

# Implantation Failure, Etiology, Diagnosis and Treatment

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## ABSTRACT

Embryonic implantation is a complex interaction between the embryo and the endometrium. Despite great investigative effort this process is still obscure. Contrary to the great advancement in patient care, follicular recruitment, oocyte quality and aspiration, embryo quality, culture and cryopreservation, our understanding of the implantation process did not enhance as much, and the tools to intervene within this process are limited. The implantation of the transferred embryos still remains the major limiting factor in IVF. Here we will review the current literature on the maternal (uterine, hematologic, immunologic and others) and embryonic factors that are associated with repeated implantation failure (RIF) and describe the various therapeutic approaches to cope with them. In addition, we will present our conclusive recommendations on how to investigate and manage RIF based on the literature and our own experience.

**Keywords:** *In vitro* fertilization, Implantation, Repeated implantation failure.

## INTRODUCTION

Repeated implantation failure (RIF) is defined as the failure to achieve a pregnancy following repeated IVF cycles. Though no formal definition exists, it is accepted that 2 to 6 IVF cycles, in which at least 10 high-grade embryos were transferred to the uterus is defined as RIF.<sup>1</sup> However in most currently operating IVF programs, three unsuccessful ART cycles in which 1 to 2 reasonably good embryos were transferred will attract a special investigative attention.<sup>2</sup>

The process of implantation depends on the communication between the embryo and the endometrium, which produces numerous factors and signals required for successful implantation and pregnancy outcome after IVF. Despite great investigative effort, this process largely remained an enigmatic 'black box'. Patient care, follicular recruitment, oocyte quality and aspiration, embryo quality culture, and cryopreservation have greatly improved since the emergence of IVF more than three decades ago. Stimulation protocols, embryo culture and transfer techniques have been optimized. However, our understanding of the implantation process did not enhance at the same rate, and the tools to intervene within it are extremely limited. Despite a significant increase in IVF success rates up to more than double the spontaneous fecundity of young fertile couples, the implantation of the transferred embryos still remains the major success limiting factor. Although the investigation

for a RIF cause is sometimes fruitful, the vast majority of the cases remain obscure or 'idiopathic'. Here, we will describe the maternal (uterine, hematologic, immunologic and others) and embryonic factors which are associated with implantation failure and describe the various therapeutic approaches to cope with them.

## MATERNAL FACTORS

The maternal factors contributing to decreased receptivity are gross uterine anomalies, such as septa, submucous fibroids, endometrial scarring, resulting in thin estrogen unresponsive endometrium with or without adhesions, altered expression of adhesive molecules, states of hypercoagulability and immunological factors.

### Uterine Anatomical Anomalies

Hysteroscopically visible uterine anomalies can be diagnosed in up to a quarter of the patients with a normally appearing cavity in their initial hysterosalpingogram or hysteroscopy.<sup>3</sup> The contribution of such findings to implantation failure is variable. The impact of lesions minimally, or not distorting the uterine cavity on implantation, remains controversial. However, the surgical correction of gross intracavitary anomalies, such as protruding submucous fibroids, adhesion or long septa was found to be beneficial.<sup>3</sup> The postsurgical pregnancy rates were higher than initially observed in the same patients,<sup>4</sup> but no appropriate prospective controlled studies have been performed. It is therefore appropriate, though not entirely evidence based, to re-evaluate the uterine cavity once the diagnosis of RIF is established, and to surgically correct any significant anomaly found.

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## Thin Endometrium

The evidence regarding the importance of endometrial thickness, as measured by ultrasonographic examination, to implantation is equivocal. While some authors have shown a strong association between this parameter to implantation,<sup>5-12</sup> others have failed to show such a relationship.<sup>13-18</sup> In some studies, the endometrial thickness was related to the IVF outcome, but only in correlation with other parameters.<sup>19,20</sup> Different minimal endometrial thickness thresholds were suggested as essential for successful implantation. In most published studies, no pregnancy was achieved when the thickness of the preovulatory endometrium was < 6 mm.<sup>21</sup> Nevertheless, Sundstrom et al<sup>22</sup> have reported a successful outcome of an IVF cycle in a patient with an endometrial thickness of no more than 4 mm.

Several therapeutic approaches have been suggested to overcome the problem of thin endometrium. Low-dose aspirin,<sup>23</sup> vaginal sildenafil<sup>24</sup> in addition to stimulation with high-dose oral and vaginal estrogens.<sup>25</sup> The purpose of these strategies is to increase the global and implantation site endometrial blood flow. Low-dose aspirin was found to have no effect in the general IVF patient population,<sup>26</sup> but none of these approaches was adequately studied in the IVF patients with RIF. We have published the reproductive results of 99 IVF cycles of a patient cohort with thin unresponsive endometrium who had RIFs. Even if some improvement was achieved employing one of these strategies, the reproductive outcome was still very poor. The pregnancy rate was low, the miscarriage rate was high, and the live birth rate was close to null.<sup>27</sup> Therefore, it is our belief that this problem has no effective treatment and other solutions like surrogacy or adoption should be sought.

