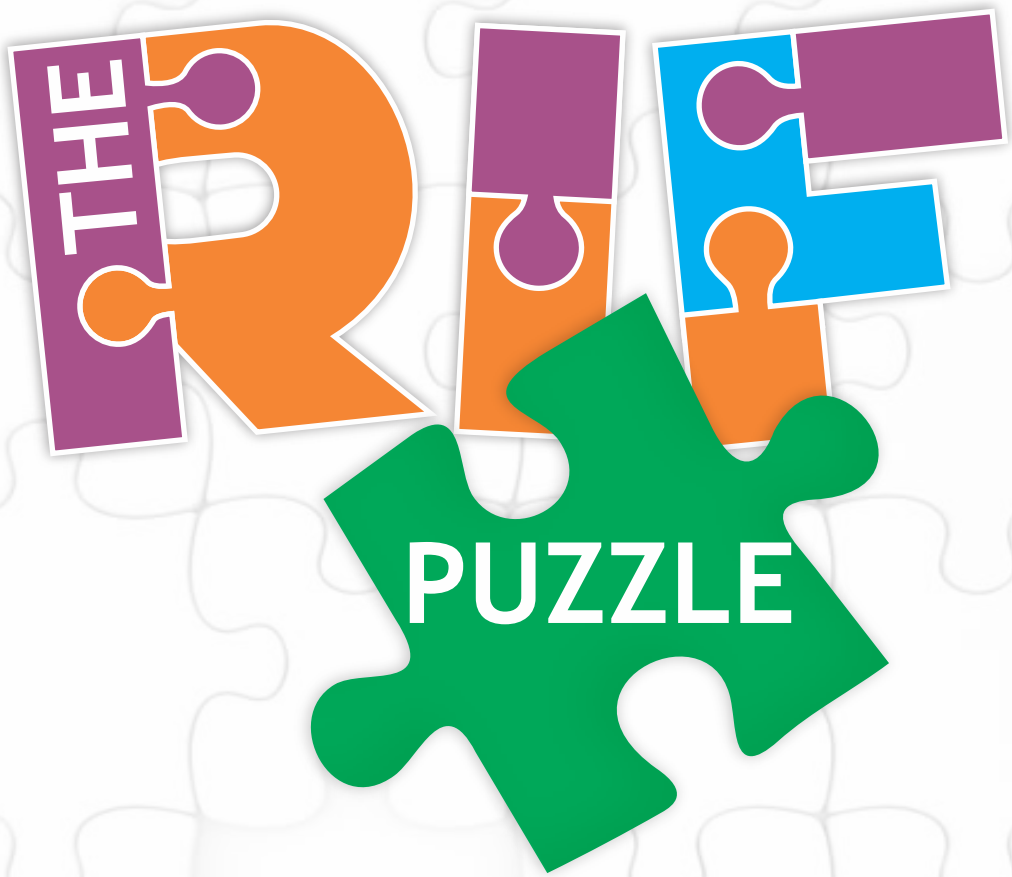
Indian Society for Assisted Reproduction

The Newsletter
EXPRESS

# SPECIAL ISSUE ON DECODING THE RIF PUZZLE




- Overview of Recurrent Implantation Failure
- Emerging Adjuvant Therapies in Recurrent Implantation Failure
- Immune Regulation at the Maternal-Foetal Interface
- Thymosin alpha in RIF

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# Memories



RIF

MASTERCLASS

Science Beyond Implantation

Indian Society for Assisted Reproduction

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# *Presidential Address*


## Prof. Ameet Patki

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Recurrent implantation failure (RIF) is a poorly defined clinical condition encountered in assisted reproductive technology (ART), characterized by repeated failure to achieve pregnancy despite transfer of embryos considered to have adequate developmental potential. Both the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) emphasize the absence of a universally accepted definition and recommend restraint in labelling patients with RIF.

I welcome you to this unique Masterclass dedicated to this rather intriguing topic. This 2-day meeting will review current concepts regarding the definition, contributing factors, diagnostic evaluation, and management of recurrent implantation failure.

We have handpicked the Faculty who will review the contemporary literature and society guidelines , focusing on evidence-based assessment and treatment strategies for patients experiencing repeated implantation failure following ART.

We have a special session on Thymosin alpha , a rather new entrant in the pharmacological array of treatment protocols. Recent studies have shown promising results and we are excited to share them with the delegates on this masterclass.

Certain established facts associated with RIF are most commonly related to embryonic factors, particularly chromosomal aneuploidy, especially in women of advanced maternal age. Uterine pathology, including fibroids, endometrial polyps, adhesions, adenomyosis, and chronic endometritis, may contribute to implantation failure and are part of the armamentarium of every clinician when treating RIF. However newer insights into Endometrial receptivity defects, immunological factors, inherited thrombophilia, and many proposed “add-on” interventions lack consistent evidence to support routine clinical use.

At the end of the 2 day sojourn, I hope the attendees have a clearer concept on RIF and will help to draw out Good Practice Guidelines (GCPR) to share with the rest of the ISAR family.

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## *From the Editorial Desk: Recurrent Implantation Failure - From Frustration to Fine-Tuning*


# Dr. Madhuri Patil

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Under the leadership of ISAR president Dr. Ameet Patki we have had a lot of educational activities over the last 2 years, which include webinars and masterclasses apart from the three annual national congresses. We have had a total of 11 masterclasses, and one of them was on Recurrent Implantation Failure.

Recurrent Implantation Failure (RIF) remains one of the most challenging and emotionally charged areas in assisted reproduction. It is a complex issue involving embryo quality, uterine factors (like polyps/fibroids), maternal health, immunology, or lab errors, with no single definition but generally after 3+ failed attempts. Key areas to explore include genetic testing (PGT-A) for embryos, assessing the endometrium for issues, optimizing transfer techniques, and considering lifestyle factors, requiring a multidisciplinary, individualized approach for effective management. Despite advances in laboratory technology, embryo culture systems, and stimulation protocols, a subset of patients continues to experience repeated failure of embryo implantation, often with good-quality embryos and optimal transfer conditions. For clinicians and embryologists alike, RIF is a reminder that implantation is not a single event, but a finely orchestrated dialogue between embryo, endometrium, and the maternal environment.

Clear communication, realistic expectations, and empathetic care are as important as scientific precision. As research continues to unravel the molecular and immunological underpinnings of implantation, the future of RIF management lies in personalised and precision reproductive medicine where clinical insight meets laboratory excellence. Thus, in RIF, success is rarely about doing more it is about doing what is right for the right patient as one size does not fit all.

This issue of our newsletter explores current concepts, controversies, and evidence-based strategies in RIF, aiming to move the conversation from frustration toward focused, informed, and compassionate care.

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## *Overview of Recurrent Implantation Failure*


# Dr. Madhuri Patil

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### 1. Introduction

Implantation failure is a major dilemma, since its potential causes are often complex & poorly understood. Even after 50 years of IVF treatment and research, major progress is made in improving stimulation protocols and fertilization procedures, optimizing embryo culture conditions & preventing premature luteinization. However, only marginal improvement is seen in implantation and pregnancy rates (IR and PR). Low implantation rates are an impediment to more efficient assisted reproductive technology.

Clinically reproductive failure may manifest in 3 different ways

- Failure to conceive
- Repeated failure to establish implantation in women undergoing IVF
- Recurrent miscarriage in women who do not have difficulty in conceiving.

