

Review

Narrative Review of Multifaceted Approaches to Managing Recurrent Implantation Failure: Insights and Innovations

Xi Luo^{1,2,3,4,5,*}, Zhenlin He^{3,4,5,†}, Rui Ma^{3,4,5,†}, Na Lin^{3,4,5}, Lei Li^{3,4,5}, Yunxiu Li^{3,4,5}, Yang Ke^{1,2,3,4,5}, Xia Meng^{1,2,3,4,5}, Ze Wu^{3,4,5,*}

¹Faculty of Life science and Technology, Kunming University of Science and Technology, 650032 Kunming, Yunnan, China

²Medical School, Kunming University of Science and Technology, 650032 Kunming, Yunnan, China

³Department of Reproductive Medicine, The First People's Hospital of Yunnan Province, 650032 Kunming, Yunnan, China

⁴Reproductive Medical Center of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, 650032 Kunming, Yunnan, China

⁵NHC Key Laboratory of Healthy Birth and Birth Defect Prevention in Western China (Co-building), First People's Hospital of Yunnan Province, 650032 Kunming, Yunnan, China

*Correspondence: luoxi_ypfph@163.com (Xi Luo); wuzes2010@163.com (Ze Wu)

†These authors contributed equally.

Academic Editor: Paolo Ivo Cavoretto

Submitted: 7 January 2024 Revised: 21 February 2024 Accepted: 28 February 2024 Published: 27 March 2024

Abstract

Objective: We aimed to explore the multifaceted etiology of recurrent implantation failure (RIF) and evaluate the efficacy of various management strategies, with a focus on refining examination protocols and treatment pathways to improve implantation success rates in patients undergoing *in vitro* fertilization-embryo transfer. **Mechanism:** The development of RIF can be attributed to a complex causal network of factors. Chromosomal anomalies, such as aneuploidies, directly impact the genetic viability of embryos. Immune system dysregulation, marked by the presence of autoantibodies, disrupts the normal immunological tolerance required for successful implantation. Endocrine disruptions interfere with the hormonal balance essential for preparing the endometrium for implantation. Anatomical irregularities in the female reproductive tract can physically impede the embryo's ability to implant. Lifestyle factors, including diet, stress, and environmental exposures, influence both male and female reproductive health, affecting gamete quality and implantation potential. These diverse factors interact in a multifaceted manner, making a personalized diagnostic and therapeutic approach essential for addressing the specific causes in each case of RIF. **Findings in brief:** The review synthesizes current understanding of RIF's etiology, highlighting the need for innovative interventions and adjustments in clinical practice. It emphasizes the significance of a highly personalized approach in managing RIF, incorporating refined examination protocols and tailor-made treatment pathways to address the unique combination of factors present in each case. **Conclusions:** Effective management of RIF requires innovative interventions and a shift in clinical practice towards personalized care. Identifying gaps in the current understanding of RIF points towards a clear direction for future research, aimed at refining treatment protocols and improving outcomes for patients. This contributes significantly to the broader field of reproductive medicine, aiming to alleviate the clinical and psychological burdens of RIF.

Keywords: recurrent implantation failure; etiology; personalized treatment; diagnostic and therapeutic strategies; clinical outcomes

1. Introduction

Recurrent implantation failure (RIF) is a significant impediment to achieving successful conception for couples undergoing assisted reproductive technology (ART), affecting roughly 10% of *in vitro* fertilization-embryo transfer (IVF-ET) patients globally [1]. This condition not only presents clinical challenges but also imposes substantial emotional and financial stress on affected individuals. The complexity of RIF is broad and multifaceted, with its etiology not fully understood, highlighting the urgent need for ongoing research to explore its underlying causes and develop effective treatment strategies.

The definition of RIF has evolved in response to advancements in *in vitro* fertilization (IVF) technology and clinical understanding. Historically, RIF was defined by the number of unsuccessful IVF cycles or the number of

high-quality embryos transferred without resulting in pregnancy [2]. However, the shift towards single embryo transfer practice to reduce multiple gestation risks has necessitated a reevaluation of RIF criteria. In 2023, the Chinese expert consensus introduced a refined definition that considers both the quantity and the quality, including the developmental potential of transferred embryos [3]. This redefinition reflects the dynamic nature of ART, where evolving technological and clinical insights require adaptable definitions.

Adding to the complexity of defining RIF, major reproductive health organizations such as the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) have established their criteria. ESHRE's guidelines suggest defining RIF as a condition observable only in IVF patients,



characterized by a series of embryo transfers deemed viable failing to result in a positive pregnancy test often enough to warrant further investigation and/or interventions [4]. A key aspect of their recommendation is the adoption of a cumulative predicted chance of implantation greater than 60% as a threshold for initiating further investigation and treatment options. This nuanced approach by ESHRE underscores the importance of individualized care and the necessity for definitions to evolve with technological and clinical advancements.

Understanding the etiology of RIF is crucial for developing personalized treatment strategies. Factors such as immune system anomalies, thrombotic processes, endometrial receptivity, anatomical abnormalities in the reproductive tract, infections, and hormonal imbalances all contribute to the condition. The collective impact of these factors adds to the overall complexity of RIF, necessitating individualized diagnostic and therapeutic approaches.

This review endeavors to explore the complex etiology of RIF, drawing on the latest research and the guidelines provided by ESHRE and ASRM. It aims to synthesize current understandings and management strategies, emphasizing the need for personalized treatment plans. By presenting a detailed examination of RIF's multifaceted aspects and the varied effectiveness of treatment approaches, this paper contributes to the ongoing dialogue on optimizing care for individuals grappling with RIF, setting the stage for future research directions and clinical application.

2. Methods

The narrative review meticulously identifies and selects relevant literature using a method that is both thorough and aligned with scientific principles, ensuring the transparency and reproducibility of findings. We conducted an exhaustive literature search across a variety of databases to encompass a wide range of studies pertinent to RIF.

2.1 Search Strategy

The databases searched include PubMed, MEDLINE, Embase, the Cochrane Library, Google Scholar, Web of Science, and key Chinese databases such as CNKI, Wanfang, and Weipu Database, with the literature search through December 2023. Our search strategy employed a combination of Medical Subject Headings (MeSH) and free-text term variants to ensure comprehensive coverage of the topic.

Additional search terms covered areas related to fertility treatments and outcomes, such as “embryo transfer”, “fertility”, “infertility”, “assisted reproductive technology”, “pregnancy”, “miscarriage”, “implantation”, “intracytoplasmic sperm injection (ICSI)”, and “*in vitro* fertilization”. This extensive and detailed search strategy was designed to be inclusive, and not restricted by language, publication status, or study design. The aim was to provide a comprehensive overview of current knowledge and

emerging insights into managing RIF. Moreover, a manual review of reference lists from identified articles was also conducted to ensure no significant study was overlooked, further broadening the scope of our literature review.

2.2 Inclusion and Exclusion Criteria

Inclusion criteria were established to identify studies that specifically address the multifaceted approaches to managing RIF, including clinical research, reviews, and guidelines that contribute significantly to understanding and treating this condition. Exclusion criteria were applied to omit studies that did not directly relate to RIF, were not within the scope of reproductive medicine, or lacked substantial evidence or relevance to the review's focus areas.

This methodological approach enhances the credibility of our narrative review by providing a clear, reproducible framework for literature selection and analysis. It underscores our commitment to a thorough examination of the multifaceted approaches to managing RIF, contributing valuable insights and innovations to the field.

3. Etiology of RIF

3.1 Biological and Physiological Factors

The endometrium, as the site for embryonic development, is regulated by steroid hormones, notably estrogen and progesterone. These hormones are essential in controlling the growth and differentiation of the endometrium, making the endocrine balance critical for successful embryo implantation.

Progesterone is key in embryo implantation. Studies have shown that patients with RIF have significantly lower progesterone levels during early pregnancy compared to those with normal pregnancies [5]. However, the link between luteal phase defects and implantation failure remains to be conclusively established, highlighting the need for further research in this area. There is debate over the effect of elevated estradiol levels during the artificial preparation of frozen embryo transfer on luteal transformation and embryo implantation, but studies suggest that neither normal nor increased estradiol levels significantly alter the process [6].

Polycystic ovary syndrome (PCOS) patients often exhibit insulin resistance and metabolic abnormalities, including altered blood lipid and glucose levels, which can hinder embryo implantation [7]. Concurrent thyroid dysfunction is also observed in patients with RIF [8,9].

Chromosomal abnormalities in embryos, such as translocations, inversions, insertions, and deletions, are significant contributors to implantation and pregnancy failure. Although more common in RIF patients than in the general population, the incidence of such abnormalities is about 2%, with translocations being the most frequent [10,11]. The incidence of chromosomal abnormalities in embryos increases with age; reports indicate that up to 76% of early pregnancy spontaneous miscarriages are due to these abnormalities [12]. It is recommended to perform high-

resolution chromosomal karyotyping analysis and provide genetic counseling for both partners in RIF cases.

3.2 Endometrial Receptivity and Embryo Implantation

Successful embryo implantation is crucially dependent on the synchronization of the embryo with the uterine endometrium's development, specifically during the optimal "implantation window". Studies demonstrate that approximately 66% of RIF patients experience impaired endometrial receptivity and altered interactions between the embryo and endometrium [13]. Transcriptomic analyses have revealed distinct expression profiles in about 25% of RIF patients, varying throughout the menstrual cycle and potentially affecting the implantation window's timing [14,15]. Furthermore, dysregulation in prostaglandin synthesis may contribute to decreased endometrial receptivity in these patients [16].