Stimulation of the endometrium by local injury using an endometrial biopsy catheter was reported to be beneficial to patients with normal endometrial thickness who had otherwise unexplained RIF.<sup>28,29</sup> However in the published studies, the good results of the poststimulation cycles were compared to the results achieved in the same patients before. This methodology is suboptimal. The effectiveness of endometrial stimulation in patients with otherwise unexplained RIF or with thin unresponsive endometrium is to be determined the performance of prospective randomized controlled trials. Another benefit of the endometrial biopsy is the availability of tissue for histological diagnosis. Significant subclinical conditions, such as chronic endometritis can be diagnosed and eventually treated. Another similar approach to RIF (with or without thin endometrium) is to remove the functional endometrium entirely by performing a formal dilatation and curettage, followed by estrogen therapy for the purpose of achieving growth and regeneration of a better endometrium. The benefit of this procedure is not supported by evidence at all, and its performance might even be detrimental.<sup>27</sup>

## PELVIC FACTORS

Patients with hydrosalpinges have lower implantation rates presumably because of the detrimental effect the hydrosalpinx

fluid has on the endometrium and possibly on the embryo as well.<sup>30,31</sup> A systematic review of three RCTs shows that tubal surgery, such as laparoscopic salpingectomy significantly increased live birth rate and pregnancy rate in women with hydrosalpinges before IVF when compared with no treatment.<sup>32</sup> It is therefore the recommendation of both the American Society for Reproductive Medicine (ASRM, USA) and the National Health Service (NHS, UK), to surgically remove fluid filled distally occluded tubes prior to any IVF treatment.<sup>33</sup>

## Altered Expression of Adhesive Molecules

Local dysregulation of cytokine expression was related to RIF. Elevated endometrial NK cells, dysregulation of interleukins (IL)<sup>12,15</sup> and high IL-1 $\beta$  and low interferon- $\gamma$  and IL-10 (Inagaki et al, 2003) were all found in the endometriae of patients suffering from RIF.<sup>34,35</sup> High levels of aromatase p450 transcription and alterations in pinopode expression have been associated with RIF as well.<sup>36,37</sup> Although research in this direction is most relevant to resolving the unexplained cases of RIF, no assay or therapeutic strategy of clinical significance based on these studies exists up to date.

## States of Hypercoagulability

The role of inherited and acquired hypercoagulable states (thrombophilia) in RIF is presumed to be in a mechanism similar to recurrent miscarriages. Antiphospholipid and other autoantibodies were associated with RIF has been shown in some early studies, but later large prospective studies performed in the late 90's failed to reveal an association between antiphospholipid antibodies and RIF.<sup>38,39</sup> Part of this ambiguity is caused by the use of different assays to determine the presence of the antibodies tested in different places and times. However, more recent studies, in which up to date examinations including genetic tests for hereditary thrombophilias were used, had associated both inherited and acquired thrombophilias with RIF and poor IVF outcome. In some studies, inherited and acquired thrombophilias were found to be more abundant among the patients with RIF, especially in those defined as having "unexplained infertility" when compared to control populations; the general population and succeeding IVF patients.<sup>40,41</sup> Moreover, the effectiveness of low molecular weight heparin in increasing the implantation, pregnancy, and live birth rates was proven in some small prospective randomized controlled trials.<sup>42</sup> On the other hand an equal amount of studies ruling out such a connection or the therapeutic effectiveness of heparin also exists.<sup>43-45</sup> Thus, screening for thrombophilia in RIF is still controversial, but is performed routinely by many practitioners.<sup>1</sup> It is our opinion that thrombophilia screening should be a part of the evaluation of RIF. It is our personal impression that once thrombophilia is diagnosed and prophylactic low dose LMW heparin is administered, the ART success rate increase. In addition, heparin prophylaxis is also important for patient safety during the hyper-estrogenic state created by controlled ovarian stimulation (COH) and pregnancy.