**It has been observed that**

- 30% embryos lost at pre-implantation stage
- 30% lost after embryos implant but prior to missed period - detectable by positive beta HCG
- 10% lost after the missed period

Implantation involves the embryo, with its inherent molecular programme of cell growth and differentiation, and the temporal differentiation of endometrial cells to attain receptivity. This entire process is governed by an array of endocrine, paracrine and autocrine modulators, of embryonic and maternal origin. Thus a reciprocal interaction between blastocyst and endometrium is required for implantation and establishment of pregnancy.[1]

### 2. What is recurrent implantation failure (RIF)?

In women with unexplained RIF, despite good hormonal response, good-quality embryos, satisfactory endometrial development and no identifiable pathology, suboptimal endometrial receptivity is considered a key factor in inhibiting embryo implantation. During the implantation window, there is a cross-talk between the embryo and the endometrium to allow attachment, adhesion and invasion of the embryo. There is no consistent definition of RIF. In 2023 ESHRE defined RIF as a scenario in which the transfer of embryos considered to be viable

has failed to result in a positive pregnancy test sufficiently often in a specific patient to warrant consideration of further investigations and/or interventions.[2]

**Etiology: Grouped into three categories[3]: (Table 1)**

| Maternal factors | | | Sperm quality | Lab Factor |
|---|---|---|---|---|
| Poor Endometrial Receptivity | Poor Oocyte /Embryo Quality & Embryo Aneuploidy | Multifactorial effectors | | |
| Abnormal endometrial thickness and morphology | Poor oocyte quality due to abnormalities in the cytoplasm and nuclear division or mitochondrial dysfunction can affect fertilization, cleavage, blastocyst formation, thus the embryo quality. All these factors can then affect implantation | Immunological factors & Thrombophilia | High DNA fragmentation - affect embryogenesis and can compromise 'embryo viability' preventing implantation. | Sub-optimal lab conditions |
| Uterine cavity abnormalities - adhesions, polyps, Hyperplasia fibroids & septae | Genetic abnormalities in the oocyte and thus embryos | Endometriosis | Abnormal morphology or aneuploidy | Increased zona thickness and Zona hardening induced by in-vitro culture or by in vivo ageing, affect hatching and is associated with lower Irs |
| Chronic endometritis? | Disruption of the normal sequence chromosome replication & segregation in early human embryos, caused by maternal cytoplasmic factors or mutations in cell cycle control genes has been seen in ART. This result in chromosomally abnormal embryos that fail to implant despite good morphology and developmental rate | Adenomyosis | Chromosomal abnormalities in male partner | |
| Abnormal microbiota? | Retarded embryonic development | Hydrosalpinx | | |
| Altered expression of adhesive molecule | | Suboptimal ovarian stimulation | | |

| | | | | |
|---|---|---|---|---|
| Abnormal proteins and endometrial gene expression profiles during WOI | | Suboptimal embryo transfer | | |
| Disorders of embryonic endometrial synchrony | | Advanced Age - due to dysfunctio-nal cytoplasmic factors, ageing oocytes & mutation in mitochondrial DNA | | |
| | | Increased female chromosomal abnormalities in the form of translocations, mosaics, inversion, deletion and chromosomal breakages, particularly at the centromere region are responsible for RIF. | | |

Table 1: Etiology Factors in Recurrent Implantation Failure

Apart from the above etiological factors controlled ovarian stimulation (COS) can also affect endometrial receptivity and thus implantation. Changes in both gonadotrophins and sex hormones is detrimental to the endometrium by altering endometrial secretome, endometrial gene expression, immune cell recruitment and glandular development.4 This in turn result in abnormal endometrial maturation and synchronization resulting in disturbance in embryo-maternal dialogue and implantation.[4]

**Factors during COS that affect Endometrial Receptivity and Embryo Endometrial Synchrony**[5]

- COS in ART result in supraphysiological E2 and P4 with abnormal concentrations of autocrine and Paracrine factors that affect endometrial receptivity (ER) with disturbance in embryo-maternal dialogue
- COS also affects mural granulosa and cumulus cell gene expression, oocyte spindle and metabolism and can result in oocyte dysmorphism (increased vacuoles, granularity, SER aggregates and course granules in PVS). It also increases ROS in the follicular fluid making it proinflammatory.
- If the oocytes are outside maturation window (immature or overmature) the embryogenesis may be affected (Figure 1)

- Time of administration of hCG is important and late administration is associated with endometrial advancement
- Supraphysiological E2 levels, premature expression of pinopodes & integrins & abnormal luteal phase steroid secretion
- Elevated P4 concentration on day of trigger can accelerate endometrial maturation
- Use of GnRH agonist for trigger significantly decrease PR due to low Luteal Phase LH which affects ER
- Blastocyst formation on day 6 or 7 results in loss of Embryonic-Endometrial Synchrony
- Even if the oocytes are outside the maturation window, they affect embryogenesis and consequently implantation.[6] (Figure 1)

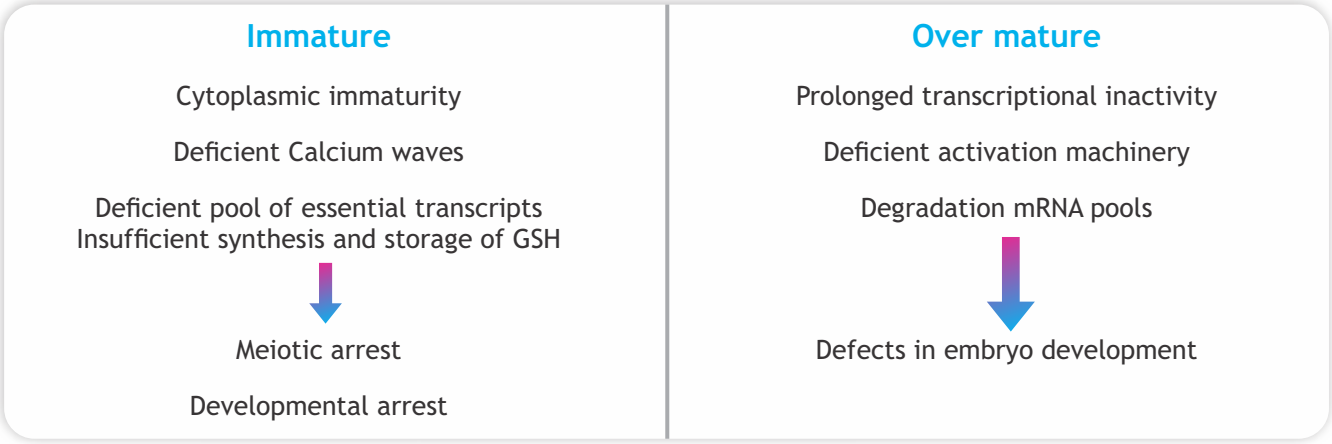


Figure 1: Known effects of being outside maturation window

Change in Metabolism Affecting Oocyte Competence: Table 2 below shows effect of PCOS[7], obesity[7], insulin resistance[8] and high fat diet[8] on oocyte competence

| | |
|---|---|
| PCOS | Extra-and intra-ovarian factors cause abnormalities during folliculogenesis, Follicular growth and oocyte meiotic maturation |
| Obesity or Insulin-resistance | exhibit abnormalities in many different mitochondrial parameters, including mitochondrial morphology and membrane potential of oocytes |
| | contribute to the inter-generational programming of metabolic disease. |
| | developmental origins of obesity and insulin resistance may be established at the time of conception |
| High fat diet | causes reduced MT membrance potential at both the germinal vesicle (GV) and metaphase II (MII) stages as well as a clustered pattern of mitochondrial distribution, indicative of altered mitochondrial activity with reports of poor oocyte and embryo quality in obese women |

Table 2: Effect if metabolic abnormalities on oocyte competence

### 3. When and how to investigate?

After failure of three cycles in which reasonably good embryos were transferred, further investigation should be initiated. This can occur in 10% of cases in IVF. However, majority of cases with recurrent implantation failure are idiopathic with no identifiable cause in either partner. A woman with recurrent implantation failure needs to be investigated to identify that group, which will benefit from some form of treatment.

A careful History and thorough examination of previous records may give some insight into the problem. General and pelvic examination with a pelvic scan may also through light on several pathologies like endometriosis, hydrosalpinx, myomas, polyps, or any other intrauterine pathology if any, which can be treated.

Ultrasound examination of the endometrium for thickness, pattern, volume and blood flow has a good sensitivity (93-100%) to predict endometrial receptivity but has a very low specificity (3-15%).[9] Hysteroscopic inspection has a sensitivity of 75% and specificity of 60%.[9] There is insufficient data available for use of natural killer cell activity and endometrial receptivity assay (ERA) in woman with RIF. ERA and pET may be effective for a subset of patients with unexplained RIF by transferring euploid embryos in a personalized WOI, with better PRs

Use of molecular tests for pinopode assessment, chronic endometritis, endometrial microbiome and abnormal expression of HOXA 10, USAG1 and EBAF gene during implantation window are still controversial.[10]

The figure 1 highlights the recommended and non-recommended investigations in a woman of RIF.[2]