The methods used for assessing endometrial receptivity, such as endometrial biopsy and the endometrial receptivity array (ERA), are still under active research. Although endometrial biopsy aids in determining the optimal implantation period, its practicality is restricted due to invasiveness, the delay in results, and its inapplicability during embryo transfer cycles.

3.3 Anatomical Abnormalities and Reproductive Health

Anatomical abnormalities within the reproductive system, including Müllerian duct anomalies, uterine fibroids, adenomyosis, endometrial polyps, intrauterine adhesions, and hydrosalpinx, have significant implications for IVF outcomes [17]. For patients with RIF, the presence of uterine anomalies such as septate or bicornuate uterus should be carefully evaluated. Both partial and complete septate uteri are linked to reduced fertility outcomes, including lower pregnancy rates and increased risks of early miscarriage and preterm birth [18]. Similarly, a bicornuate uterus is associated with higher risks of preterm birth and mid-trimester miscarriage.

Uterine fibroids can lead to deformation of the uterine cavity and adhesions, hindering embryo implantation. Particularly, submucosal fibroids have been found to significantly affect IVF outcomes by impacting implantation and pregnancy rates through various mechanisms, such as increased uterine muscle contractions, abnormal blood vessel formation, and changes in the endocrine environment within the uterine cavity [19]. However, the influence of intramural and subserosal fibroids is relatively minor [20].

Adenomyosis, characterized by an abnormal immune response due to altered sex steroid levels, negatively impacts implantation success [21]. Elevated levels of pro-inflammatory cytokines and increased anti-inflammatory mediators have been observed in patients with adenomyosis, along with a decrease in uterine natural killer (uNK) cell functionality, which is also observed in endometriosis and linked to reduced implantation success [22]. Endometrial polyps and intrauterine adhesions, often

resulting from procedures such as uterine curettage, can obstruct embryo implantation by causing uterine cavity deformities and endometrial damage [23–25]. Furthermore, hydrosalpinx may compromise IVF-ET outcomes due to factors such as insufficient nutrient supply and the presence of inflammatory agents [26].

The female reproductive tract is home to distinct bacterial populations, extending from the vagina to the ovaries [27]. The role of the female reproductive tract's microbiome is also pivotal, with the vaginal microbiota, particularly lactic acid bacteria such as *Lactobacillus crispatus*, *L. jensenii*, *L. iners*, and *L. gasseri*, showing a positive correlation with pregnancy outcomes [28]. A decrease in vaginal lactobacillus and increased microbial alpha diversity have been noted in unexplained RIF patients, contrasting with those who achieved pregnancy after a thaw cycle transplant [29]. Chronic endometritis, associated with alterations in the uterine endometrial microbiota, is predominantly diagnosed through histopathology. Studies have indicated a significant prevalence of chronic endometritis in RIF patients, along with an altered immune status within the endometrium [30,31].

3.4 Immune System and Inflammatory Response

Thrombophilia, indicated by prothrombin time shortening (PTS), significantly influences the management of recurrent spontaneous abortion (RSA) and plays a crucial role in treatments for RIF [32]. The hypercoagulable state associated with PTS can present as early as the first trimester of pregnancy, leading to the formation of microthrombi within the uterine spiral arteries or the villous capillaries. This condition can disrupt maternal-fetal circulation, potentially impairing the process of embryo implantation and consequently, increasing the incidence of RIF. It is important to note that this hypercoagulable state can manifest shortly after embryo implantation, underscoring the intricate relationship between thrombophilia and reproductive challenges [33]. This early occurrence of a hypercoagulable state, even in the initial stages of pregnancy, necessitates tailored management strategies for individuals with PTS, to address the potential for implantation issues and to mitigate the risk of pregnancy failures associated with RIF.

Hereditary PTS, or hereditary thrombophilia, encompasses genetic disorders characterized by defects in anti-coagulant factors or fibrinolytic activity genes, elevating blood clot formation risk. This includes deficiencies in anti-coagulant proteins (protein C, protein S, antithrombin), factor V Leiden mutation, hereditary Hyperhomocysteinemia (Hhcy), and prothrombin gene mutations. While strongly associated with deep vein thrombosis and late pregnancy loss, the link between hereditary PTS and early RSA is less clear. Emerging evidence suggests that ART-related embryo implantation failure may involve trophoblast or chorionic villus vascular injury and reduced nutritive layer invasiveness [34].

Acquired thrombophilia, also known as acquired PTS, encompasses a range of conditions that increase the risk of thrombosis. These conditions include antiphospholipid syndrome (APS), acquired Hhcy, and connective tissue diseases that predispose individuals to thrombosis such as systemic lupus erythematosus (SLE). Other factors contributing to acquired thrombophilia include uncontrolled hypertension, long-standing diabetes, chronic kidney disease, prolonged periods of immobilization, and the use of hormone replacement therapy. Notably, ART is a significant risk factor for acquired PTS, increasing venous thromboembolism risk [35]. While a definitive link between recurrent early implantation failure and acquired thrombophilia has not been established, ART heightens thrombotic event likelihood, adversely affecting embryo implantation. Comprehensive coagulation status assessment in fully informed patients is recommended.

The immunomodulatory mechanisms in embryo implantation are complex and diverse, forming a focal point in both basic and clinical medicine research. Decidual stromal cells in the endometrium are pivotal in regulating trophoblast cell invasion and local maternal immune response suppression, both crucial for successful implantation [36]. Imbalanced immune responses can lead to implantation failure. Immune factors in pregnancy failure include autoimmune and homologous immune factors.

Autoimmune abnormalities in RIF patients, characterized by the presence of tissue-specific or non-specific autoantibodies, can indicate underlying autoimmune diseases such as APS and SLE [37]. These autoantibodies may directly contribute to reproductive failures by impairing implantation processes or by inducing an inflammatory environment detrimental to embryo development. These include anti-sperm antibodies (ASA), anti-endometrial antibodies (AEA), anti-ovarian antibodies (AOA), anti-thyroid antibodies (ATA), antiphospholipid antibodies (APL), antinuclear antibodies (ANA), and anti-DNA antibodies. Concurrent autoimmune diseases are common and include APS, SLE, undifferentiated connective tissue disease (UCTD), sjögren's syndrome (SS), rheumatoid arthritis (RA), and systemic sclerosis (SSc). These conditions can often coexist, indicating a complex interplay of autoimmune mechanisms within an individual, affecting multiple organ systems and leading to a variety of clinical manifestations. Profiling for ANA and APL is crucial in the clinical management of RIF patients with suspected autoimmune etiologies [38]. ANA profiling helps identify underlying systemic autoimmune conditions, such as SLE, that may affect fertility. Similarly, APL detection is pivotal for diagnosing APS, a condition directly linked to thrombotic events and miscarriage. These profiles not only assist in the initial diagnosis but also guide treatment strategies by identifying specific autoimmune targets and evaluating the efficacy of immunosuppressive therapies. Autoimmune diseases such as SLE, SSc, and RA impair

fertility through multiple mechanisms [39]. For instance, prolonged treatment may lead to advanced maternal age, a known risk factor for reduced fertility. Gonadotoxic drugs used in managing these conditions can cause ovarian failure, while the diseases themselves can lead to reproductive endocrine dysfunction. Furthermore, the hyperactive immune response, characterized by the production of autoantibodies, can interfere with the establishment of maternal-fetal immune tolerance, increasing the risk of infertility and miscarriage. Failure in establishing maternal-fetal immune tolerance contributes to infertility and miscarriage risk, underscoring the complex role of immune system dysregulation in reproductive failures [40].

APS, characterized by elevated APL, manifests in obstetrics as thrombosis, recurrent pregnancy loss, intrauterine growth restriction, stillbirth, severe preeclampsia, placental insufficiency, and infertility. It is a primary treatable condition linked to autoimmune-related pregnancy loss [41]. Anti-phosphatidylserine/prothrombin (aPS/PT) antibodies are promising markers for APS. Immunoglobulin G (IgG) type aPS/PT antibodies and IgG type anti- $\beta 2$ glycoprotein I ($\beta 2$ -GP1) domain 1 antibodies exhibit superior diagnostic and prognostic value in APS. Their presence not only aids in accurately diagnosing APS but also in predicting the clinical outcomes and guiding the management of patients with this autoimmune condition. The relationship between atypical antiphospholipid antibodies and recurrent pathological pregnancies is significant, but no randomized controlled trials (RCTs) have explored APS's role in RIF. Women with autoimmune diseases like SLE, UCTD, RA, and SSc face increased RIF risk [42–44].

In the context of RIF, the placenta-specific protein encoded by the *placenta enriched 1 (PLAC1)* gene, predominantly expressed in trophoblast cells, plays a pivotal role in the embryo implantation process. Autoantibodies generated by the immune system against this protein, particularly targeting specific regions, may impede successful embryo implantation. Comparative studies have demonstrated a significant elevation in the levels of these autoantibodies in patients experiencing RIF compared to healthy women of reproductive age who have not undergone implantation failures. This observation underscores the importance of assessing PLAC1-related immune responses in patients with RIF and may guide therapeutic strategies aimed at mitigating immune-mediated barriers to implantation [45].

