RBMO

REVIEW

Recurrent implantation failure in IVF: A Canadian Fertility and Andrology Society Clinical Practice Guideline

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KEY MESSAGE

Recurrent implantation failure (RIF) is devastating for both couples and clinicians. The definition of RIF is variable, as are the effects of adjuvant medications and techniques to improve the implantation rate. This guideline provides recommendations on the investigation and management options that may increase the chance of a live birth.

ABSTRACT

Recurrent implantation failure (RIF) after IVF is a challenging topic for clinicians and can be a devastating reality for some patients with infertility. The purpose of this guideline from the Canadian Fertility and Andrology Society (CFAS) is to provide the most relevant evidence to date for the assessment and management of RIF. This guideline was developed using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach. This guideline recognizes the presence of heterogeneity in the definition of RIF. Recommendations are offered here on the investigation of RIF and management options that may increase the chance of a live birth.

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KEY WORDS
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This Clinical Practice Guideline has been prepared with and approved by the Canadian Fertility and Andrology Society (CFAS) Clinical Practice Guideline Committee†. It has also been reviewed and approved by the CFAS membership.
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INTRODUCTION

Implantation is one of the most critical steps in reproduction (*Aplin, 2000*). Successful implantation requires a competent blastocyst, a receptive endometrium and a synchronized dialogue between the maternal and embryonic tissues (*Galan et al., 2000*). The process of implantation involves apposition, adhesion and invasion (*Enders and Nelson, 1973*). During blastocyst apposition, trophoblast cells come into contact with the endometrial epithelium. Adhesion describes the process by which the blastocyst will subsequently anchor to the endometrial basal lamina and stromal extracellular matrix. This is followed by invasion, with blastocyst penetration through the luminal epithelium (*Enders and Nelson, 1973*). In assisted reproductive technology (ART), implantation is often defined as ultrasonographic evidence of an intrauterine gestational sac (*Coughlan et al., 2014*); however, a positive pregnancy test reflects the initiation of implantation (*RPL et al., 2018*).

Recurrent implantation failure (RIF) is the absence of implantation after repeated embryo transfers. While this clinical phenomenon is commonly encountered and there is vast literature on the subject, there is no universally accepted definition (TABLE 1). Variations in the definition reflect inconsistencies in the number of failed embryo transfers, the number, stage and quality of embryo(s) transferred (cleavage stage, blastocyst and euploid status), as well as the definition of implantation (concentration of human chorionic gonadotrophin [HCG] and presence of a gestational sac). The definition of RIF is continuously evolving, and this guideline recognizes the heterogeneity of the definitions, and therefore the data available.

Certain conditions must be excluded and surgically addressed prior to IVF, such as hydrosalpinges or uterine lesions deforming the cavity. Most RIF studies exclude these conditions, and address what appears as unexplained RIF.

Regardless of the variability in definitions, the diagnosis of RIF is a difficult reality for many couples undergoing infertility treatment. While understanding the emotional and financial burden of this diagnosis, and the vulnerability of these patients, it is important that valid investigative and management efforts be made. The purpose of this guideline is to provide an evidence-based approach to the assessment and management of patients with RIF.

METHODS: GUIDELINE DEVELOPMENT

This guideline is informed by the available research data on RIF in IVF. It mainly concerns the diagnostic tests and management options in ART settings. In accordance with CFAS requirements, the guideline development working group adopted the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to recommendation development, which provides a framework for the guideline development process including assessment of the quality of evidence and recommendations. An initial literature search was performed in February 2017 and updated in February 2020 to identify issues related to diagnostic interventions and therapeutic options available in case of RIF in IVF. Databases searched included Ovid

TABLE 1 DEFINITIONS OF RECURRENT IMPLANTATION FAILURE IN THE LITERATURE

Author, year of publication	Study topic	Definition
Polanski et al., 2014b	RIF review	Absence of implantation after two consecutive cycles of fresh or frozen IVF embryo transfers with a cumulative number of transferred embryos of four or more cleavage-stage embryos or two or more blastocysts, all of good quality
Coughlan et al., 2014	RIF review	Failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years
El-Toukhy et al., 2016	Hysteroscopy in RIF	Two to four previous fresh or frozen IVF treatment cycles ending in an embryo transfer but no pregnancy
Mariee et al., 2012	Endometrial immune profile in RIF	Failure of three fresh IVF cycles or two fresh IVF and two frozen embryos transfer cycles
Ledee et al., 2016	Endometrial immune profile in RIF	Failure to have an ongoing pregnancy >10 weeks after at least six embryos were transferred on day 3 or day 5 in women aged <43 years
Mitri et al., 2016	Embryo transfer technique	Failure to have a clinical pregnancy after four or more blastocysts (fresh or frozen) after ruling out malformed uterine cavity, hydrosalpinx, abnormal karyotype or persistently thin endometrium in women aged 38 year or younger
Kitaya et al., 2017	Chronic endometritis and RIF	Serial negative pregnancy tests following transfer of three or more morphologically good cleavage-stage embryos and/or blastocysts
Lensen et al., 2019	Endometrial injury	Two previous implantation failures, no precision on number of embryos
Olesen et al., 2019	Endometrial injury	Implantation failure despite top-quality embryo or blastocyst transfer(s)
Greco et al., 2014	PGT-A in RIF	Three to nine previous implantation failures after IVF (mean 4.9)
Huang, Wei & Li, 2017	HCG infusion in RIF	Two or more failed transfer of good quality embryos
Makrigiannakis et al, 2015	PBMC infusion in RIF	Three or more failed IVF cycles with a cumulative transfer of six embryos or three blastocysts of good quality
Koot et al., 2019	Prognosis of RIF	Three failed IVF or ICSI treatments, each with at least one fresh good quality embryo per transfer, or failure to achieve pregnancy after transfer of 10 good quality embryos

HCG, human chorionic gonadotrophin; ICSI, intracytoplasmic sperm injection; PBMC, peripheral blood mononuclear cell; PGT-A, preimplantation genetic testing for aneuploidies; RIF, recurrent implantation failure.