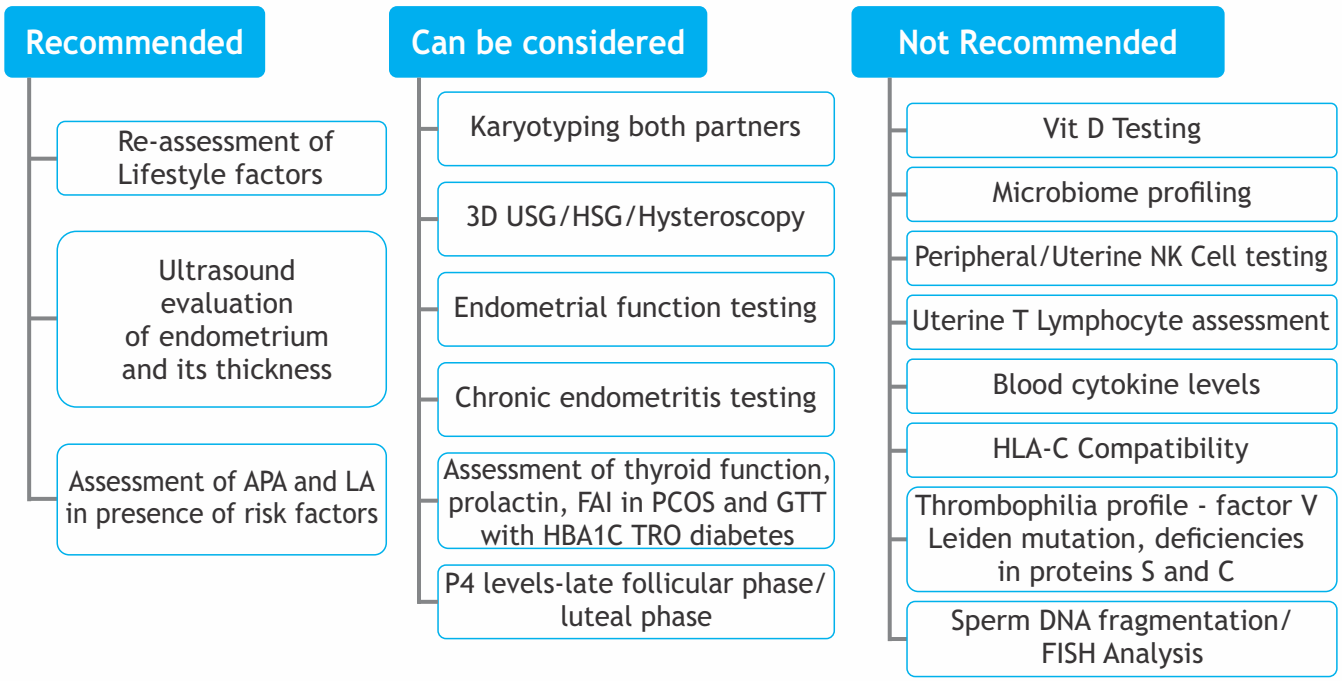


Figure 2 : Investigations for RIF

**4. What are the measures to improve the implantation rate?**

Although benefits have been reported for endocrinological and immunological problems, most treatment modalities are still controversial.

Treatment Strategies For RIF include

**i.** Improve Endometrial Receptivity by treatment of Endometrial Factors in Implantation Failure is enumerated in figure 2.[3,10]

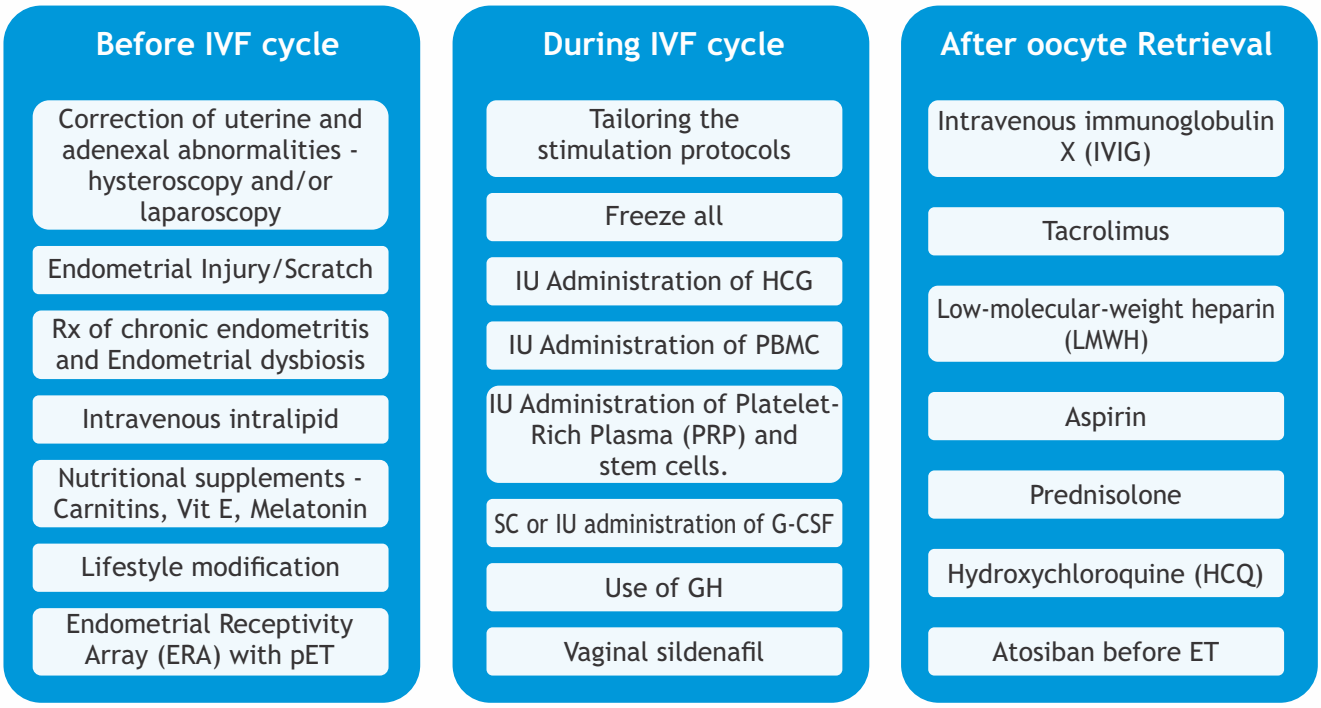


Figure 3: Treatment of Endometrial Factors in Implantation Failure

**ii.** Laboratory and procedural technologies and interventions[3]: High laboratory standards and adherence to correct procedures are critical to the outcome of ART for which robust quality management programmes essential.[11] One could use several laboratory and procedural technologies and interventions for selection of gametes and embryos like spindle view, PICSI, IMSI and MACS (Magnetic activated cell sorting) which has no evidence. There is low quality evidence for the use of microfluidics, blastocyst transfer, PGT-A and time lapse. There is also low-quality evidence to use sequential embryo transfer [i.e. sequential ET on day 2/3 and on day 5), assisted hatching (AH) and Zygote intrafallopian tube transfer (ZIFT). There is no evidence to use ET medium enriched with hyaluronic acid and treatments like autologous embryo-cumulus cells co-culture and mitochondrial transfer are not approved for clinical practice.[11]

**iii.** Management of Multifactorial effectors like hydrosalpinx, adenomyosis, endometriosis by surgery or medical treatment may improve the art outcome.[3] Apart from this psychological assistance and psychotherapy reduces anxiety and depression and enhances conception success.[3]

**iv.** Immunomodulatory therapies[12]

- Corticosteroids during COS and Peri-implantation - Not recommend as a routine in all RIF during COS and peri-implantation period. May have a role in patients with positive thyroid antiantibodies & ANA and Endometriosis Grade A
- Intravenous Immunoglobulin (IVIG) enhance the action of regulatory T cells and reduce Th1 cytotoxic reactions Insufficient evidence to recommend Grade C
- Peripheral Mononuclear Cells - Based on the theory that local immune cells at the implantation site actively contribute to embryo implantation Insufficient contradictory evidence to recommend. Grade C
- Intravenous Intralipid Some role in immune modulation. decrease natural killer cell activation and the production of pro-inflammatory cytokines Insufficient evidence to recommend Grade C
- Tacrolimus immunosuppressive drug and Plausible treatment for RIF with elevated Th1/ Th2 ratio
- Adalimumab - TNFa blocking antibody used for Th1/ Th2 cytokine elevation Insufficient evidence to recommend Grade C
- Daily administration of hydroxychloroquine (HCQ) Downregulate Th17 cytokines and upregulate Treg cytokines Influencing the Th17/Treg ratio and improving IR

**v.** Use of Autologous peripheral blood Platelet rich Plasma (PRP)13 and CD133+ Bone marrow stem cells[11,14] and Umbilical cord MSC15 as investigational treatment in woman with RIF as they are rich source of growth factors and can suppress immune response. They have effective regenerative and anti-inflammatory agent.