Imbalances in maternal-fetal immunity, characterized by abnormalities in the numbers, functions, and interactions of immune cells at the maternal-fetal interface, are associated with RIF [46]. These immune cells include natural killer (NK) cells, T cells, macrophages, myeloid-derived suppressor cells (MDSCs), decidual stromal cells (DSCs), and trophoblast cells. Disruptions in the equilibrium of these cell types can impede the establishment of a conducive environment for embryo implantation, highlighting the complexity of immune system involvement

in successful pregnancy outcomes. Alterations in the expression patterns of specific immune cells or associated mediators, including cytokines and chemokines, may contribute to RIF, yet the precise mechanisms underlying these changes remain an active area of investigation [47]. NK cells, abundant around uterine trophoblasts during early pregnancy, may regulate physiological trophoblast invasion, immune tolerance, embryonic development, and various immune/metabolic pathways [48]. Patients with RIF exhibit increased levels of pro-inflammatory cytokines, including interferon-gamma (IFN- γ), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and interleukin-4 (IL-4), alongside decreased levels of the anti-inflammatory cytokine transforming growth factor-beta 1 (TGF- β 1). This imbalance between pro-inflammatory and anti-inflammatory cytokines may contribute to the pathophysiology of RIF, highlighting the importance of cytokine regulation in maintaining a conducive environment for embryo implantation [49]. Studies on endometrial cytokines prior to implantation have revealed that there are positive correlations between Interferon gamma-induced protein 10 (IP-10) and tumor necrosis factor-alpha (TNF- α) with implantation success and clinical outcomes. Conversely, monocyte chemoattractant protein-1 (MCP-1) and IL-1 β demonstrate negative correlations with these parameters. These findings underscore the complex roles that specific cytokines play in the endometrial environment, influencing the likelihood of implantation success and subsequent clinical outcomes [50].

3.5 External Influences and Lifestyle Factors

Maternal age significantly influences embryo quality in IVF procedures [51]. Studies have demonstrated a correlation between increased maternal age and a higher incidence of non-diploid embryos, which adversely affects embryo quality and increases implantation failure rates [52]. Specifically, IVF success rates for women aged ≥ 35 are lower than those for women aged < 35 [53].

Studies show that in IVF treatments, both age and body mass index (BMI) indicators independently reduce success rates. Specifically, women aged over 40 and those with elevated BMI face increased challenges, notably affecting the effectiveness and leading to conditions like RIF [54,55]. An elevated BMI, particularly above 30 kg/m², is associated with lower embryo implantation rates in IVF procedures. This decrease is likely due to obesity-induced changes in endometrial receptivity and follicular function [56,57].

The smoking history of both partners is also a significant factor in RIF [58]. In female patients with over five years of smoking history, negative impacts include reduced egg retrieval, increased cycle cancellation rates, and altered ovarian response to stimulation [59]. This effect is primarily due to cigarette toxins interfering with estradiol production during the follicular phase, impacting corpus luteum formation and embryo implantation [60,61]. Further-

more, high serum cotinine levels in female smokers correlate with fewer retrieved eggs and reduced availability of high-quality embryos, consequently increasing the likelihood of negative pregnancy outcomes [62]. In males, smoking leads to increased reactive oxygen species in seminal plasma, altered sperm microRNA expression, and increased sperm DNA fragmentation, all contributing to diminished sperm quality [63].

3.6 Partner-Related Factors

The understanding of RIF has expanded to encompass the significant role of paternal factors alongside maternal contributions. Among these, paternal thrombophilia, particularly the M2/ANXA5 haplotype, has garnered attention for its potential impact on RIF [64]. This genetic predisposition to increased clot formation, more prevalent in fathers of RIF patients, suggests its influence on key reproductive processes including placental development and embryo-maternal interactions. Such insights into paternal genetic factors urge a comprehensive evaluation in the context of RIF.

While the genetic aspects draw considerable focus, the broader spectrum of male fertility, such as sperm quality, has also been under investigation for its role in RIF [65]. Emerging research has explored the relationship between suboptimal sperm quality and its effects on embryo development and early placental function. However, the link between specific male fertility issues, like sperm DNA fragmentation, and RIF outcomes remains ambiguous. A notable study involving a small patient cohort indicated no significant correlation between sperm DNA fragmentation index and RIF outcomes [66]. This finding suggests that, despite the intuitive connection between sperm quality and reproductive success, the direct association of certain male fertility variables with RIF is not definitively supported by the current body of research.

Given this landscape, the routine assessment of sperm DNA fragmentation index as a predictive measure for RIF is currently not recommended [67]. This stance reflects a cautious approach, acknowledging the complex and multifactorial nature of RIF, where the interplay of numerous paternal and maternal factors contributes to the condition. It underscores the necessity for further research to unravel the intricate dynamics between male fertility issues and RIF, aiming to enhance the understanding and management of this challenging condition.

In conclusion, the exploration of partner-related factors in RIF highlights the importance of considering both paternal genetic predispositions, such as the M2/ANXA5 haplotype, and broader aspects of male fertility in the comprehensive assessment and management of RIF. The nuanced interplay between these factors and their impact on successful implantation and pregnancy emphasizes the need for ongoing research to clarify their roles and inform effective interventions.

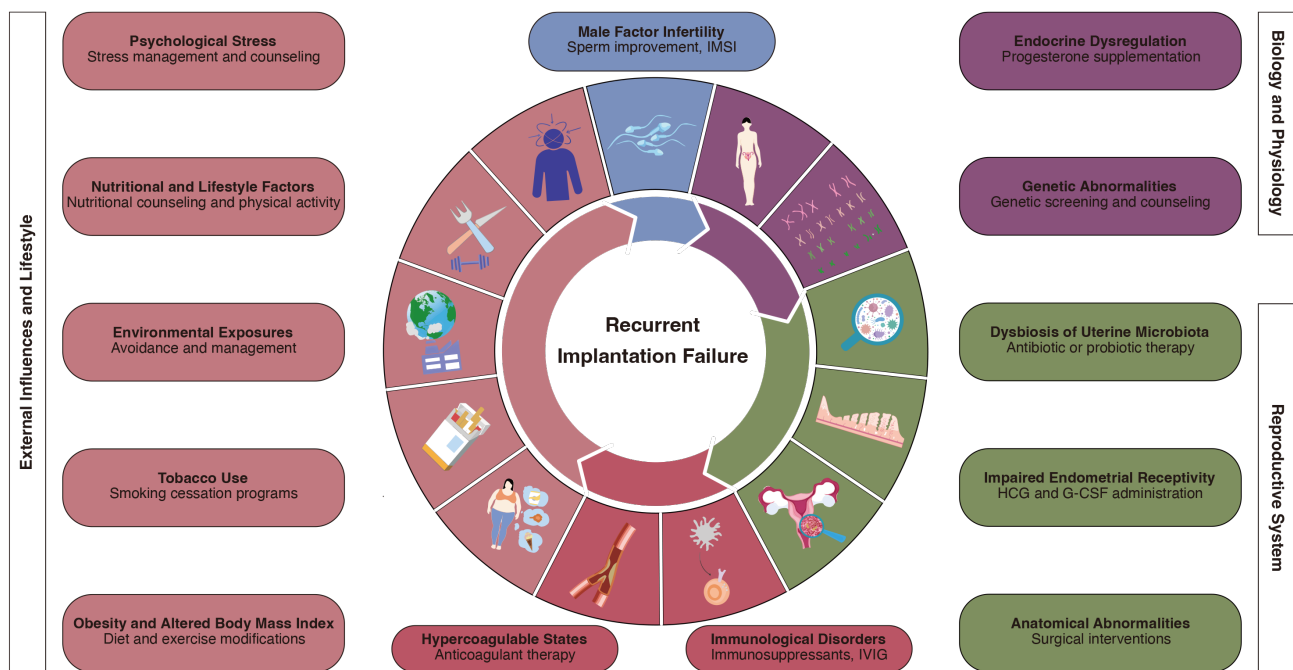


Fig. 1. Graphical overview of recurrent implantation failure (RIF) etiology and treatment approaches. IMSI, intracytoplasmic morphologically selected sperm injection; IVIG, intravenous immunoglobulin; hCG, human chorionic gonadotropin; G-CSF, granulocyte colony-stimulating factor.

4. Advanced Approaches in RIF Management

In summarizing the various factors contributing to RIF and their corresponding optimized treatment approaches, Table 1 (Ref. [7,9,12,13,18,28,40,51,58,61,66,68–70]) and Fig. 1 provide comprehensive overviews. Table 1 serves as a quick reference, detailing the etiology, clinical profile factors, and recommended treatment strategies for each identified cause of RIF. Complementing this, Fig. 1 offers a graphical representation of these elements, visually demonstrating the complex interplay between the etiology and treatment of RIF. Together, these resources emphasize the necessity for individualized treatment plans, catering to the multi-faceted nature of RIF.

4.1 General & Drug Therapy

Couples are advised to regulate their weight, adhere to a nutritious diet, maintain consistent sleep patterns, participate in suitable physical activities, abstain from smoking, moderate alcohol intake, focus on psychological well-being, and pursue psychological support when warranted [58]. Multidisciplinary consultation is recommended for patients with comorbidities to effectively manage their health conditions.

The inclusion of progesterone support is critical in IVF protocols. Saccone G *et al.* [71] systematic review and meta-analysis highlighted the substantial benefits of progesterone in early pregnancy, particularly for women with recurrent pregnancy loss. Dydrogesterone, especially when

combined with ART, outperforms other progesterone types. A comparison of oral desogestrel and vaginal medications revealed that the former is associated with fewer side effects and greater patient compliance [72]. Thus, the integration of progesterone, especially oral gestrol into IVF protocols, is pivotal in enhancing pregnancy and live birth rates among RIF patients, particularly during the luteal phase of treatment. Nonetheless, additional research is required to confirm these results.