## Immunological Factors

The association between RIF and immune dysregulation is the most difficult to establish and few studies have actually shown that this association is possible. Carp et al suggested that couples sharing HLA alleles are at high risk of RIF and recurrent very early preclinical pregnancy losses.<sup>46</sup> In this study, performed in HLA similar couples, antipaternal complement-dependent antibodies were assayed and mixed culture with the male partners' lymphocytes was performed. The (female) patients were immunized with the male partners' lymphocytes if both humoral and cellular antipaternal assays were negative. The study population included IVF patients with recurrent biochemical pregnancies, RIF and early miscarriages. Other IVF cycles were attempted after antipaternal immune response was produced, and resulted in a significantly higher than expected number of viable ongoing pregnancies in all patient groups.<sup>46</sup> However, this preliminary report has never been confirmed and alloimmunization with another person's lymphocytes might be risky and detrimental. Elram et al have also established an association between RIF and immune dysfunction.<sup>47</sup> In this study, couples with at least seven unsuccessful ETs that were found to share at least three HLA loci, and had a negative cross-match test were included. The therapeutic intervention was the administration of nonspecific intravenous immunoglobulins (IVIG); 30 gm before oocyte retrieval, and a second dose as soon as a fetal heart beat was identified. The 10 couples that participated in this study had undergone a total of 98 prior IVF cycles without any implantations occurring. Following a total of 18 IVIG courses, seven women conceived, resulting in six deliveries and one second trimester miscarriage. These results suggest that couples high order RIF, HLA similarity and maternal tolerance to paternal antigens have an immunological basis to their problem and might benefit from an immunomodulatory treatment, such as IVIG. Unlike partner alloimmunization, IVIG therapy is non-hazardous in most cases. The commercially available IVIG preparations originating from pooled blood donations, but are subject to strict preparation and safety regulations assuring they are pathogen free, safe, and of high quality. Due to the paucity of studies establishing an immune etiology to RIF and the high cost of IVIG therapy, we believe that the immune investigation for a RIF cause should be performed last, only in couples with high order RIF after other causes have been ruled out or treated. The tests to be performed are determination of HLA loci and cross-match with the male partner's lymphocytes. In those patients meeting the criteria specified by Carp et al<sup>46</sup> or Elram et al,<sup>47</sup> IVIG therapy is safe and potentially beneficial and should therefore be considered. Partner alloimmunization is not as safe as IVIG therapy and offers no additional advantages.

## EMBRYONIC FACTORS

Despite the significant advancement in human extracorporeal embryo culture, the existing knowledge and tools to investigate and treat RIF due to embryonic causes is limited. The recognized

embryonic factors to RIF are genetic abnormalities, suboptimal growth in culture and zona hardening.

## Genetic Abnormalities

Chromosomal abnormalities are not infrequent in human embryos cultured *in vitro* and such embryos have a reduced implantation potential. The percentage of embryonic aneuploidy was found to be higher in RIF cases than in controls.<sup>48,49</sup> The disruption of chromosome replication and segregation in a greater than anticipated fraction of the cultured early human embryos might be a common cause for RIF. In most cases the parental karyotypes are normal and the embryonic chromosomal aberrations found are incidental or secondary to disturbed gametogenesis. In a minority of cases, a parental balanced translocation is the cause for the generation of aneuploid gametes and embryos. An increased incidence of sperm chromosomal abnormalities was reported in patients with a normal (systemic) karyotype and RIF.<sup>50</sup> An increased frequency of female (systemic) chromosomal translocations, mosaics, inversions and deletions were observed in young women with high-order RIF.<sup>51,52</sup>

Preimplantation genetic screening (PGS) is the performance of FISH on biopsied blastomeres with probes for the centromeres of the 3 to 8 chromosomes responsible most frequently for aneuploidies. Thus by selecting only the chromosomally normal embryos for transfer, PGS was initially presumed to significantly increase the implantation rates.<sup>48,53,54</sup> However when a larger prospective study was performed, the use of PGS did not increase but instead significantly reduced the rates of ongoing pregnancies and live births after IVF.<sup>55</sup> Although the patients enrolled in this study were of advanced age and not necessarily defined as having RIF, the results are relevant to patients with RIF as well as screening for aneuploidy does not increase the take-home baby rate.

Parental karyotype determination should be a part of the RIF investigation, especially if a history of miscarriages exists. If a parental translocation or other anomaly is discovered, than preimplantation genetic diagnosis (PGD) is warranted like any other inherited condition. On the other hand, if the parental karyotype is normal, the performance of genetic screening is of no benefit.

## Embryo Culture and Transfer

Presently a large variety of high-quality standard commercially available IVF media for different purposes exists. In some cases, patient specific culture conditions are required for optimal embryonic development. In some RIF cases it might be beneficial to empirically alter the culture media and conditions used when *in vitro* embryo culture is suboptimal. The evolving proteomic and metabolomic methods assisting with the selection of the embryo with the best implantation potential are not designed for RIF cases, but might prove to be of some benefit in these cases too.

