298C and MTHFR C677T. Oocyte and embryo development are negatively impacted by decreased MTHFR activity [91]. Evidence suggested a connection between MTHFR 677C>T and ovarian reserve, oocyte maturation, and embryo aneuploidy. The MTHFR gene polymorphism might play a role in the etiology of patients with recurrent miscarriage (RM) or RIF [92].

In a study by Choi et al., the findings showed that the combination MTHFR 677/MTHFR 1298 genotype might be linked to an elevated risk of RIF [93]. Enciso et al. explained that, "the elevated rates of RM and IF are caused by MTHFR mutations, which also affect the aneuploidy levels of the embryo" [94]. In a study done by Rotondo et al., it was observed that idiopathic infertile women exhibit an increased frequency of MTHFR 677C>T polymorphisms when compared to the control women of that study [95]. Safdarian et al. also found that recurrent IVF failures were associated with homozygous MTHFR C677T mutations [96]. Guo et al. revealed that the MTHFR 677 T genotype was associated with a higher incidence of trisomies in chromosomes 18 and 21 [97].

4.5 Human Progesterone Receptor (hPR) Gene

Kastner et al. describe another critical genetic variation in the human progesterone receptor gene that is associated with the probability of implantation failure [98]. The human progesterone receptor (hPR) gene is a dual function gene that encodes two distinct isoforms with distinct transcriptional factor activity, hPR-A and hPR-B. Sartorius et al. states that "the longer isoform, hPR-A, has 165 additional amino acid residues on its amino terminus end, which leads to the change of hPR-B conformation and significant difference between the target genes and physiologic effects of the two isoforms" [99]. Cramer et al. noted that "the imbalance between these isoforms' expression leads to severe abnormalities in ovarian and uterine function and defective implantation" [100].

4.6 HLA-G Gene

The non-classical HLA class Ib protein known as human leukocyte antigen (HLA-G) is essential for the mother to accept the semi-allogeneic fetus is located within the major histocompatibility complex (MHC) at 6p21.3 [101,102]. In contrast to the highly variable conventional HLA Ia genes, the HLA-G gene has limited tissue expression and modest allelic variation. HLA-G is mostly expressed in immunological organs and in the maternal-fetal interface [103]. The suppression of cytolysis by natural killer (NK) cells, enrichment of regulatory T (Treg) cells, and encouragement of a switch from a T-helper (Th)1 to a Th2 cytokine profile are all crucial roles of HLA-G at the fetal-maternal interface [104]. Hackmon et al. denoted that, "higher expression of HLA-G by blastocysts has a significant concordance with a higher success rate of implantation" [105]. HLA-G is essential for immunological tolerance at the maternal-fetal interface.

The crucial component determining embryo implantation is maternal immunological tolerance, which is brought on by interactions between soluble HLA-G and uterine lymphocytes. It is necessary for embryo implantation that HLA-G be soluble. However, research on the function of parental sHLA-G expression before conception is limited [106,107]. The study done by Lashley et al. showed that, although 14-bp ins/del polymorphism is linked to recurrent implantation failure, the immunological role of HLA-G and its genetic impact are unclear [108]. According to a meta-analysis by Fan et al., the HLA-G 14-bp insertion allele may enhance the incidence of RIF in Caucasians [109]. Implantation failure may be attributed to the high expression of sHLA-Gtot and sHLA-GEV as well as the 14-bp deletion allele [110]. In the study of Lashley et al., it was shown that "the −14bp/+14bp or +14bp/+14bp genotype was more common in women with RIF, nearly 92% compared to 64.6% in the IVF control (sIVF) and 58% in the fertile control (SP) group" [109]. Enghelabifar et al. confirmed the relationship between ins/del HLA-G genotype and increased risk of implantation failure [111].

5. Treatment Strategies

Once an anomaly related with implantation failure is identified, therapeutic options such as uterine septectomy, intra-uterine adhesion removal, endometrial polypectomy or myomectomy (particularly the submucous variety), and hydrosalpinx excision should be considered [19]. It is believed that intrauterine injection of a patient's own lymphocytes may increase endometrial receptivity and implantation rates while restoring the immunological balance in individuals with RIF, who may be unable to recruit the requisite lymphocytes for successful implantation [112]. New research on intrauterine infusion of platelet-rich plasma has also demonstrated a benefit in IVF transfers for women with thin endometriums [113,114,115]. Granulocyte colony-stimulating factor has been investigated as an in vitro fertilization adjunct treatment given locally or systemically to women with a thin endometrial lining, a history of recurrent pregnancy loss (RPL), or RIF [116,117,118]. Other immune therapies for RIF under investigation include intrauterine hCG infusion, intravenous immunoglobulin (IVIG), intravenous intralipid therapy and heparin [119]. The above reports signify the various treatment strategies available to achieve a successful pregnancy [120-139].

6. Conclusion

Recurrent implantation failure is the process of failure to attain a pregnancy following 2-6 IVF cycles, in which more than 10 high-grade embryos were transferred to the uterus. There are several factors that cause failure of implantation, especially the genetics of parents and the embryo. There is growing evidence that genetic variables governing invasion and angiogenesis processes are important in embryo implantation. The present review is a pointer of various research studies and genetic factors involved in implantation failure. The review also highlights invasion and angiogenesis as a critical process behind implantation failure. By genotyping RIF suffered couples, the reasons and risk of IVF failure can be predicted in order to provide appropriate therapeutic options. The review also emphasizes further in-depth clinical trials on IVF to overcome infertility in the near future.

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Conflict of Interest

The Authors declare that there is no conflict of interest.

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