4.2 Reproductive & Antimicrobial Treatment

In managing RIF, effective intervention in chronic endometritis (CE) and abnormalities of reproductive anatomy are crucial. Patients diagnosed with CE through hysteroscopy and culture benefit significantly from antibiotic treatment, which has shown efficacy in resolving the majority of infections and thus improving success rates in future IVF cycles [68]. Patients experiencing pathogen clearance post-antimicrobial treatment exhibit markedly higher clinical pregnancy and live birth rates compared to those with persistent infections [73]. Recommended empirical antibiotic treatments include doxycycline (100 mg, twice daily, orally for 14 days) and a combination of levofloxacin (400 mg) with metronidazole (500 mg, once daily, orally for 14 days). Ciprofloxacin (500 mg, twice daily, orally for 10 days) is often prescribed for gram-negative bacteria, while amoxicillin-clavulanate (1 g, twice daily, orally for 8 days) targets gram-positive bacteria. In cases of persistent CE, minocycline (100 mg, twice daily, orally for 12 days) is administered. Current advancements, including non-invasive

Table 1. Comprehensive fertility treatment strategies and corresponding etiologies.

Etiology	Clinical profile factors	Optimized treatment	Reference
General health risks	Advanced maternal age (≥ 35 years)	Oocyte cryopreservation, personalized controlled ovarian stimulation, enhanced embryo selection, and oocyte donation are advanced fertility treatments aimed at preserving eggs, stimulating optimal egg production, selecting the best embryos, and utilizing donor eggs for assisted reproduction, respectively.	Ubaldi FM <i>et al.</i> (2019) [51].
	Obesity (BMI > 30 kg/m ²)	Non-pharmacological interventions: weight management and nutritional supplementation—lifestyle changes including diet and exercise to optimize body weight and nutritional status.	Collins GG <i>et al.</i> (2015) [58].
	Long-term smoking history (≥ 5 years)	Lifestyle modification—changes in daily habits to improve overall health.	Collins GG <i>et al.</i> (2015) [58].
	Inappropriate endometrial receptivity	Intrauterine hCG, PRP, PBMCs, G-CSF infusions, and ERA—a combination of treatments involving hormones, plasma, cells, and endometrial receptivity analysis to enhance implantation success.	Craciunas L <i>et al.</i> (2019) [13].
Endocrine system disorders	Genetic factors in fertility	PGT-A—preimplantation genetic testing for aneuploidies, a technique to assess embryos for chromosomal normalcy before transfer.	Sciorio R <i>et al.</i> (2020) [12].
	Corpus luteum insufficiency	Additional progesterone supplementation—use of progesterone to support the uterine lining and encourage implantation.	Duncan WC (2021) [61].
	Thyroid disorders impacting fertility	Levothyroxine supplementation for hypothyroidism—hormonal supplementation to treat underactive thyroid, which can affect fertility.	Velkeniers B <i>et al.</i> (2013) [9].
	Insulin resistance and hyperinsulinemia	Insulin-sensitizing agents: metformin and troglitazone—medications to improve insulin sensitivity.	Sakumoto T <i>et al.</i> (2010) [7].
Reproductive microbiome environment	Reproductive tract dysbiosis	Administration of antibiotics or probiotics—use of antibiotics to treat infections and probiotics to balance the microbiome.	Han Y <i>et al.</i> (2021) [28].
	Chronic endometritis as a fertility factor	Antibiotics therapy—specific antibiotic treatments to address bacterial infections that could hinder implantation.	Cheng X <i>et al.</i> (2022) [68].
Anatomical factors in fertility	Anatomical fertility impediments: müllerian duct malformations, uterine fibroids, adenomyosis, endometrial polyps, intrauterine adhesions, hydrosalpinx	Hysteroscopy, surgical intervention—diagnostic and corrective surgery to examine and treat uterine abnormalities.	Chan YY <i>et al.</i> (2011) [18].
Prothrombotic conditions	Abnormal thrombophilia screening outcomes	LMWH treatment—low molecular weight heparin therapy to reduce the risk of blood clots, which can affect implantation.	Nelson SM <i>et al.</i> (2008) [69].
Immunological factors affecting fertility	Autoimmune diseases affecting fertility: APS, SLE, UCTD, SS, RA, SSc	Aspirin, low-dose unfluorinated glucocorticoids, hydroxychloroquine, sulfamazine, tacrolimus, and cyclosporine, supplemented with low molecular weight heparin—a regimen of medications to manage inflammation and immune response, alongside blood-thinning agents.	Andreoli L <i>et al.</i> (2017) [40].
	Maternal-fetal immunological imbalance	IVIG or plasma exchange therapy—intravenous immunoglobulin or plasma exchange procedures to modify immune system activity.	Abdolmohammadi-Vahid S <i>et al.</i> (2019) [70].
Male reproductive factors	Poor sperm quality	IMSI—intracytoplasmic morphologically selected sperm injection, a technique to select the best sperm for injection into an egg.	Coughlan C <i>et al.</i> (2015) [66].

BMI, body mass index; hCG, human chorionic gonadotropin; PRP, platelet-rich plasma; PBMCs, peripheral blood mononuclear cells; G-CSF, granulocyte colony-stimulating factor; ERA, endometrial receptivity array; PGT-A, preimplantation genetic testing for aneuploidy; LMWH, low molecular weight heparin; APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease; SS, sjögren's syndrome; RA, rheumatoid arthritis; SSc, systemic sclerosis; IVIG, intravenous immunoglobulin; IMSI, intracytoplasmic morphologically selected sperm injection.

techniques like sequencing and quantitative polymerase chain reaction (PCR) detection of endometrial fluid, are increasingly employed for diagnosing CE, enabling more precise guidance on antibiotic therapy [74].

Concurrently, addressing submucosal fibroids, uterine polyps, intrauterine adhesions, and hydrosalpinx, frequently associated with RIF, is essential. Hysteroscopy is the preferred method for evaluating these anatomical issues. Patients with submucosal fibroids generally have lower implantation and live birth rates. Treatment considerations for these fibroids, especially during hysteroscopic myomectomy, involve assessing the size of the fibroid and its impact on the uterine cavity [75]. Removing endometrial polyps has been shown to significantly increase clinical pregnancy rates in intrauterine insemination [76]. Although the impact of intrauterine adhesion separation on implantation rates is not definitively established, severe adhesions are known to negatively influence pregnancy outcomes [77]. Postoperative administration of estrogen and progesterone aids in endometrial growth and prevents re-adhesion in patients with severe intrauterine adhesions. For moderate to severe hydrosalpinx, surgical interventions such as tubal resection or occlusion are shown to enhance embryo implantation success in IVF [78]. Notably, patients with bilateral hydrosalpinx undergoing tubal resection demonstrate significant improvements in clinical pregnancy and live birth rates in subsequent IVF cycles [79,80].

4.3 IVF & Endometrial Optimization

In the management of RIF, optimizing IVF protocols and enhancing endometrial receptivity are integral. Selecting a tailored controlled ovarian hyperstimulation protocol is essential in RIF treatment, involving decisions on the optimal stimulation regimen, evaluating the necessity for pre-treatment, determining the appropriate starting dose, timing and method for trigger administration, and devising effective embryo transfer strategies. Additionally, special attention should be given to luteal phase support. It's noteworthy that high-dose gonadotropin may increase the risk of early embryonic aneuploidy and reduce successful implantation rates [81]. Assisted hatching (AH), which involves thinning of the zona pellucida using chemical, mechanical, or laser methods, may improve outcomes for patients over 38 years old [82]. However, a rigorous RCT of 796 RIF patients showed no significant difference in clinical pregnancy rates post-AH [83].

The selection of ovarian stimulation protocols directly impacts the number and quality of retrieved oocytes and embryos, thus influencing reproductive outcomes. Individualized treatment plans for RIF patients should be based on their medical history and previous ovarian stimulation outcomes. In scenarios where a sufficient number of embryos are available, employing frozen-thawed blastocyst transfer is recommended [84]. Sequential embryo transfer has been

shown to improve endometrial receptivity [85], though the associated risks of multiple pregnancies should not be overlooked. Given the mixed results regarding the efficacy of AH in RIF, cautious application is advised.

In the realm of RIF, alterations in endometrial receptivity play a crucial role, with innovative therapeutic approaches like intrauterine infusion emerging to address this challenge [86]. This technique involves administering agents such as human chorionic gonadotropin (hCG), autologous platelet-rich plasma (PRP), peripheral blood mononuclear cells (PBMCs), and granulocyte colony-stimulating factor (G-CSF) directly into the uterus, aiming to enhance its receptivity [87–90]. The underlying principle of intrauterine infusion focuses on modifying the endometrial environment to foster conditions favorable for implantation by promoting endometrial regeneration, thickening, and enhancing immunological tolerance at the maternal-fetal interface.

For instance, hCG is thought to stimulate the secretion of growth factors essential for successful implantation, while PRP's growth factors may regenerate and repair the endometrium. Similarly, PBMCs could modulate the immune environment to support implantation, and G-CSF might improve endometrial thickness and quality. Despite the potential of hCG intrauterine infusion to increase embryo implantation rates in infertility cases, its efficacy in RIF patients is under debate, largely due to the lack of standardized protocols regarding dosage and infusion duration [91,92].

The effectiveness of intrauterine infusions in RIF, therefore, remains a controversial topic, underscoring the need for further research to establish standardized treatment protocols. Such research would elucidate the mechanisms of action of these treatments and refine their clinical application, potentially offering new avenues for overcoming RIF.

4.4 Complex Therapies

In the treatment of RIF, the use of antithrombotic therapy and immunotherapy plays a crucial role, particularly for women with predispositions to certain conditions. Women undergoing ART-assisted reproduction with a tendency for thrombosis may benefit from low molecular weight heparin (LMWH) therapy under fully informed consent, as LMWH has shown to reduce trophoblast apoptosis, promote angiogenesis and trophoblast invasion, thereby improving embryo implantation success rate and live birth rate [69,93,94].

