

RECURRENT IMPLANTATION FAILURE

Abstract

Implantation failure refers to the failure of the embryo to reach a stage when an Intrauterine gestational sac is recognized by ultrasonography. From the clinical point of view, it is worthy to note that the term 'implantation failure' refers to two different types of situation, those in whom there has never been evidence of implantation (no detectable HCG production) and those who have evidence of implantation (detectable HCG production) but it did not proceed to beyond the formation of a gestational sac visible on ultrasonography. In the present era where the field of ART has been so advanced, the unexplained recurrent implantation failure poses a great challenge. Still there are so much of debates regarding the ideal approach towards these cases that there is no definite evidence based proven approach about dealing with these cases. In this review we have tried to simplify the approach towards RIF cases and to reach to possible conclusion keeping in mind the current treatment options available.

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I. INTRODUCTION

Implantation failure refers to the failure of the embryo to reach a stage when an intrauterine gestational sac is recognized by ultrasonography. From the clinical point of view, it is worthy to note that the term ‘implantation failure’ refers to two different types of situation, those in whom there has never been evidence of implantation (no detectable HCG production) and those who have evidence of implantation (detectable HCG production) but it did not proceed to beyond the formation of a gestational sac visible on ultrasonography. In the present era where the field of ART has been so advanced, the unexplained recurrent implantation failure poses a great challenge. Still there are so much of debates regarding the ideal approach towards these cases that there is no definite evidence based proven approach about dealing with these cases. In this review we have tried to simplify the approach towards RIF cases and to reach to possible conclusion keeping in mind the current treatment options available.

II. DEFINITION

The term RIF is applicable to only those patients undergoing ART. There is no universally accepted definition of RIF. The following table depicts various definitions of RIF in the literature.

Definitions of RIF In The Literature	
<i>Polanski et al., 2014b</i>	Absence of implantation after two consecutive cycles of fresh or frozen IVF embryo transfers with a cumulative number of transferred embryos of four or more cleavage-stage embryos or two or more blastocysts, all of good quality
<i>Coughlan et al., 2014</i>	Failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years
<i>El-Toukhy et al., 2016</i>	Two to four previous fresh or frozen IVF treatment cycles ending in an embryo transfer but no pregnancy
<i>Mariee et al., 2012</i>	Failure of three fresh IVF cycles or two fresh IVF and two frozen embryos transfer cycles
<i>Ledee et al., 2016</i>	Failure to have an ongoing pregnancy >10 weeks after at least six embryos were transferred on day 3 or day 5 in women aged <43 years
<i>Mitri et al., 2016</i>	Failure to have a clinical pregnancy after four or more blastocysts (fresh or frozen) after ruling out malformed uterine cavity, hydrosalpinx, abnormal karyotype or persistently thin endometrium in women aged 38 year or younger
<i>Kitaya et al., 2017</i>	Serial negative pregnancy tests following transfer of three or more morphologically good cleavage-stage embryos and/or blastocysts
<i>Lensen et al., 2019</i>	Two previous implantation failures, no precision on number of embryos

<i>Olesen et al., 2019</i>	Implantation failure despite top-quality embryo or blastocyst transfer(s)
<i>Greco et al., 2014</i>	Three to nine previous implantation failures after IVF (mean 4.9)
<i>Huang, Wei & Li, 2017</i>	Two or more failed transfer of good quality embryos
<i>Koot et al., 2019</i>	Three failed IVF or ICSI treatments, each with at least one fresh good quality embryo per transfer, or failure to achieve pregnancy after transfer of 10 good quality embryos

Regardless of the variability in definitions, the diagnosis of RIF is a difficult reality for many couples undergoing infertility treatment. There is as yet no universally accepted definition for RIF, despite many publications on this topic.

- 1. Incidence:** Due to variability in definition of RIF, there are no accurate data available on exact incidence or prevalence of RIF. Biochemical pregnancy is reported to vary from 8 to 33% in general population which includes spontaneous conception also.
- 2. Causes of RIF:** The causes of RIF can be broadly divided into following three categories. Maternal factors, embryonic factors and paternal factors.