Coculture of embryos with homologous endometrial cells was suggested to improve culture conditions due to the secretion



of embryotrophic factors, such as nutrients, growth factors and cytokines, and neutralization of harmful substances.<sup>56,57</sup> Using this method, an impressive pregnancy rate was reported in a large patient group with RIF.<sup>58</sup> However, most IVF units are not equipped and do not have facilities and personnel required for routine performance of coculture.

Embryo culture (and eventual transfer) to the blastocyst stage harbors several benefits. The blastocyst is placed in the endometrial cavity 5 to 6 days after fertilization, as in natural conception. Culturing the embryos to the blastocyst stage examines the activation of the entire embryonic genome and biologically selects *in vitro* the embryos with the highest implantation potential. However, *in vitro* embryo loss is inherent to blastocyst culture and might jeopardize the entire treatment cycle. Two large RCTs have shown that blastocyst culture after RIF following cleavage stage transfers resulted in significantly higher implantation and live birth rates.<sup>59,60</sup> However, this approach should be adopted with caution considering the high embryonic aneuploidy rate in RIF cases,<sup>48,61</sup> and the risk of losing most or even all the embryos.

Use of the best transfer technique is mandatory in each cycle and obvious in RIF. Embryo transfer (ET) with soft atraumatic catheters under ultrasound guidance to assure midcavity placement is the superior and almost universally accepted standard in ART.<sup>62</sup> Revision of the transfer history is mandatory in each RIF case in order to assure that no anatomical or other problem have precluded optimal smoothly performed ETs. In special cases, measures like cervical dilation or ET under general anesthesia are necessary to assure this goal. Many clinicians transfer large number of embryos after RIF. Even in countries in which the number of transferred embryos is limited by voluntary or mandatory regulations, the limit is lifted once RIFs occur. However, there is little evidence that the transfer of more than three embryos is beneficial in such case.

### Zona Hardening

The zona pellucida (ZP) surrounding the oocyte hardens after fertilization, depolarization and spillage of the cortical granules, in order to prevent polyspermy and protect the integrity of the migrating embryo until its implantation. The appearance of a breach in the hard and nonadhesive ZP and blastocyst hatching is part of normal implantation. Increased ZP thickness and hardness was associated with lower implantation rates.<sup>63,64</sup> Thus, failure of the ZP to rupture has been suggested as a possible cause of RIF, and different mechanical, chemical and optical techniques were used in order to regionally weaken the ZP or even create an opening in it in order to assist hatching (AH) and implantation. AH is not hazardous. A single report of an association between AH and monozygotic twinning was published but was not sustained by others.<sup>65</sup> AH was not found to improve the overall success rate of ART.<sup>66-68</sup> However, evidence to the benefit of performing AH in selected RIF cases does exist. Cohen et al performed a randomized, prospective trial in which mechanical and chemical AH was found to be

beneficial only if performed on embryos with a poor implantation potential (thick ZP, poor development, advanced maternal age).<sup>69</sup> Obruca and associates used an Er:Yag laser system for AH in RIF cases resulting in increased implantation and pregnancy rates (14.4% vs 6% and 40% vs 16.2% respectively).<sup>70</sup> Chao, Magli and Nakayama et al have shown AH, performed by different techniques, to increase pregnancy and implantation rates when selectively applied in patients with RIFs.<sup>71-73</sup> The latter studies do support the notion that AH, no matter how performed, might be beneficial in RIF cases. Despite the lack of uniformity in the study design and methods used, it seems that AH is beneficial in selected cases of poor prognosis, and bares no actual risk. We believe that AH by the method most familiar to the embryologist, should be performed in every RIF case.

### SUMMARY AND CONCLUSIONS

RIF is a difficult unresolved challenge in reproductive medicine and a source of endless patient frustration and despair. Though far from resolution, several investigative measures and therapeutic interventions were found to be useful in this complex condition according to the published literature and our experience.

1. A repeated evaluation of the uterine cavity and eventual correction of any significant anomaly found should be performed in all cases.
2. An endometrial biopsy might be of both diagnostic and therapeutic value.
3. Hydrosalpinges should be surgically treated.
4. The benefit of thrombophilia screening and LMW heparin therapy is supported by some works and in our opinion should be a part of the initial evaluation.
5. All couples who experience RIF should have their karyotypes examined, and targeted PGD should be performed if a parental aberration is found. If the parental karyotypes are normal, the performance of PGS is of no benefit.
6. Couple HLA typing and cross-match are warranted in high order RIF if no other anomaly was detected. IVIG therapy should be offered if 3 or more HLA loci are common and the cross-match is negative.
7. The historical embryo culture reports should be investigated and condition changes should be considered. Blastocyst culture should be considered if embryos of adequate number and quality are available.
8. A procedure for assisted hatching should be performed in all RIF cases prior to embryo transfer.

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