Immune dysfunction, categorized into autoimmune and alloimmune factors, significantly contributes to RIF. For women with rheumatic immune diseases, such as systemic lupus erythematosus, adherence to guidelines, including routine low-dose aspirin and specific immunosuppressive agents during pregnancy, is essential. Tacrolimus, in particular, shows promise in managing RIF resulting from immune dysregulation [95]. In treating RIF-associated an-

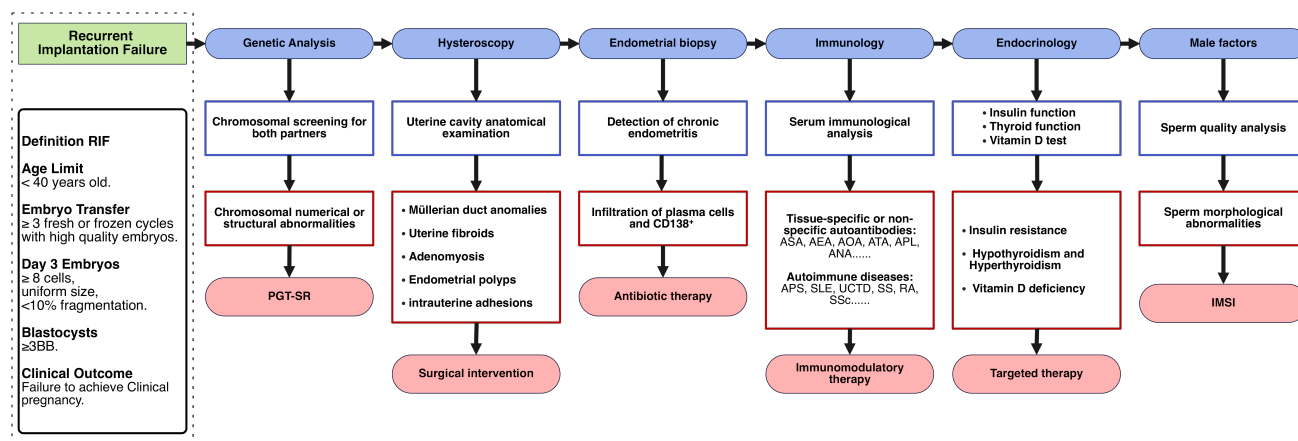


Fig. 2. Diagnostic and treatment process flowchart for recurrent implantation failure patients. PGT-SR, preimplantation genetic testing-structural rearrangements; ASA, anti-sperm antibodies; AEA, anti-endometrial antibodies; AOA, anti-ovarian antibodies; ATA, anti-thyroid antibodies; APL, antiphospholipid antibodies; ANA, antinuclear antibodies; APS, antiphospholipid syndrome; SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease; SS, sjögren's syndrome; RA, rheumatoid arthritis; SSc, systemic sclerosis.

antiphospholipid syndrome, an approach similar to obstetric antiphospholipid syndrome is applied, involving low-dose aspirin and LMWH, with hydroxychloroquine recommended for more complex cases. Secondary antiphospholipid syndrome requires addressing the underlying primary disease.

The role of uNK in facilitating normal trophoblast invasion and the formation of the placenta is crucial. Treatments such as intravenous immunoglobulin (IVIG) are known to enhance the function of regulatory T cells (Tregs) and reduce the cytotoxicity of T helper 1 (Th1) cells, proving to be effective in cases of RIF. This approach highlights the significance of modulating immune cell interactions within the uterine environment to support successful implantation and pregnancy outcomes [70,96–99]. However, treatments such as Intralipid, a 20% fat emulsion, have shown mixed results, with a 2019 study reporting no significant improvement in outcomes following its administration [100]. The use of PBMCs infusion, based on enhancing embryo implantation through locally implanted immune cells, has demonstrated varied clinical outcomes [101–103]. G-CSF, important for neutrophil proliferation and differentiation, has been associated with increased implantation rates when used intrauterine [104]. Despite the potential benefits of these treatments, due to limitations in randomized controlled trial data, small sample sizes, and study heterogeneity, routine clinical implementation of immunotherapy in RIF is not recommended without conducting standardized clinical trials with informed patient consent.

Genetic screening is advised for RIF couples with identified chromosomal anomalies, as chromosomal irregularities in embryos are a significant factor contributing to RIF. While preimplantation genetic testing for aneuploidy (PGT-A) can notably enhance implantation rates in cases

where both partners have normal chromosomes [105,106], its use in RIF remains a subject of debate. This debate centers not only on the challenges in identifying mosaic embryos and the risk of false positives but also on the recognition that PGT-A, despite proving embryos to be euploid, cannot guarantee successful implantation [107]. Furthermore, it is imperative to consider the potential adverse effects associated with the embryo biopsy procedure utilized in PGT-A. Alteri *A et al.* [108] underscore the lack of definitive evidence fully addressing the potential obstetric, neonatal, or long-term consequences of embryo biopsy. While the evidence is limited and subject to controversy, there are indications that embryo biopsy at the cleavage stage may be associated with an increased risk of low birth weight and small for gestational age neonates compared to infants born from non-biopsied embryos. An increase in preterm deliveries and birth defects has also been suggested in cases of trophectoderm biopsy. For both cleavage and blastocyst stage biopsies, an elevated risk for hypertensive disorders of pregnancy was observed. However, these associations may be confounded by other embryo manipulation procedures or inherent patient or population characteristics. Given the insufficient evidence to assess the obstetric, neonatal, and long-term health outcomes following embryo biopsy, the development of invasive preimplantation genetic testing (PGT) strategies should proceed with caution. The pursuit of non-invasive methods, based on the analysis of embryo cell-free DNA, is advocated to circumvent the potential limitations and risks associated with embryo biopsy procedures.

4.5 Male Factor Treatment

In the context of RIF, male factors, especially sperm quality, play a crucial role in the early development of the

embryo and the success of implantation. Intracytoplasmic morphologically selected sperm injection (IMSI), a technique that involves the selection of high-quality sperm for injection into the oocyte, has been demonstrated to significantly enhance implantation, clinical pregnancy, and live birth rates in patients experiencing RIF. This method underscores the importance of sperm quality in reproductive success and offers a targeted approach to overcome certain male factor infertility issues contributing to implantation failures [109]. However, the criteria for sperm selection in IMSI remain under debate, and there is a lack of conclusive evidence supporting its advantages [110]. As such, this area necessitates further research to establish standardized criteria and validate its efficacy.

Building upon the comprehensive exploration of treatment strategies in previous sections, we present a vital tool for clinical application. Fig. 2, titled ‘Diagnostic and Treatment Process Flowchart for Recurrent Implantation Failure Patients’, offers a succinct and structured pathway for diagnosing and treating RIF. This flowchart is designed to assist clinicians in navigating the complexity of RIF management, ensuring a streamlined and effective approach to patient care. It delineates a step-by-step process, from initial patient consultation through to specific therapeutic interventions, optimizing the clinical decision-making process.

5. Conclusions

In addressing RIF, it is paramount to understand the patient’s comprehensive medical history. Chromosomal abnormalities in embryos are a prevalent cause of implantation failure, necessitating investigations into endometrial factors such as injury, adhesion, inflammation, or abnormal hyperplasia. Additionally, conditions like thromboembolic predispositions and immune-related disorders, including antiphospholipid syndrome, require targeted interventions. The emerging focus on endometrial receptivity testing and reproductive tract microbiome analysis offers personalized treatment insights, though these methods still demand further validation.

Considering the complexity of RIF’s etiology, a multi-pronged treatment approach is essential. Clinicians must continuously refine their strategies based on the latest research to enhance treatment efficacy, aiming to increase the chances of successful pregnancies for RIF patients. The intricate nature of RIF’s causes underscores the need for future treatments to incorporate multi-faceted diagnostic approaches, tailoring individualized plans for patients. While advancements in diagnostic technologies show promise, further studies are necessary to validate their effectiveness and precision in understanding the mechanisms behind potential pathogenic factors.

RIF remains a multi-etiological and heterogeneous disorder. The challenge in achieving a unified understanding of its etiology lies partly in the difficulties of pinpointing specific causes in patients and partly in the lack of high-

quality, evidence-based diagnostic methods. As research progresses, our comprehension and management of RIF are expected to evolve, becoming more precise and effective. This advancement is critical for the field of reproductive medicine and offers hope to those grappling with this complex condition.

Abbreviations

AEA, anti-endometrial antibodies; AH, assisted hatching; AOA, anti-ovarian antibodies; aPS/PT, antiphospholipid/prothrombin complex antibodies; APS, antiphospholipid syndrome; APL, anti-phospholipid antibodies; ART, assisted reproductive technologies; ASA, anti-sperm antibodies; ATA, anti-thyroid antibodies; BMI, body mass index; CE, chronic endometritis; ERA, endometrial receptivity array; G-CSF, granulocyte colony-stimulating factor; hCG, human chorionic gonadotropin; Hhcy, hyperhomocysteinemia; IMSI, intracytoplasmic morphologically-selected sperm injection; IVF, *in vitro* fertilization; IVIG, intravenous immunoglobulin; LMWH, low molecular weight heparin; PCOS, polycystic ovary syndrome; PBMCs, peripheral blood mononuclear cells; PGT-A, preimplantation genetic testing for aneuploidy; PRP, platelet-rich plasma; PTS, prethrombotic status; RA, rheumatoid arthritis; RCT, randomized controlled trial; RIF, recurrent implantation failure; RSA, recurrent spontaneous abortion; SS, sjögren’s syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; uNK, uterine natural killer cells; UCTD, undifferentiated connective tissue disease.