Maternal Factors	
Maternal Age	As age increases so does the frequency of aneuploidy. There are higher rates of embryo-endometrial asynchrony with increasing maternal age.
BMI	Increased BMI leads to decrease in implantation rate. In patients undergoing IVF, Class I, II, and III obese patients (BMI > 30 kg/m ²) had the highest chance of implantation failure.
Smoking	Cigarette toxins might play a role in disrupting corpus luteum formation and implantation of the embryo.
Stress	Elevated levels of cortisol, also known as “the stress hormone,” lead to a 2.7 times greater chance (95% CI = 1.2–6.2) of miscarriage within the first 3 weeks after conception in comparison with women with low cortisol levels.
Uterine Factors & Endometrial Factors	Uterine fibroids, Endometrial polyps, Congenital uterine anomalies, intrauterine synechiae, Adenomyosis, Hydrosalpinx.
Thrombophilias	Antiphospholipid antibody syndrome
Connective Tissue Disease	Some of the disorders associated with RIF are Systemic Lupus Erythematosus, Rheumatoid Arthritis, Systemic Sclerosis, Primary Sjogren’s Syndrome, Inflammatory Myositis.
Immunological Factors	Desregulation of uterine NK cells, untreated Hypothyroidism, Cytokine Imbalance.
Embryonic Factors	
Chromosomal Abnormalities	Translocations, Mosaicism, Inversions and deletions are more common in RIF patients. The most common abnormality in RIF patients is translocation. Abnormalities in hatching process.

Paternal Factors
Paternal factors associated with RIF are Varicocele, Infections, Leucocytospermia, Testicular torsion, Testicular cancer, Systemic infections, Diabetes Sperm Chromosomal Aneuploidy, Sperm DNA fragmentation

3. Investigations in case of RIF: There are ‘n’ numbers of investigations which are offered to cases of RIF worldwide. The following list of investigations is as per the recent Canadian Fertility and Andrology Society (2).

Level of Evidence	
Strong	Weak
Hysteroscopy-routine use is not recommended	Parental Karyotyping
Thrombophilia testing is not recommended	DFI testing should not be routinely used. The use of DFI testing should be as such limited to research settings and counseling purpose.
Immunological testing-only for research purpose	Screening for chronic endometritis should not be routinely offered.
Endometrial Receptivity Array-limited to research settings	
PGT-A:insufficient data to recommend for or against the case of RIF.	

Practically speaking, if the patient arrives after even one IVF failure in today’s era it is always advisable to counsel the patient to undergo PGT testing. The decision of ERA is a dynamic one. It depends on the number of PGT normal embryos one has. If there are less number of PGT normal embryos it is always beneficial to go for hysteroscopy and ERA testing and then to go for transfer.

III. HOW TO PROCEED IN CASE OF RIF?

Counseling: The counseling should be stressed on following important points in previous one or more IVF failure:

- Possibility of Multiple ovum pick up in borderline ovarian reserve
- PGT A should be recommended
- Hysteroscopy as and when required
- The decision of ERA will be based on the number of PGT normal embryos.
- The possibility of donor oocytes in case of previous embryo arrest, consistently aneuploid embryos and consistently empty follicle syndrome.
- The possibility of surrogacy in idiopathic RIF.

IV. OVARIAN STIMULATION

Regarding ovarian stimulation if the history of previous stimulation is available, we can modify the dose accordingly. We will prefer to do genetic polymorphism testing of FSH and LH receptor before deciding the dose of stimulation. Otherwise we will give the standard dose of gonadotropins depending on whether the patient belongs to normal, hyper or hyporesponder group. We will prefer adding adjuvant in hypo responders along with gonadotropins. For further details, kindly refer to the chapter on ovarian stimulation.

V. EMBRYO TRANSFER

The current trend is towards blastocyst frozen embryo transfer in case of RIF patients. According to the current study published in 2019, the clinical pregnancy rate, implantation rate, and ongoing pregnancy rate were higher in the SBT group compared with the DET group (41.15% vs. 27.11%, $p < 0.001$; 41.15% vs. 19.28%, $p < 0.001$; 40.03% vs. 25.9%, $p < 0.001$)(3). They concluded that blastocyst stage transfer could be a proffered strategy in RIF patients.

VI. ROLE OF PGT

Performing preimplantation genetic screening for aneuploidy detection is believed to improve the implantation rates in RIF. Trophectoderm biopsy is preferred as risk of mosaicism is lower. Blastocyst analysis was associated with high pregnancy rates. Hence, the application of comprehensive chromosome screening is likely to assist a subset of RIF patients (those capable of producing blast cysts) in achieving pregnancies(4).