## 5. Conclusion

RIF is distressing both to the infertile couple & physician responsible for treatment. As ART is considered as the last step in the armamentarium of infertility treatments, couple are faced with the cold reality of having tried everything & failed. Moreover, etiology of RIF in ART complex and ill understood and treatment options are vague and therefore maximum effort should be deployed to isolate the potential correctable factors that may be responsible. There is no evidence from RCTs that any of the treatments has a significant value, but on the contrary, everyone agrees that trying different approaches may help in achieving a pregnancy in many cases that failed repeatedly.

An improved understanding of the molecular mechanisms responsible for abnormal implantation will likely improve clinicians' abilities to treat disorders that result in repeated implantation failure.

Further unraveling of molecules involved in intricate mechanism of implantation are needed for better comprehension of link between altered endometrial receptivity and implantation failure.

Treatment modalities and the evidence to use them for RIF is summarized in figure 4

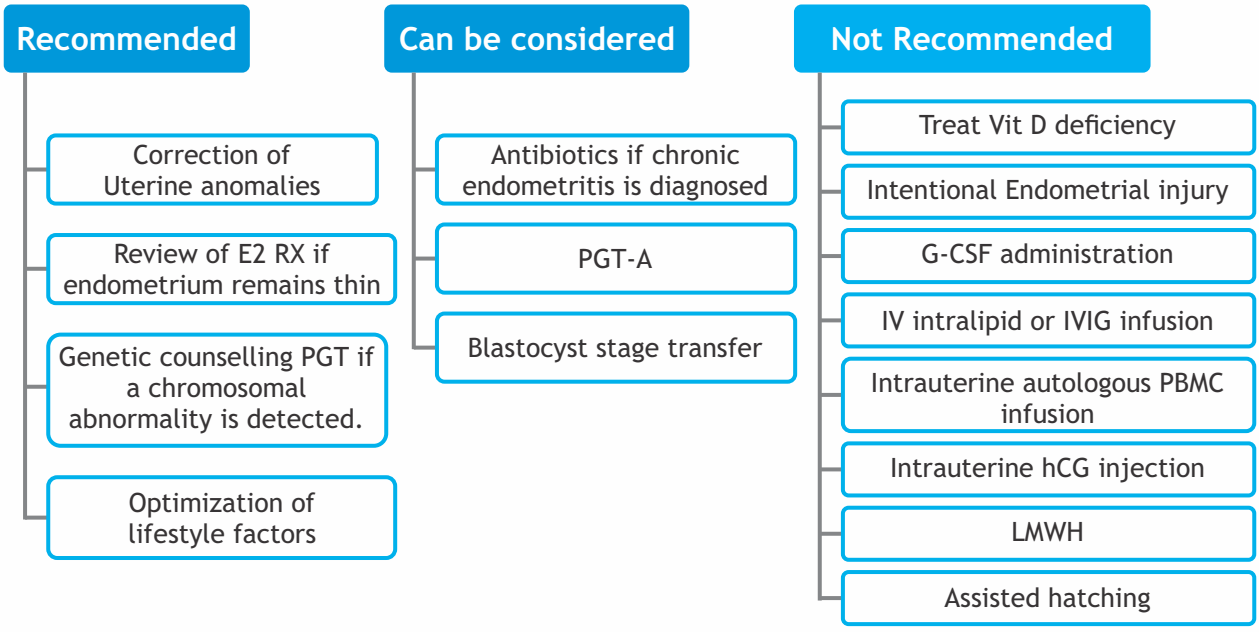


Figure 4: Interventions for RIF

Despite high-quality research in the rapidly expanding area of management of RIF, improving embryo implantation continues to pose a major challenge to clinicians. The growing trend towards transferring fewer embryos further increases the need to improve implantation rates.

## 6. Key Points

- Precise pathogenesis of RIF has not been elucidated butmay result from implantation checkpoint failures and could also be the cause for euploid embryos to fail to implant. This could render implantation failure a “too selective” disorder.
- Bi-directional regulation of infiltrating fetal cells and maternal immune response is necessary for establishment of a successful pregnancy
- Establishment of set of standardized tests for preliminary evaluation would direct the approach of treatment for each individual couple
- Tissue sampling, which is often required for the direct assessment of markers of endometrial receptivity, is inherently impossible in actual ET cycles
- Adjuvants are either not associated with improved outcome in IVF or insufficiently studied to make definitive conclusions regarding benefits and risks
- Patients must be informed of uncertain benefits and risks for use of adjuvants in RIF
- Lack of evidence does not allow incorporation of various treatment modalities in routine clinical practice
- Empirical therapies should, whenever possible, be considered only in the setting of carefully conducted clinical trials
- Adaptation and personalization of fertility therapy based on individual patient

characteristics may help to optimize efficacy and safety outcomes for individual couples

- While new techniques are the way to progress, proper assessment is a prerequisite before general use
- They should not be marketed prematurely based on inconclusive trials

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# *Emerging Adjuvant Therapies in Recurrent Implantation Failure: A Review of Recent Evidence*


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**Abstract**

Recurrent implantation failure (RIF) remains a formidable challenge in assisted reproductive technology, characterized by the failure to achieve a clinical pregnancy after multiple embryo transfers of good-quality embryos. Despite advances in in vitro fertilization (IVF), the complex and multifactorial nature of RIF encompassing immunological, thrombophilic, endometrial, hormonal, and embryonic factors has necessitated the exploration of adjunctive therapies beyond standard protocols. This review aims to consolidate and critically assess the current evidence surrounding adjuvant therapies used in the management of RIF. A wide range of interventions has been proposed to enhance endometrial receptivity, modulate immune responses, and improve implantation success. These include immunomodulators such as intralipids, corticosteroids, intravenous immunoglobulin (IVIG), and thymosin alpha-1; endometrial receptivity enhancers like granulocyte colony-stimulating factor (G-CSF), platelet-rich plasma (PRP), and endometrial scratching; anti-thrombotic agents such as low-dose aspirin and low molecular weight heparin; antioxidants like Coenzyme Q10 and vitamin D; and emerging approaches including probiotics, sildenafil, growth hormone, and stem cell therapy. Although promising, the clinical application of these adjuvants remains limited by heterogeneity in study designs, inconsistent definitions of RIF, and the absence of universally accepted protocols. This review underscores the need for well-powered randomized controlled trials, validated biomarkers for patient stratification, and personalized treatment strategies. Future research should focus on mechanistic insights and precision medicine to optimize outcomes in this challenging patient population.


## 1. Introduction

Recurrent implantation failure (RIF) is a distressing clinical entity in reproductive medicine, defined as the failure to achieve a clinical pregnancy after the transfer of at least three high-quality embryos in a minimum of three fresh or frozen cycles in women under the age of 40[2]. However, definitions vary across studies, contributing to inconsistencies in diagnosis and management.

Given this multifactorial aetiology, a "one-size-fits-all" approach is often inadequate. Individualized treatment strategies that address patient-specific aetiologies are essential for improving implantation success. Adjuvant therapies interventions used alongside standard ART protocols have emerged as potential tools to enhance endometrial receptivity, modulate

immune tolerance, and support embryo implantation. These include immunomodulators, hormonal agents, antioxidants, anticoagulants, and regenerative approaches. Exploring the role of adjuvants in RIF holds promise for refining therapeutic strategies and optimizing outcomes in this challenging cohort.