Author Contributions

ZH and RM were the primary analysts for this review. They conducted comprehensive literature searches and synthesized the findings, and were responsible for drafting the initial manuscript. NL, LL and YL, as the lead clinicians for recurrent implantation failure, provided valuable insights that significantly shaped the core content of this review. YK and XM contributed extensively to the organization and compilation of research for this study. ZW and XL contributed to the conceptualization and oversight of the review. XL also significantly revised and approved the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

We extend my sincere gratitude to the team at the Department of Reproductive Medicine, the First People’s Hospital of Yunnan Province, for their invaluable clinical insights into recurrent implantation failure. Their contribu-

tions, encompassing a wealth of practical experiences and expertise, have been instrumental in shaping the perspectives and methodologies employed in this review. Their guidance in interpreting clinical data and refining research approaches significantly enhanced the rigor and depth of this study.

Funding

This review was supported by grants from the National Natural Science Foundation of China (82260292, 82360305); the Open Project of Yunnan Provincial Reproductive and Obstetrics and Gynecology Clinical Medicine Center (2022LCZXKF-SZ16); the Open Project of Yunnan Provincial Key Specialty of Gynecology (2022FKZDZK-13); the Social development projects of Yunnan Province (202302AA310044); the Reproductive Obstetrics and Gynecology Clinical Center of Yunnan Province (2022LCZXKF-SZ05); the Yunnan Revitalization Talent Support Program (XDYC-MY-2022-0055); and the Kunming University of Science and Technology Medical School research development fund project (SRDP-2023-003). The funders had no role in study design; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to submit the article for publication.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Busnelli A, Reschini M, Cardellicchio L, Vegetti W, Somigliana E, Vercellini P. How common is real repeated implantation failure? An indirect estimate of the prevalence. *Reproductive Biomedicine Online*. 2020; 40: 91–97.
- [2] Coughlan C, Ledger W, Wang Q, Liu F, Demirolo A, Gurgan T, *et al*. Recurrent implantation failure: definition and management. *Reproductive Biomedicine Online*. 2014; 28: 14–38.
- [3] Chinese Association of Reproductive Medicine, Professional Committee of Reproductive Medicine, China Medical Women's Association. Expert consensus on diagnosis and treatment of recurrent implantation failure. *Zhonghua Yi Xue Za Zhi*. 2023; 103: 89–100. (In Chinese)
- [4] ESHRE Working Group on Recurrent Implantation Failure, Cimadomo D, de Los Santos MJ, Griesinger G, Lainas G, Le Clef N, *et al*. ESHRE good practice recommendations on recurrent implantation failure. *Human Reproduction Open*. 2023; 2023: hoac023.
- [5] Baird DD, Weinberg CR, Wilcox AJ, McConnaughey DR, Musey PI, Collins DC. Hormonal profiles of natural conception cycles ending in early, unrecognized pregnancy loss. *The Journal of Clinical Endocrinology and Metabolism*. 1991; 72: 793–800.
- [6] Osman EK, Wang T, Zhan Y, Juneau CR, Morin SJ, Seli E, *et al*. Varying levels of serum estradiol do not alter the timing of the early endometrial secretory transformation. *Human Reproduction* (Oxford, England). 2020; 35: 1637–1647.
- [7] Sakumoto T, Tokunaga Y, Tanaka H, Nohara M, Motegi E, Shinkawa T, *et al*. Insulin resistance/hyperinsulinemia and reproductive disorders in infertile women. *Reproductive Medicine and Biology*. 2010; 9: 185–190.
- [8] Bellver J, Soares SR, Alvarez C, Muñoz E, Ramírez A, Rubio C, *et al*. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. *Human Reproduction* (Oxford, England). 2008; 23: 278–284.
- [9] Velkeniers B, Van Meerhaeghe A, Poppe K, Unuane D, Tourayne H, Haentjens P. Levothyroxine treatment and pregnancy outcome in women with subclinical hypothyroidism undergoing assisted reproduction technologies: systematic review and meta-analysis of RCTs. *Human Reproduction Update*. 2013; 19: 251–258.
- [10] De Sutter P, Stadhouers R, Dutré M, Gerris J, Dhont M. Prevalence of chromosomal abnormalities and timing of karyotype analysis in patients with recurrent implantation failure (RIF) following assisted reproduction. *Facts, Views & Vision in ObGyn*. 2012; 4: 59–65.
- [11] Raziel A, Friedler S, Schachter M, Kasterstein E, Strassburger D, Ron-El R. Increased frequency of female partner chromosomal abnormalities in patients with high-order implantation failure after in vitro fertilization. *Fertility and Sterility*. 2002; 78: 515–519.
- [12] Sciorio R, Dattilo M. PGT-A preimplantation genetic testing for aneuploidies and embryo selection in routine ART cycles: Time to step back? *Clinical Genetics*. 2020; 98: 107–115.
- [13] Craciunas L, Gallos I, Chu J, Bourne T, Quenby S, Brosens JJ, *et al*. Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis. *Human Reproduction Update*. 2019; 25: 202–223.
- [14] Tapia A, Gangi LM, Zegers-Hochschild F, Balmaceda J, Pommer R, Trejo L, *et al*. Differences in the endometrial transcript profile during the receptive period between women who were refractory to implantation and those who achieved pregnancy. *Human Reproduction* (Oxford, England). 2008; 23: 340–351.
- [15] Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, *et al*. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertility and Sterility*. 2013; 100: 818–824.
- [16] Achache H, Tsafir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. *Fertility and Sterility*. 2010; 94: 1271–1278.
- [17] Kim MA, Kim HS, Kim YH. Reproductive, Obstetric and Neonatal Outcomes in Women with Congenital Uterine Anomalies: A Systematic Review and Meta-Analysis. *Journal of Clinical Medicine*. 2021; 10: 4797.
- [18] Chan YY, Jayaprakasan K, Tan A, Thornton JG, Coomarasamy A, Raine-Fenning NJ. Reproductive outcomes in women with congenital uterine anomalies: a systematic review. *Ultrasound in Obstetrics & Gynecology: the Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. 2011; 38: 371–382.
- [19] Eldar-Geva T, Meagher S, Healy DL, MacLachlan V, Breheny S, Wood C. Effect of intramural, subserosal, and submucosal uterine fibroids on the outcome of assisted reproductive technology treatment. *Fertility and Sterility*. 1998; 70: 687–691.
- [20] Bonanni V, Reschini M, La Vecchia I, Castiglioni M, Muzii L, Vercellini P, *et al*. The impact of small and asymptomatic intramural and subserosal fibroids on female fertility: a case-control study. *Human Reproduction Open*. 2022; 2023: hoac056.
- [21] Bourdon M, Santulli P, Jeljel M, Vannuccini S, Marcellin L, Doridot L, *et al*. Immunological changes associated with adenomyosis: a systematic review. *Human Reproduction Update*. 2021; 27: 108–129.
- [22] Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women