VII. ROLE OF ERA

The altered window of implantation (WOI) is one of the proposed cause of RIF. The ERA is the tool designed to find out this displaced WOI by studying the expression of genes of the proteins in the endometrial. According to one recent retrospective study published in 2017 that evaluated the role of ERA in RIF patients, 41% of them were having non receptive ERA on which personalized embryo transfer was performed. When analyzing implantation rates and pregnancy outcomes (live births plus ongoing second trimester pregnancies), both were notably higher in the receptive group compared to the non-receptive group even after pET(5). The decision of ERA should be a dynamic one depending on the number of PGT normal embryo one has.

VIII. ROLE OF IMMUNOTHERAPY

There are several immunological factors responsible for RIF. But the results of immunotherapy are contradictory in RIF (6). The subcutaneous injection of G-CSF can improve the outcome of patients with recurrent miscarriage (7) and the intrauterine infusion of G-CSF in women with a thin endometrium increases the thickness of the endometrium and pregnancy rate. A thin endometrium is detrimental to ET and may cause RIF (8). The IVF success rate after the administration of IVIG increases in women with previous IVF failure, a high NK cell number, and/or an elevated Th1/ Th2 ratio(9). The optimal protocol for the administration of IVIG is undefined and the overall benefits of IVIG are still controversial. In

2006, Japanese scientists first reported that administration of autologous PBMCs(peripheral blood mononuclear cells) could significantly promote clinical pregnancy rate, implantation rate, and live birth rate in patients with repeated failure of IVF–ET(10).Then, Okitsu *et al*(11)demonstrated that only patients who had three or more implantation failures, the clinical pregnancy rate, and the implantation rate in the PBMC-treated group were significantly higher than those in the nontreated group. It is better to recommend the immunotherapy after two or more implantation failures with euploid embryos. Still further research is required to design the exact protocol of immunotherapy in patient selection of RIF.

IX. ROLE OF INTRAUTERINE PRP INSTILLATION

Two RCTs investigated whether administration of intrauterine PRP could improve IVF outcomes in women with RIF. Pooling of results showed a significantly increased chance of clinical pregnancy in treated women (12, 13). In RIF’s patients with good endometrium the role of intrauterine PRP is controversial. After PGT and ERA, the cases of implantation failures can have some beneficial effect of intrauterine PRP instillation but still more research is required to prove its exact role in idiopathic RIF.

X. SURROGACY IN RIF

Factors responsible for RIF have important implication regarding treatment, however in many couples a definitive cause cannot be found (14).In those couples in whom no etiology is found after investigation of RIF and in whom empirical therapy was not effective ,surrogacy is a the only option(15).The role of intrauterine and intraarterial stem cell therapy is still experimental and more research is still required in this field. But it is always advisable to give the option of stem cell therapy for thin endometrium before going for surrogacy. Then it should be left on the patient to decide depending on their financial and psychological status.

XI. LAB PERSPECTIVES TO IMPROVE THE RATE OF QUALITATIVE BLASTOCYST IN RIF

Chromosomal abnormalities, sperm DNA damage, zona hardening, inadequate culture conditions, and suboptimal embryo development all play a significant role in the etiology of recurrent implantation failure. Optimal culture conditions and blastocyst transfer could contribute toward improving implantation and pregnancy rates. Novel embryo assessment and selection procedures, such as time-lapse imaging and metabolomics, may help in better evaluation of embryo quality and viability and help in selecting embryos with the highest implantation potential (16).Following are the management options to improve the rate of blast cyst formation:

1. Chromosomal Abnormalities

- Preimplantation genetic screening
- Comparative genomic hybridization array
- Single nucleotide polymorphisms

2. Zona Hardening

- Assisted Hatching

3. Suboptimal Culture

- Optimal culture media
- Blastocyst transfer
- Coculture
- ZIFT

4. Assessment of Embryo Quality and Viability

- Time-lapse imaging -EmbryoScope
- Metabolomics
- Proteomics

5. Improving ET Technique

XII. CONCLUSION

RIF with unknown cause significantly hampers IVF success. It can be considered as an evolving field with a significant need for a well defined research. In the current generation of ART recipients and practitioners, the temptation to intervene after two or more failed embryo transfers is significant. Women with RIF should be offered appropriate investigations to rule out an underlying cause for the repeated failure. The main treatment strategy in couples with RIF is to improve the quality of the embryos transferred and the receptivity of the endometrium. Empirical treatments should be offered only after aneuploidy cause of embryo has been ruled out and they should be evidence based. Counseling plays an important role in these cases which should be stressed on possibility of multiple ovum pickups, embryo arrest, PGT-A and the role of ERA and other empirical treatments if all of these fails.

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