**2. Established and Emerging Adjuvants**

Recurrent implantation failure (RIF) is a distressing challenge in assisted reproductive technology (ART), typically defined as the failure to achieve a clinical pregnancy after the transfer of at least three good-quality embryos across multiple IVF cycles [2]. Affecting up to 10-15% of women undergoing ART in the Indian subcontinent, RIF has complex, multifactorial underpinnings. The factors implicated range from uterine anomalies and immunological imbalances to thrombophilia, hormonal dysfunction, oxidative stress, and endometrial receptivity issues. Given this complexity, adjuvant therapies interventions used alongside conventional ART are increasingly employed to improve outcomes. This article reviews current evidence on various adjuvants used in RIF. (Table 1)

| Before IVF cycle | During IVF cycle | After oocyte Retrieval |
|---|---|---|
| Rx of chronic endometritis and Endometrial dysbiosis | IU Administration of HCG | Intravenous immunoglobulin (IVIG) |
| Intravenous intralipid | IU Administration of PBMC | Tacrolimus |
| Nutritional supplements | IU Administration of Platelet-Rich Plasma (PRP) and stem cells | Low-molecular-weight heparin (LMWH) |
| Lifestyle modification | SC or IU administration of G-CSF | Aspirin |
| Thymosin alpha-1 | Use of GH | Prednisolone |
| Endometrial Injury/Scratch | | Hydroxychloroquine (HCQ) |

**Table 1: Adjuvants in Recurrent Implantation Failure**

Intralipids are fat emulsions, rich in soybean oil and egg phospholipids, modulate natural killer (NK) cell activity and reduce pro-inflammatory cytokines[5,9]. Several studies suggest their benefit in women with elevated NK cell activity or autoimmune markers, although standardized protocols and large trials are still lacking. Prednisolone or dexamethasone may suppress inflammatory cytokines and modulate immune tolerance at the maternal-foetal interface. Evidence is mixed, but corticosteroids are commonly used in patients with autoimmune or inflammatory profiles. A synthetic thymic peptide, thymosin alpha-1 has immunoregulatory properties that enhance T-regulatory cell activity. Preliminary studies show improved implantation and pregnancy rates, especially in women with immune dysfunction. IVIG modulates immune responses by neutralizing autoantibodies and downregulating cytotoxic T-cell activity [10]. Though costly, it has shown promise in women with repeated ART failures and

immunological abnormalities. As a calcineurin inhibitor, tacrolimus selectively suppresses T-cell activation. Its use in RIF patients with elevated Th1/Th2 ratios has shown encouraging outcomes, though nephrotoxicity and dosage monitoring are critical considerations. Lymphocyte Immune Therapy (LIT) involves injecting paternal or donor lymphocytes into the maternal body to induce immune tolerance. Though controversial and not universally recommended, some clinics report improved implantation in highly selected cases.

Granulocyte Colony-Stimulating Factor (G-CSF) promotes endometrial proliferation and enhances angiogenesis [1,8]. Intrauterine administration has been associated with improved endometrial thickness and clinical pregnancy rates in thin endometrium and RIF cases.

Autologous PRP contains growth factors that aid endometrial regeneration and vascularization [1]. Several small studies show improved implantation and live birth rates in women with thin or non-receptive endometrium.

Endometrial Scratching: This mechanical injury, usually performed in the luteal phase prior to embryo transfer, is thought to trigger a pro-repair and inflammatory cascade that enhances receptivity. Evidence is inconsistent, and recent trials suggest limited benefit in unselected populations [6].

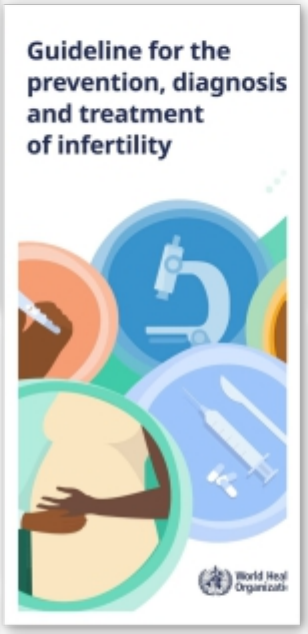
Low-Dose Aspirin (LDA) is used to improve uteroplacental blood flow and reduce micro thrombosis. It may benefit patients with antiphospholipid syndrome or thrombophilia, though its routine use in RIF without thrombophilic disorders remains debated. Low Molecular Weight Heparin (LMWH) like enoxaparin possesses anticoagulant and immunomodulatory effects [3]. Beneficial in women with known thrombophilia or implantation failure, some studies support its use even in women without diagnosed clotting disorders. Pentoxifylline , a methylxanthine derivative improves microcirculation and reduces inflammation. While mainly used in male infertility, emerging data suggest it may benefit endometrial perfusion and receptivity. CoQ10 improves mitochondrial function and oocyte quality. In women with poor ovarian reserve and RIF, CoQ10 supplementation may improve embryo quality and implantation potential. Deficiency in vitamin D is common and associated with poor reproductive outcomes [4]. Supplementation has been linked with improved endometrial receptivity and IVF success in some studies, although data are not uniform. As a potent antioxidant, melatonin enhances oocyte quality and luteal support. Some studies report improved implantation and clinical pregnancy rates, particularly in older women or those with oxidative stress. Omega-3 fatty acids exert anti-inflammatory and vasodilatory effects.

Though evidence in RIF is limited, their general role in improving reproductive and metabolic health supports their use as adjuncts. Intrauterine infusion of autologous stem cells (e.g., bone marrow-derived or menstrual blood-derived) is under investigation for regenerating non-receptive or thin endometrium. Early studies show potential, particularly in cases of Asherman syndrome or refractory thin lining. Vaginal sildenafil citrate enhances endometrial blood flow via vasodilation. Though data are mixed, it may be beneficial in women with persistent thin endometrium or poor perfusion. Growth Hormone (GH) may improve endometrial thickness and modulate implantation pathways. It has shown promise in women with thin endometrium or poor responders, though high cost and limited data constrain its use.

Novel agents targeting specific immune pathways such as anti-TNF agents, IL-6 inhibitors, and

# ISAR Goes Interna







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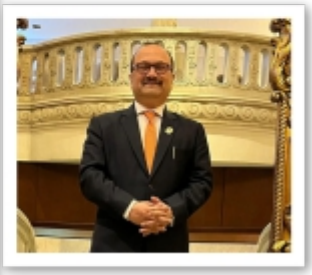






checkpoint inhibitors are under early-phase evaluation[7]. These therapies aim to fine-tune immune responses during implantation and may herald a new era in precision immunomodulation.

Recurrent implantation failure represents a multifaceted barrier to successful conception, often requiring a tailored and multidisciplinary approach. Adjuvant therapies offer hope, especially when guided by individualized assessment of underlying pathophysiology. While some adjuvants like corticosteroids, G-CSF, or PRP are gaining clinical traction, others remain investigational[1,3,8]. The heterogeneity of patient populations and study designs underscores the urgent need for robust, well-controlled clinical trials [2]. As understanding of endometrial biology and immune interactions deepens, adjuvants hold the potential to transform the RIF landscape through targeted, patient-specific interventions.

## 3. Discussion

The growing body of literature on adjuvants in recurrent implantation failure (RIF) reflects increasing interest in overcoming this complex reproductive challenge[2,3]. While several adjuvants have demonstrated potential in improving implantation and pregnancy rates, the strength of current evidence remains variable and often inconclusive. Agents such as corticosteroids, low molecular weight heparin (LMWH), intralipids, G-CSF, PRP, and vitamin D supplementation are supported by small randomized controlled trials (RCTs) and observational studies[1,3,4,5,8]. However, these findings often lack replication in larger, multicentre trials. Notably, many studies suffer from methodological limitations such as small sample sizes, inconsistent definitions of RIF, lack of blinding, and heterogeneous outcome measures. This variability makes it difficult to draw definitive conclusions about efficacy and generalizability.

A significant challenge in this field lies in the absence of a universally accepted definition of RIF [2]. Criteria vary in terms of the number and quality of embryos transferred, number of failed cycles, and age of the patient. This inconsistency complicates patient selection for studies and leads to heterogeneous populations, ultimately limiting comparability across trials. Moreover, diverse protocols in timing, dosing, and delivery methods of adjuvants further contribute to the lack of standardization.

There is also a gap in understanding the underlying aetiology of RIF in individual patients, making empiric use of adjuvants common practice. This "trial-and-error" approach leads to over-treatment or use of costly and invasive therapies without proven benefit. The development of reliable, non-invasive biomarkers for endometrial receptivity and immune profiling is crucial to guide personalized adjuvant therapy. Integration of tools such as endometrial receptivity assays, immune function testing, and microbiome profiling may enable more targeted and effective interventions.

In conclusion, while adjuvants hold promise in managing RIF, widespread clinical use must be tempered by caution due to limited high-quality evidence [2,3]. Future research must focus on refining diagnostic criteria, standardizing treatment protocols, and developing biomarker-guided strategies to optimize patient outcomes.

## 4. Conclusion and Future Directions

Recurrent implantation failure (RIF) is a multifactorial condition, and while medical interventions remain central, growing evidence highlights the importance of lifestyle

modification and a holistic approach in optimizing outcomes. Modifiable factors such as body weight, nutrition, physical activity, and stress have significant influence on reproductive health. Achieving an optimal body mass index (BMI) improves hormonal balance, endometrial receptivity, and oocyte quality. A balanced diet rich in antioxidants, omega-3 fatty acids, and micronutrients supports implantation by reducing oxidative stress and systemic inflammation. Regular moderate exercise enhances insulin sensitivity and cardiovascular health, while excessive or strenuous activity may be detrimental.