- with adenomyosis and endometriosis: a systematic review and meta-analysis. *Human Reproduction Update*. 2019; 25: 592–632.
- [23] Richlin SS, Ramachandran S, Shanti A, Murphy AA, Parthasarathy S. Glycodelin levels in uterine flushings and in plasma of patients with leiomyomas and polyps: implications for implantation. *Human Reproduction (Oxford, England)*. 2002; 17: 2742–2747.
- [24] Elbehery MM, Nouh AA, Mohamed ML, Alanwar AA, Abd-Allah SH, Shalaby SM. Insulin-like growth factor binding protein-1 and glycodelin levels in uterine flushing before and after hysteroscopic polypectomy. *Clinical Laboratory*. 2011; 57: 953–957.
- [25] Demiroglu A, Gurgan T. Effect of treatment of intrauterine pathologies with office hysteroscopy in patients with recurrent IVF failure. *Reproductive Biomedicine Online*. 2004; 8: 590–594.
- [26] Kodaman PH, Arici A, Seli E. Evidence-based diagnosis and management of tubal factor infertility. *Current Opinion in Obstetrics & Gynecology*. 2004; 16: 221–229.
- [27] Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, *et al.* The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nature Communications*. 2017; 8: 875.
- [28] Han Y, Liu Z, Chen T. Role of Vaginal Microbiota Dysbiosis in Gynecological Diseases and the Potential Interventions. *Frontiers in Microbiology*. 2021; 12: 643422.
- [29] Fu M, Zhang X, Liang Y, Lin S, Qian W, Fan S. Alterations in Vaginal Microbiota and Associated Metabolome in Women with Recurrent Implantation Failure. *mBio*. 2020; 11: e03242-19.
- [30] Kitaya K, Matsubayashi H, Takaya Y, Nishiyama R, Yamaguchi K, Takeuchi T, *et al.* Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2017; 78: e12719.
- [31] Li Y, Yu S, Huang C, Lian R, Chen C, Liu S, *et al.* Evaluation of peripheral and uterine immune status of chronic endometritis in patients with recurrent reproductive failure. *Fertility and Sterility*. 2020; 113: 187–196.e1.
- [32] Yang W, Sun Q, Zhou Z, Gao Y, Shi F, Wu X, *et al.* Coagulation parameters predictive of repeated implantation failure in Chinese women: A retrospective study. *Medicine*. 2020; 99: e23320.
- [33] Samfireag M, Potre C, Potre O, Tudor R, Hoinoiu T, Anghel A. Approach to Thrombophilia in Pregnancy-A Narrative Review. *Medicina (Kaunas, Lithuania)*. 2022; 58: 692.
- [34] Ata B, Urman B. Thrombophilia and assisted reproduction technology-any detrimental impact or unnecessary overuse? *Journal of Assisted Reproduction and Genetics*. 2016; 33: 1305–1310.
- [35] Steinvil A, Raz R, Berliner S, Steinberg DM, Zeltser D, Levran D, *et al.* Association of common thrombophilias and antiphospholipid antibodies with success rate of in vitro fertilisation. *Thrombosis and Haemostasis*. 2012; 108: 1192–1197.
- [36] Murata H, Tanaka S, Okada H. The Regulators of Human Endometrial Stromal Cell Decidualization. *Biomolecules*. 2022; 12: 1275.
- [37] Motak-Pochrzest H, Malinowski A. Does autoimmunity play a role in the risk of implantation failures? *Neuro Endocrinology Letters*. 2018; 38: 575–578.
- [38] Simopoulou M, Sfakianoudis K, Maziotis E, Grigoriadis S, Giannelou P, Rapani A, *et al.* The Impact of Autoantibodies on IVF Treatment and Outcome: A Systematic Review. *International Journal of Molecular Sciences*. 2019; 20: 892.
- [39] Merz WM, Fischer-Betz R, Hellwig K, Lamprecht G, Gembruch U. Pregnancy and Autoimmune Disease. *Deutsches Arzteblatt International*. 2022; 119: 145–156.
- [40] Andreoli L, Bertias GK, Agmon-Levin N, Brown S, Cervera R, Costedoat-Chalumeau N, *et al.* EULAR recommendations for women's health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. *Annals of the Rheumatic Diseases*. 2017; 76: 476–485.
- [41] Garcia D, Erkan D. Diagnosis and Management of the Antiphospholipid Syndrome. *The New England Journal of Medicine*. 2018; 378: 2010–2021.
- [42] Marder W. Update on pregnancy complications in systemic lupus erythematosus. *Current Opinion in Rheumatology*. 2019; 31: 650–658.
- [43] Marder W, Littlejohn EA, Somers EC. Pregnancy and autoimmune connective tissue diseases. *Best Practice & Research. Clinical Rheumatology*. 2016; 30: 63–80.
- [44] Radin M, Schreiber K, Cuadrado MJ, Cecchi I, Andreoli L, Franceschini F, *et al.* Pregnancy outcomes in mixed connective tissue disease: a multicentre study. *Rheumatology (Oxford, England)*. 2019; 58: 2000–2008.
- [45] Matteo M, Greco P, Levi Setti PE, Morengi E, De Rosario F, Massenzio F, *et al.* Preliminary evidence for high anti-PLAC1 antibody levels in infertile patients with repeated unexplained implantation failure. *Placenta*. 2013; 34: 335–339.
- [46] Feng X, Meng X, Guo S, Li K, Wang L, Ai J. Identification of key genes and immune cell infiltration in recurrent implantation failure: A study based on integrated analysis of multiple microarray studies. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2022; 88: e13607.
- [47] Vacca P, Vitale C, Munari E, Cassatella MA, Mingari MC, Moretta L. Human Innate Lymphoid Cells: Their Functional and Cellular Interactions in Decidua. *Frontiers in Immunology*. 2018; 9: 1897.
- [48] Moffett A, Shreeve N. First do no harm: uterine natural killer (NK) cells in assisted reproduction. *Human Reproduction (Oxford, England)*. 2015; 30: 1519–1525.
- [49] Liang PY, Diao LH, Huang CY, Lian RC, Chen X, Li GG, *et al.* The pro-inflammatory and anti-inflammatory cytokine profile in peripheral blood of women with recurrent implantation failure. *Reproductive Biomedicine Online*. 2015; 31: 823–826.
- [50] Boomsma CM, Kavelaars A, Eijkemans MJC, Lentjes EG, Fauser BCJM, Heijnen CJ, *et al.* Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. *Human Reproduction (Oxford, England)*. 2009; 24: 1427–1435.
- [51] Ubaldi FM, Cimadomo D, Vaiarelli A, Fabbrozi G, Venturella R, Maggiulli R, *et al.* Advanced Maternal Age in IVF: Still a Challenge? The Present and the Future of Its Treatment. *Frontiers in Endocrinology*. 2019; 10: 94.
- [52] Cimadomo D, Fabbrozi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of Maternal Age on Oocyte and Embryo Competence. *Frontiers in Endocrinology*. 2018; 9: 327.
- [53] Shapiro BS, Daneshmand ST, Desai J, Garner FC, Aguirre M, Hudson C. The risk of embryo-endometrium asynchrony increases with maternal age after ovarian stimulation and IVF. *Reproductive Biomedicine Online*. 2016; 33: 50–55.
- [54] Yan J, Wu K, Tang R, Ding L, Chen ZJ. Effect of maternal age on the outcomes of in vitro fertilization and embryo transfer (IVF-ET). *Science China. Life Sciences*. 2012; 55: 694–698.
- [55] Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: an updated systematic review and meta-analysis. *Reproductive Biomedicine Online*. 2011; 23: 421–439.
- [56] Moragianni VA, Jones SML, Ryley DA. The effect of body mass index on the outcomes of first assisted reproductive technology cycles. *Fertility and Sterility*. 2012; 98: 102–108.
- [57] Schulte MMB, Tsai JH, Moley KH. Obesity and PCOS: the effect of metabolic derangements on endometrial receptivity at the

- time of implantation. *Reproductive Sciences* (Thousand Oaks, Calif.). 2015; 22: 6–14.
- [58] Collins GG, Rossi BV. The impact of lifestyle modifications, diet, and vitamin supplementation on natural fertility. *Fertility Research and Practice*. 2015; 1: 11.
- [59] Klonoff-Cohen H, Natarajan L, Marrs R, Yee B. Effects of female and male smoking on success rates of IVF and gamete intra-Fallopian transfer. *Human Reproduction* (Oxford, England). 2001; 16: 1382–1390.
- [60] Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. *Nicotine & Tobacco Research: Official Journal of the Society for Research on Nicotine and Tobacco*. 2004; 6: S125–S140.
- [61] Duncan WC. The inadequate corpus luteum. *Reproduction & Fertility*. 2021; 2: C1–C7.
- [62] Fuentes A, Muñoz A, Barnhart K, Argüello B, Díaz M, Pommer R. Recent cigarette smoking and assisted reproductive technologies outcome. *Fertility and Sterility*. 2010; 93: 89–95.
- [63] Fullston T, McPherson NO, Zander-Fox D, Lane M. The most common vices of men can damage fertility and the health of the next generation. *The Journal of Endocrinology*. 2017; 234: F1–F6.
- [64] Rogenhofer N, Markoff A, Ennerst X, Bogdanova N, Thaler C. Maternal and paternal carriage of the annexin A5 M2 haplotype: a possible risk factor for recurrent implantation failure (RIF). *Journal of Assisted Reproduction and Genetics*. 2021; 38: 235–242.
- [65] Simon A, Laufer N. Assessment and treatment of repeated implantation failure (RIF). *Journal of Assisted Reproduction and Genetics*. 2012; 29: 1227–1239.
- [66] Coughlan C, Clarke H, Cutting R, Saxton J, Waite S, Ledger W, *et al.* Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. *Asian Journal of Andrology*. 2015; 17: 681–685.
- [67] Malhotra V. Should sperm DNA fragmentation testing be routinely used in assessing male infertility? *Translational Andrology and Urology*. 2017; 6: S699–S701.
- [68] Cheng X, Huang Z, Xiao Z, Bai Y. Does antibiotic therapy for chronic endometritis improve clinical outcomes of patients with recurrent implantation failure in subsequent IVF cycles? A systematic review and meta-analysis. *Journal of Assisted Reproduction and Genetics*. 2022; 39: 1797–1813.
- [69] Nelson SM, Greer IA. The potential role of heparin in assisted conception. *Human Reproduction Update*. 2008; 14: 623–645.
- [70] Abdolmohammadi-Vahid S, Pashazadeh F, Pourmoghaddam Z, Aghebati-Maleki L, Abdollahi-Fard S, Yousefi M. The effectiveness of IVIG therapy in pregnancy and live birth rate of women with recurrent implantation failure (RIF): A systematic review and meta-analysis. *Journal of Reproductive Immunology*. 2019; 134–135: 28–33.
- [71] Saccone G, Schoen C, Franasiak JM, Scott RT, Jr, Berghella V. Supplementation with progestogens in the first trimester of pregnancy to prevent miscarriage in women with unexplained recurrent miscarriage: a systematic review and meta-analysis of randomized, controlled trials. *Fertility and Sterility*. 2017; 107: 430–438.e3.
- [72] Tournaye H, Sukhikh GT, Kahler E, Griesinger G. A Phase III randomized controlled trial comparing the efficacy, safety and tolerability of oral dydrogesterone versus micronized vaginal progesterone for luteal support in in vitro fertilization. *Human Reproduction* (Oxford, England). 2017; 32: 1019–1027.
- [73] Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, *et al.* Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Human Reproduction* (Oxford, England). 2015; 30: 323–330.
- [74] Moreno I, Garcia-Grau I, Perez-Villaroya D, Gonzalez-Monfort M, Bahçeci M, Barrioune MJ, *et al.* Endometrial microbiota composition is associated with reproductive outcome in infertile patients. *Microbiome*. 2022; 10: 1.
- [75] Pritts EA, Parker WH, Olive DL. Fibroids and infertility: an updated systematic review of the evidence. *Fertility and Sterility*. 2009; 91: 1215–1223.
- [76] Hu Y, Xiang Y, Huang W. Endometrial polyps effect on pregnancy outcomes in infertile women with minimal/mild endometriosis: A retrospective study. *The Journal of Obstetrics and Gynaecology Research*. 2023; 49: 2946–2951.
- [77] March CM. Management of Asherman's syndrome. *Reproductive Biomedicine Online*. 2011; 23: 63–76.
- [78] Practice Committee of American Society for Reproductive Medicine in collaboration with Society of Reproductive S. Salpingectomy for hydrosalpinx prior to in vitro fertilization. *Fertility and sterility*. 2008; 90: S66–S68.
- [79] Strandell A, Lindhard A, Waldenström U, Thorburn J, Janson PO, Hamberger L. Hydrosalpinx and IVF outcome: a prospective, randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. *Human Reproduction*. 1999; 14: 2762–2769.
- [80] Kontoravdis A, Makrakis E, Pantos K, Botsis D, Deligeoroglou E, Creasas G. Proximal tubal occlusion and salpingectomy result in similar improvement in in vitro fertilization outcome in patients with hydrosalpinx. *Fertility and Sterility*. 2006; 86: 1642–1649.
- [81] Sachdeva K, Upadhyay D, Discutido R, Varghese MM, Albuz F, Almekosh R, *et al.* Low Gonadotropin Dosage Reduces Aneuploidy in Human Preimplantation Embryos: First Clinical Study in a UAE Population. *Genetic Testing and Molecular Biomarkers*. 2018; 22: 630–634.
- [82] Stein A, Rufas O, Amit S, Avrech O, Pinkas H, Ovadia J, *et al.* Assisted hatching by partial zona dissection of human pre-embryos in patients with recurrent implantation failure after in vitro fertilization. *Fertility and Sterility*. 1995; 63: 838–841.
- [83] Valojerdi MR, Eftekhari-Yazdi P, Karimian L, Ashtiani SK. Effect of laser zona pellucida opening on clinical outcome of assisted reproduction technology in patients with advanced female age, recurrent implantation failure, or frozen-thawed embryos. *Fertility and Sterility*. 2008; 90: 84–91.
- [84] Guerif F, Bidault R, Gasnier O, Couet ML, Gervereau O, Lansac J, *et al.* Efficacy of blastocyst transfer after implantation failure. *Reproductive Biomedicine Online*. 2004; 9: 630–636.
- [85] Zhang J, Wang C, Zhang H, Zhou Y. Sequential cleavage and blastocyst embryo transfer and IVF outcomes: a systematic review. *Reproductive Biology and Endocrinology: RB&E*. 2021; 19: 142.
- [86] Benkhalifa M, Joao F, Duval C, Montjean D, Bouricha M, Cabry R, *et al.* Endometrium Immunomodulation to Prevent Recurrent Implantation Failure in Assisted Reproductive Technology. *International Journal of Molecular Sciences*. 2022; 23: 12787.
- [87] Nobijari FF, Arefi SS, Moini A, Taheripannah R, Fazeli E, Kharazi H, *et al.* Endometrium immunomodulation by intrauterine insemination administration of treated peripheral blood mononuclear cell prior frozen/thawed embryos in patients with repeated implantation failure. *Zygote* (Cambridge, England). 2019; 27: 214–218.
- [88] Li S, Wang J, Cheng Y, Zhou D, Yin T, Xu W, *et al.* Intrauterine administration of hCG-activated autologous human peripheral blood mononuclear cells (PBMC) promotes live birth rates in frozen/thawed embryo transfer cycles of patients with repeated implantation failure. *Journal of Reproductive Immunology*. 2017; 119: 15–22.
- [89] Bakry MS, Eldesouky E, Alghazaly MM, Farag E, Sultan EEK, Elazzazy H, *et al.* Granulocyte colony stimulating factor versus human chorionic gonadotropin for recurrent implantation failure