Chronic stress and anxiety can disrupt hypothalamic pituitary ovarian axis function and impair implantation. Mind body interventions, including mindfulness, yoga, meditation, and counselling, have shown benefits in reducing stress and improving pregnancy rates in assisted reproduction. Lifestyle interventions such as smoking cessation, moderation of alcohol, and adequate sleep hygiene further contribute to favourable reproductive outcomes.

Adopting a holistic strategy that integrates lifestyle optimization with conventional medical treatments may not only improve endometrial receptivity and embryo uterine synchrony but also enhance overall patient well-being, empowering couples to actively participate in their fertility journey.

Despite encouraging preliminary data, the evidence base for many of these adjuvants is limited by small sample sizes, inconsistent methodologies, and a lack of standardized protocols [2,3]. The heterogeneity in defining RIF and variability in treatment responses underscore the need for a more tailored approach.

Intracrinology or local steroid metabolism also plays an important role in the process of implantation (Figure 1) and should be taken into consideration when we deal with RIF.

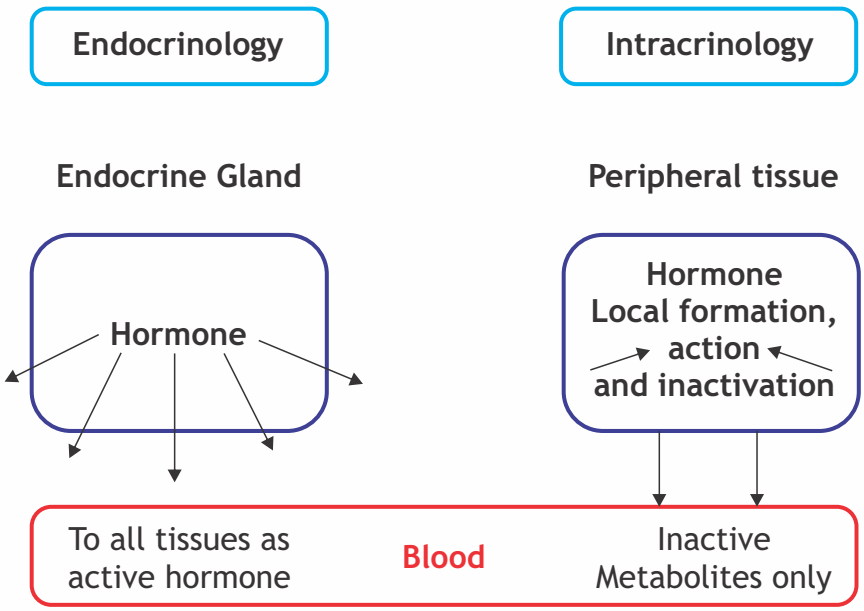


**Figure 1: Intracrinology (Local Steroid Metabolism)**

Ultimately, the future of RIF management lies in the ability to match the right adjuvant to the right patient. By transitioning from empirical treatments to evidence-based, personalized interventions, the promise of improving implantation success and live birth rates becomes a more attainable goal.

**5. Key Messages:**

- Empirical therapies should, whenever possible, be considered only in the setting of carefully conducted clinical trials
- Lack of evidence does not allow incorporation of various treatment modalities in routine clinical practice
- Patients with infertility are particularly vulnerable to trying new treatments in hopes of conceiving
- Properly designed RCTs are therefore needed to clarify which therapy could be beneficial in RIF and RPL treatment
- RIF and RPL patients should be properly informed regarding potential benefits and risks of each therapy used
- While new techniques are the way to progress, proper assessment is a prerequisite before general use
- They should not be marketed prematurely based on inconclusive trials
- Adaptation and personalization of fertility therapy based on individual patient characteristics may help to optimize efficacy and safety outcomes for individual couples

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Indian Society for Assisted Reproduction

# *Immune Regulation at the Maternal-Foetal Interface: Implications for Recurrent Implantation Failure and Pregnancy Loss*


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### Abstract

The maternal-fetal interface represents a unique immunological environment where the maternal immune system must tolerate the semi-allogeneic fetus while retaining the capacity to defend against pathogens. This balance is maintained by complex interactions between trophoblasts, decidual stromal cells, and specialized immune subsets, including uterine natural killer (uNK) cells, macrophages, dendritic cells, and regulatory T cells (Tregs). Dysregulation of these mechanisms has been linked to recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL), major challenges in reproductive medicine. This review synthesizes current evidence on the immunology of early pregnancy, the consequences of aberrant immune responses, and emerging immunomodulatory interventions aimed at restoring tolerance and reproductive success.


### 1. Introduction

Implantation and early pregnancy represent one of the most striking immunological paradoxes: the maternal immune system must recognize and accommodate the semi-allogeneic fetus while simultaneously preserving effective host defence. Far from being a state of generalized immune suppression, pregnancy involves finely tuned immune adaptation at the maternal-fetal interface, orchestrated by decidual immune cells and fetal trophoblasts [1,2]. Aberrations in these processes can contribute to infertility, recurrent implantation failure (RIF), and recurrent pregnancy loss (RPL), all of which impose profound psychological and clinical burdens [3-5]. Understanding the immunological dynamics that underlie reproductive success therefore holds major implications for diagnosis, prevention, and therapy in assisted reproduction and beyond.

### 2. Dynamic Immunological Stages of Pregnancy

Pregnancy can be conceptualized as a sequence of immunological stages, each characterized by specific inflammatory or tolerogenic responses that facilitate implantation, fetal growth, and ultimately parturition.

#### 2.1 Implantation: A Controlled Pro-Inflammatory Event

Implantation resembles a wound-healing response, characterized by localized tissue breakdown, cytokine release, and immune cell infiltration. Pro-inflammatory mediators

including IL-6, IL-15, TNF-α, CXCL10, and CCL2 drive recruitment of immune cells that support trophoblast invasion and vascular remodelling[5,6]. Dendritic cells play a central role in this phase, promoting decidualization and endometrial receptivity. Perturbations in this early pro-inflammatory milieu can result in defective implantation and early pregnancy failure.

### 2.2 Establishment and Maintenance: Transition to Immune Tolerance

Once implantation is achieved, the local immune environment shifts toward anti-inflammatory dominance. Expansion of $FOXP3^+$ Tregs, polarization of macrophages toward an M2 phenotype, and modulation of uNK activity foster tolerance and tissue remodelling [7,8]. Tregs produce IL-10 and TGF-ß, suppressing effector T-cell responses, while uNK cells secrete angiogenic factors such as VEGF and PlGF to facilitate spiral artery remodelling [9]. Dendritic cells adopt a semi-mature phenotype, promoting Treg induction while restraining pro-inflammatory T-cell priming [10].

### 2.3 Parturition: Reinstatement of Inflammation

Toward term, pregnancy transitions back to a pro-inflammatory state, marked by elevated IL-1ß and IL-6, prostaglandin synthesis, and immune cell activation, all of which contribute to cervical ripening and uterine contractions [11]. Premature or dysregulated activation of this inflammatory program underlies complications such as preterm labour.

This cyclical orchestration of inflammation and tolerance underscores the immune system's adaptability throughout pregnancy, and deviations at any stage may precipitate RIF or RPL

## 3. Cellular Mediators of Maternal-Fetal Immune Tolerance

The decidua harbours a unique leukocyte composition optimized for implantation and fetal development, distinct from that of peripheral blood.

### 3.1 Uterine Natural Killer (uNK) Cells

uNK cells constitute the majority of decidual leukocytes in early pregnancy. Unlike peripheral NK cells, they display low cytotoxicity but high production of angiogenic factors and cytokines, enabling vascular remodelling and trophoblast regulation [12]. Their function is modulated by interactions between maternal killer immunoglobulin-like receptors (KIRs) and fetal HLA-C molecules, with certain combinations predisposing to abnormal placentation and pregnancy loss. Single-cell studies have revealed multiple subpopulations of uNKs, suggesting functional heterogeneity with both supportive and potentially deleterious roles [13].

### 3.2 Regulatory T Cells (Tregs)

Tregs, defined by $CD4^+CD25^+FOXP3^+$ expression, are pivotal for tolerance to fetal antigens. They suppress effector T-cell responses, promote trophoblast survival, and regulate local macrophage and NK cell activity. Expansion of Tregs correlates with successful implantation, whereas reduced numbers or impaired function are consistently observed in women with RIF or RPL [15-17]. In murine models, Treg depletion induces fetal resorption, while adoptive transfer rescues implantation [18].

### 3.3 Macrophages

Decidual macrophages constitute 20–25% of immune cells and exhibit plasticity between M1 (pro-inflammatory) and M2 (anti-inflammatory, pro-remodelling) states. During normal pregnancy, M2 dominance supports tissue repair, angiogenesis, and tolerance [19]. Excessive skewing toward M1 polarization, however, is associated with miscarriage, preeclampsia, and impaired trophoblast invasion [20].

### 3.4 Dendritic Cells

Dendritic cells in the decidua adopt a tolerogenic phenotype, characterized by low co-stimulatory molecule expression and high secretion of IL-10 and IDO. They promote Treg induction and cross-talk with NK cells to regulate angiogenesis and trophoblast behaviour [14,21]. Aberrant maturation of dendritic cells shifts the immune milieu toward inflammation, disrupting implantation and placentation.

Collectively, these cellular players cooperate to establish a permissive environment for fetal development. Their dysregulation forms the basis of many immune-mediated reproductive failures.

## 4. The Maternal-Fetal Interface and HLA System

The semi-allogeneic fetus must evade maternal immune attack despite expressing paternal antigens. This is achieved through unique HLA expression by extravillous trophoblasts, which lack highly polymorphic HLA-A and HLA-B, but express HLA-C, HLA-E, HLA-F, and HLA-G[22,23]. HLA-C engages maternal KIR receptors on uNK cells, influencing cytokine secretion and vascular remodelling. Specific KIR/HLA-C combinations, such as maternal KIR AA with fetal HLA-C2, increase the risk of RPL and preeclampsia[24]. HLA-G exerts tolerogenic effects by inhibiting NK and $CD8^+$ T-cell cytotoxicity and promoting regulatory dendritic and macrophage phenotypes[25]. HLA-E interactions with CD94/NKG2 receptors further suppress cytotoxic responses[26]. Disruption of these HLA-mediated pathways whether by excessive parental allele sharing or altered antigen presentation compromises tolerance, resulting in implantation failure or miscarriage [27,28].

## 5. Immune Dysregulation in RIF and RPL

Women with RIF or RPL exhibit consistent patterns of immune disturbance:

1. Pro-inflammatory cytokine excess: Elevated TNF-α, IFN-γ, and IL-1β with reduced IL-10 and TGF-β create a hostile Th1/Th17-dominant environment[29,30].
2. uNK cell alterations: Abnormal numbers or hyperactivation of uNKs impair vascular remodelling. Meta-analyses confirm their pivotal role in RIF and RPL [31].
3. Treg deficiency and instability: Reduced Treg numbers and impaired function, alongside Th17 expansion, are strongly associated with poor outcomes [16,17,32].
4. Macrophage dysfunction: A shift toward M1 polarization, with pro-inflammatory cytokine release and reduced trophoblast support, exacerbates immune imbalance [20,33].
5. Genetic predispositions, including FOXP3 polymorphisms and KIR/HLA mismatches, as well

as chronic endometritis or autoimmune processes, further contribute to these abnormalities [34-36].

### 6. Immunomodulatory Interventions

Given the immunological underpinnings of RIF and RPL, multiple interventions have been explored:

Intralipid therapy: Intravenous lipid emulsions reduce NK cell cytotoxicity and may enhance tolerance. Some studies suggest improved implantation, though evidence remains heterogeneous.

Corticosteroids: Agents such as prednisolone suppress inflammation and promote Treg expansion but carry risks with prolonged use. Clinical outcomes remain mixed.

Granulocyte colony-stimulating factor (G-CSF): G-CSF promotes angiogenesis, endometrial receptivity, and Treg expansion. Trials have reported variable benefits in RIF.

Treg-boosting strategies: Experimental approaches, including low-dose IL-2 therapy and adoptive transfer of *ex vivo* expanded Tregs, hold promise but remain investigational.

These therapies underscore the therapeutic potential of immune modulation but highlight the need for individualized, phenotype-driven strategies.

### 7. Future Perspectives

Emerging technologies such as single-cell RNA sequencing, mass cytometry, and spatial transcriptomics are refining our understanding of immune heterogeneity at the maternal-fetal interface. Stratifying patients based on immune profiles, genetic markers, or placental sex-specific immune responses could enable precision immunotherapy in RIF and RPL. Furthermore, integrating immunological markers into routine reproductive evaluation may improve patient selection for immunomodulatory treatments, reducing unnecessary interventions and enhancing outcomes.

### 8. Conclusion

Pregnancy is sustained by intricate immune adaptations that balance tolerance and defence. Disruption of these mechanisms contributes to recurrent implantation failure and pregnancy loss, conditions of major clinical relevance. Advances in mechanistic understanding are paving the way for immune-based diagnostics and therapeutics. While interventions such as intralipids, corticosteroids, and G-CSF show promise, future progress will depend on personalized approaches that account for genetic, immunological, and sex-specific factors.

### 8. Key points:

- Pregnancy is a state of immunological balance during which the mother and the developing fetus must tolerate each other while maintaining sufficient immunocompetence to ward off potential threat
- Maternal immune adaption to pregnancy, adequate placentation and fetal development and maintenance of fetal immune tolerance are necessary for an normally progressing pregnancy
- Bi-directional regulation of infiltrating fetal cells and maternal immune response is

necessary for a successful pregnancy

- There may be challenges to pregnancy adaption and failure to maintain immunbe tolerance that result in RIF and RPL
- Exogenous factors include stress, medication, infection, exposure to toxiv agents and others while the endogenous factors are age, KIRs and HLA - C mismatch, low progesterone, insufficient memory Treg cells and other unknown factors.
- Pro-inflammatory microenvironment is crucial for normal implantation and parturition, whereas a tolerogenic environment is induced during the course of pregnancy to enable normal placentation and fetal growth
- After delivery, the immunomodulatory effects mediated by fetal antigens and pregnancy hormones disappear, giving rise to T cell activity together with persisting monocyte activity
- Any defect of this elegant and complex process of reciprocal immunological adaptation between the fetus and the mother might potentially translate into an obstetric failure
- New insights into feto-maternal immune cross-talk may help us to understand the pathogenesis of pregnancy complications as well as poor postnatal health.

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*Thymosin alpha in RIF*


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**Abstract**

Recurrent implantation failure (RIF) remains a significant challenge in assisted reproductive technology (ART), affecting approximately 10–15% of couples undergoing such treatments.[1,2]

This review explores the potential of Thymosin Alpha-1 (TA1) as an immunomodulatory adjuvant to improve implantation outcomes in RIF. We discuss RIF definitions, immunological mechanisms, existing clinical evidence including an Indian open-label study and a compelling case report from Chennai and the need for rigorous randomized trials.

Given its favourable safety profile, TA1 may represent a viable therapeutic option warranting further investigation.


## 1. Introduction

Recurrent implantation failure (RIF) is an enigmatic condition characterized by failure to establish a clinical pregnancy despite multiple embryo transfers involving high-quality embryos. Definitions vary but a commonly referenced one describes RIF as failure after transfer of ≥4 good-quality embryos across at least 3 fresh or frozen IVF cycles in women under 40.[1,3] Prevalence estimates range from 10–15% of couples undergoing ART. [1,2] Factors implicated in RIF include uterine anomalies (e.g., fibroids, adhesions), endometrial receptivity, embryo quality, thrombophilia, male factor, endocrine/metabolic disturbances, microbiome issues, and immune dysregulation.

## 2. The Immunological Dimension in RIF

Successful implantation requires a delicate balance between pro-inflammatory (Th1) and anti-inflammatory (Th2/Treg) immune responses. A successful implantation requires a shift from a Th1-dominated inflammatory state vital for early trophoblast invasion to a Th2-dominant anti-inflammatory environment supportive of ongoing pregnancy. In RIF, elevated levels of Th1-associated cytokines (e.g., TNF-α, IFN-γ), reduced Th2 cytokines (e.g., IL-4, IL-10), and decreased regulatory T cells (Tregs) have been documented. These immune disturbances compromise endometrial receptivity during the narrow “window of implantation”. Dysregulation especially Th1 dominance can impede implantation.[4,5,6]

RIF may also be associated with elevated inflammatory cytokines (e.g., TNF-α, IFN-γ) and decreased regulatory factors such as IL-10 and TGF-ß.[4]

Furthermore, a recent study classified RIF endometrial subtypes into immune-driven (RIF-I) and metabolic-driven (RIF-M) patterns, underscoring the heterogeneity of its pathogenesis

**3. Thymosin Alpha-1: Mechanism and Clinical Rationale**

Thymosin Alpha-1 (TA1) is a 28-amino-acid thymic peptide with immunomodulatory properties. It has been used clinically in 35 countries (including India) for decades, especially in viral immunotherapy and to modulate T-cell responses, via subcutaneous administration.