- in intra cytoplasmic sperm injection: a randomized clinical trial. *BMC Pregnancy and Childbirth*. 2022; 22: 881.
- [90] Li M, Kang Y, Wang Q, Yan L. Efficacy of Autologous Intrauterine Infusion of Platelet-Rich Plasma in Patients with Unexplained Repeated Implantation Failures in Embryo Transfer: A Systematic Review and Meta-Analysis. *Journal of Clinical Medicine*. 2022; 11: 6753.
- [91] Gao M, Jiang X, Li B, Li L, Duan M, Zhang X, *et al.* Intrauterine injection of human chorionic gonadotropin before embryo transfer can improve in vitro fertilization-embryo transfer outcomes: a meta-analysis of randomized controlled trials. *Fertility and Sterility*. 2019; 112: 89–97.e1.
- [92] Conforti A, Longobardi S, Carbone L, Iorio GG, Cariati F, Campitiello MR, *et al.* Does Intrauterine Injection of hCG Improve IVF Outcome? A Systematic Review and a Meta-Analysis. *International Journal of Molecular Sciences*. 2022; 23: 12193.
- [93] Shomer E, Katzenell S, Zipori Y, Rebibo-Sabbah A, Brenner B, Aharon A. Microvesicles of pregnant women receiving low molecular weight heparin improve trophoblast function. *Thrombosis Research*. 2016; 137: 141–147.
- [94] Noci I, Milanini MN, Ruggiero M, Papini F, Fuzzi B, Artini PG. Effect of dalteparin sodium administration on IVF outcome in non-thrombophilic young women: a pilot study. *Reproductive Biomedicine Online*. 2011; 22: 615–620.
- [95] Nakagawa K, Kwak-Kim J, Ota K, Kuroda K, Hisano M, Sugiyama R, *et al.* Immunosuppression with tacrolimus improved reproductive outcome of women with repeated implantation failure and elevated peripheral blood TH1/TH2 cell ratios. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2015; 73: 353–361.
- [96] Hviid MM, Macklon N. Immune modulation treatments-where is the evidence? *Fertility and Sterility*. 2017; 107: 1284–1293.
- [97] Moraru M, Carbone J, Alecsandru D, Castillo-Rama M, García-Segovia A, Gil J, *et al.* Intravenous immunoglobulin treatment increased live birth rate in a Spanish cohort of women with recurrent reproductive failure and expanded CD56(+) cells. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2012; 68: 75–84.
- [98] Ramos-Medina R, García-Segovia A, Gil J, Carbone J, Aguarón de la Cruz A, Seyffert A, *et al.* Experience in IVIg therapy for selected women with recurrent reproductive failure and NK cell expansion. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2014; 71: 458–466.
- [99] Li J, Chen Y, Liu C, Hu Y, Li L. Intravenous immunoglobulin treatment for repeated IVF/ICSI failure and unexplained infertility: a systematic review and a meta-analysis. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2013; 70: 434–447.
- [100] Singh N, Davis AA, Kumar S, Kriplani A. The effect of administration of intravenous intralipid on pregnancy outcomes in women with implantation failure after IVF/ICSI with non-donor oocytes: A randomised controlled trial. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*. 2019; 240: 45–51.
- [101] Madkour A, Bouamoud N, Louanjli N, Kaarouch I, Copin H, Benkhalifa M, *et al.* Intrauterine insemination of cultured peripheral blood mononuclear cells prior to embryo transfer improves clinical outcome for patients with repeated implantation failures. *Zygote (Cambridge, England)*. 2016; 24: 58–69.
- [102] Okitsu O, Kiyokawa M, Oda T, Miyake K, Sato Y, Fujiwara H. Intrauterine administration of autologous peripheral blood mononuclear cells increases clinical pregnancy rates in frozen/thawed embryo transfer cycles of patients with repeated implantation failure. *Journal of Reproductive Immunology*. 2011; 92: 82–87.
- [103] Yu N, Zhang B, Xu M, Wang S, Liu R, Wu J, *et al.* Intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) activated by HCG improves the implantation and pregnancy rates in patients with repeated implantation failure: a prospective randomized study. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*. 2016; 76: 212–216.
- [104] Davari-Tanha F, Shahrokh Tehraninejad E, Ghazi M, Shahraki Z. The role of G-CSF in recurrent implantation failure: A randomized double blind placebo control trial. *International Journal of Reproductive Biomedicine*. 2016; 14: 737–742.
- [105] Pantou A, Mitrakos A, Kokkali G, Petroutsou K, Tounta G, Lazaros L, *et al.* The impact of preimplantation genetic testing for aneuploidies (PGT-A) on clinical outcomes in high risk patients. *Journal of Assisted Reproduction and Genetics*. 2022; 39: 1341–1349.
- [106] Pirtea P, De Ziegler D, Tao X, Sun L, Zhan Y, Ayoubi JM, *et al.* Rate of true recurrent implantation failure is low: results of three successive frozen euploid single embryo transfers. *Fertility and Sterility*. 2021; 115: 45–53.
- [107] Cimadomo D, Rienzi L, Conforti A, Forman E, Canosa S, Innocenti F, *et al.* Opening the black box: why do euploid blastocysts fail to implant? A systematic review and meta-analysis. *Human Reproduction Update*. 2023; 29: 570–633.
- [108] Alteri A, Cermisoni GC, Pozzoni M, Gaeta G, Cavoretto PI, Viganò P. Obstetric, neonatal, and child health outcomes following embryo biopsy for preimplantation genetic testing. *Human Reproduction Update*. 2023; 29: 291–306.
- [109] Shalom-Paz E, Anabusi S, Michaeli M, Karchovsky-Shoshan E, Rothfarb N, Shavit T, *et al.* Can intra cytoplasmic morphologically selected sperm injection (IMSI) technique improve outcome in patients with repeated IVF-ICSI failure? a comparative study. *Gynecological Endocrinology: the Official Journal of the International Society of Gynecological Endocrinology*. 2015; 31: 247–251.
- [110] Lo Monte G, Murisier F, Piva I, Germond M, Marci R. Focus on intracytoplasmic morphologically selected sperm injection (IMSI): a mini-review. *Asian Journal of Andrology*. 2013; 15: 608–615.