TA1 has demonstrated the ability to increase regulatory T-cells (Tregs) and attenuate pro-inflammatory cytokine production, thereby potentially restoring immune homeostasis favourable for embryo implantation.[4] It also induces interleukin (IL)-2 production, differentiation of immature cord blood lymphocytes, production of B cell growth factors, and increased macrophage antigen presentation efficiency.

Its proposed mechanisms relevant to RIF include: (Figure 1)

a. Promotion of Immune Tolerance - Enhances Treg cell numbers and function, helping to suppress harmful immune responses against the embryo.

b. Cytokine Modulation - Shifts cytokine balance toward an anti-inflammatory profile (increased IL-10, TGF-β; decreased TNF-α, IFN-γ).

c. NK Cell Regulation – Reduces excessive NK cell cytotoxicity without compromising necessary immune defence.

d. Dendritic Cell Maturation – Encourages tolerogenic dendritic cell profiles that favour maternal-foetal immune harmony.

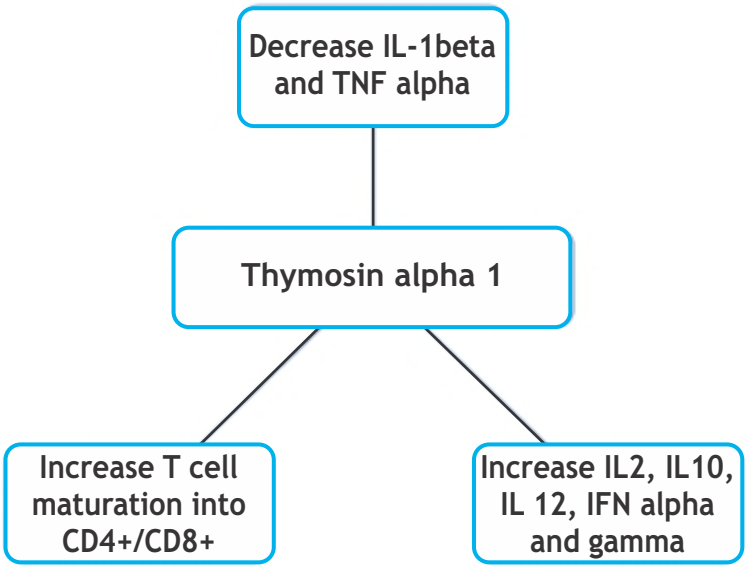


**Figure 1: Mechanism of action of Thymosin alpha on immune system**

RIF is increasingly recognised to have an immune component in a subset of patients including abnormal Treg/Th17 balance, altered NK cell phenotypes, and dysfunctional antigen

presentation in endometrium giving a theoretical window where Tα1 could restore implantation-friendly immune homeostasis. However, RIF is heterogeneous and immune abnormalities are neither universal nor uniformly defined, so patient selection is critical for trials.[1,3]

**4. Summary of clinical evidence to date**

Older work observed lower maternal serum Tα1 levels around conception in pregnancies that subsequently miscarried, suggesting an association between periconceptional Tα1 and early pregnancy maintenance. These findings are correlative and predate modern immuno-phenotyping, but they provide historical biological plausibility.

Recently published case reports and small single-centre reports describe successful pregnancy after adjuvant Tα1 in individual RIF patients or very small cohorts. These reports are hypothesis-generating but uncontrolled and susceptible to selection and publication bias.

Investigators have proposed and presented small randomized open-label pilot study designs of Tα1 as an add-on for frozen embryo transfer (e.g., 20 patients randomized; multi-dose regimens pre- and post-ET). To my knowledge there are proposals and presentations of pilot RCTs but no large, peer-reviewed randomized trials with robust live-birth endpoints published to date. Thus, clinical evidence remains preliminary.

**5. Emerging Clinical Evidence in RIF**

**5.1 Indian Exploratory Study (New Delhi)**

A small open-label study (n=14) conducted at the International Fertility Centre, New Delhi, administered a total of 3.2 mg of Alpha thymosin divided into two doses early in the cycle, with an additional dose before embryo transfer. Resulting outcomes included a positive beta-hCG rate of 64.3%, with 88.9% of these pregnancies demonstrating foetal cardiac activity. However, no significant correlation was observed between outcomes and progesterone levels, patient age, or BMI. The authors highlighted the need for randomized controlled trials due to small sample size and exploratory design.[2]

**5.2 Case Report from Chennai**

A compelling case involved a 32-year-old woman with unexplained RIF (despite multiple failed IVF, surgical interventions, PRP, and various adjuvant therapies). Immune profiling revealed Th1 predominance. She received 13 injections of TA1 (3.2 mg on alternate days, with a final 1.6 mg dose 48 hours prior to embryo transfer) alongside HRT. A single euploid embryo transfer resulted in an ongoing pregnancy at 15 weeks a promising outcome given the previous failures.[4]

**5.3 My own clinical experience**

In this cohort of 53 women undergoing IVF (January–June 2025), median age was 33 years (range 24–48); median marriage duration was 7 years. Cycle attempts included first (25), second (18), third (6), and fourth (2). Pregnancy outcomes were positive in 30 (56.6%); results were pending for 2 patients in patients who were prescribed thymosin alpha. No adverse outcomes were documented, supporting the safety profile of thymosin in this patient population. In the context of RIF/RPL patients, where baseline success rates are typically lower, this improvement is particularly significant.

## 6. Safety Profile

TA1 has a longstanding clinical history since 1979, with over 2000 publications and use across all age groups children (as young as 13 months) to elderly (up to 101 years) without notable adverse effects. Given its endogenous nature and regulatory role, supplementation is not expected to pose foetal risks; nonetheless, safety in pregnancy still requires systematic evaluation.[7]

## 7. Discussion

The immunological basis of implantation failure justifies exploration of TA1 as an adjunct in RIF, especially in immune-dysregulated subsets (e.g., RIF-I). The Indian exploratory data and case reports offer encouraging signals, but sample sizes and study designs limit generalizability.

Emerging molecular subtyping (immune vs metabolic RIF) highlights the importance of personalized approaches; TA1 would likely benefit only the immune-driven phenotype.[6]

ESHRE guidelines recommend that interventions for RIF be grounded in sound biological rationale and robust evidence currently lacking for TA1.[8]

Further, umbrella reviews stress the paucity of high-quality RCTs for interventions in RIF. [9]

## 8. Recommendations and Future Directions

- Recruit large-scale, randomized controlled trials evaluating TA1 (e.g., 3.2 mg regimen) in immune-profiled RIF patients versus placebo.
- Incorporate molecular classification (RIF-I vs RIF-M) into selection and stratification protocols.
- Standardize outcome metrics: implantation rate, clinical pregnancy rate, live birth, immunologic biomarkers.
- Monitor safety rigorously, including maternal and foetal outcomes across gestation.
- Perform comparative analyses against other immunomodulators (e.g., steroids, IVIG, intralipids).
- Cost-effectiveness studies, especially in Indian and low-resource settings.

## 9. Conclusion

Thymosin Alpha-1 presents a biologically plausible and well-tolerated immunomodulatory approach for RIF, especially immune-driven cases. Preliminary Indian data are promising, but robust, personalized RCTs are essential to establish efficacy, safety, and clinical utility. With thoughtful integration of molecular phenotyping and evidence-based protocols, TA1 could emerge as a valuable adjunct in managing the challenging cohort of RIF patients.

Indian Society for Assisted Reproduction

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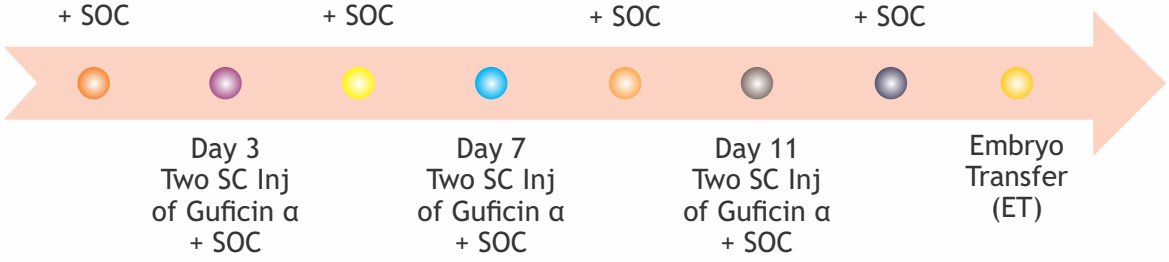


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