MEDLINE, the Cochrane Central Register of Controlled Trials and EMBASE from the time of inception to the date of completion of searches. Full search terms can be provided on request. Titles and abstracts were screened for inclusion, followed by a full-text review by two independent reviewers; a third reviewer was consulted to resolve discordance.

INVESTIGATIONS

Cavity assessment

Hysteroscopy is one of the most widely used investigational tools in women with RIF. It is considered the gold standard for diagnosing and treating intrauterine pathologies that could be missed on transvaginal ultrasound, such as fibroids, polyps or adhesions (*Soares et al., 2000*). Although cavity assessment studies of patients with RIF refer mainly to hysteroscopy, sonohysterography is an acceptable alternative (*Negm et al., 2012*; *Reda et al., 2016*). The incidence of abnormal hysteroscopic findings in women with RIF varies between 14% and 51% (*Gao et al., 2015*; *Hosseini et al., 2014*; *Lambert et al., 2016*; *Pabuccu et al., 2016*). Polyps, intrauterine adhesions and submucosal fibroids represent the most commonly detected anomalies.

A well-designed multicentre randomized clinical trial addressed the role of hysteroscopy in the workup of RIF in women with a normal baseline ultrasound. The TROPHY study compared 350 women with RIF (2–4 failed IVF cycles) having hysteroscopy prior to IVF with 352 RIF controls with no hysteroscopy. In the hysteroscopy group, an anomaly was found in 24% of patients (85/350), namely arcuate uterus, hypervascularization, mucosal elevation, endometrial and cervical polyps, partial uterine septum, cervical stenosis, submucous fibroid, hemi-uterus, micropolyps or endometrial defects. However, the yield of surgically correctable problems was only 4% (15/350). The live birth rate (LBR) in patients with an abnormal hysteroscopy was 31% (26/85). Overall, there was no difference in LBR between the two groups (29% in both groups; risk ratio [RR] 1.0; 95% confidence interval [CI] 0.79–1.25; $P = 0.96$) after surgical correction (*El-Toukhy et al., 2016*).

Recommendation:

1. In RIF patients with a normal baseline ultrasound, the routine use of hysteroscopy is not recommended.

Strength: strong.

Quality of evidence: high.

Justification: this was based on a well-conducted multicentre randomized controlled trial (RCT) comparing hysteroscopy with no hysteroscopy in women with RIF, which showed no difference in LBR.

Thrombophilia testing

Interest in thrombophilia testing in RIF patients is largely influenced by the research on recurrent pregnancy loss (RPL). A possible mechanism for pregnancy complications among inherited thrombophilia carriers is thrombosis of the maternal vessels, which could reduce perfusion of the intervillous space, leading to placental failure. It has been suggested that implantation failure in women undergoing IVF may be due to similar damage of the decidual or chorionic vessels, or reduction of trophoblast invasiveness, preventing embryo implantation (*Ata and Urman, 2016*).

Acquired thrombophilia, including antiphospholipid syndrome, has been shown to be relevant in recurrent early pregnancy loss, as increased coagulability can theoretically affect embryo implantation and early pregnancy development, possibly through vascular occlusion (*Ata and Urman, 2016*). Autoimmune factors may play an additional role in the thrombotic activity of invading trophoblasts. Several studies describe an incidence of inherited and acquired thrombophilia in RIF patients that varies between 4% and 62%. However, many of these studies were small and include findings that are not clinically relevant (e.g. heterozygous status for *MTHFR* mutation) (*Bellver et al., 2008*; *Qublan et al., 2006*; *Safdarian et al., 2014*; *Simur et al., 2009*).

The largest dataset published by Steinvil in 2012 retrospectively analysed 594 women who underwent a mean of seven failed IVF cycles and who had thrombophilia testing and were not treated with anticoagulation; it compared them with 637 fertile women and 17,337 women members at the same healthcare facility with no history of venous thromboembolism. None of the common thrombophilias tested was significantly associated with RIF (acquired activated protein C resistance and/or factor V Leiden, prothrombin mutation and lupus anticoagulant/anticardiolipin immunoglobulin G [IgG]) (*Steinvil et al., 2012*).

Recommendation:

2. Testing for inherited or acquired thrombophilia in patients with RIF is not recommended.

Strength: strong.

Quality of evidence: low.

Justification: there is insufficient evidence that either inherited or acquired thrombophilias are increased in RIF patients.

Immunological testing

The immunological aspects of implantation are many and are documented extensively in basic scientific and clinical research. The decidualized stromal cells of the endometrium, critical to implantation, are able to regulate trophoblast invasion and to dampen the local maternal immune response (*Coughlan et al., 2014*). The failure to control that immune reaction may lead to implantation failure.

Several serological immune profiles may play a role in patients with RIF. Antibodies against placenta-specific 1 (a protein expressed in the placenta and encoded by trophoblast-specific gene *PLAC1*) may impair implantation and have been shown in a small case-control study to be higher in patients with RIF compared with fertile controls (*Matteo et al., 2013*). Liang and colleagues also performed a small case-control study, and showed that pro-inflammatory factors (interferon-gamma [IFN-γ], interleukin (IL)-1β, IL-6 and IL-4) were increased, and anti-inflammatory factors (transforming growth factor-beta 1) were decreased, in the peripheral blood of RIF patients compared with control participants pregnant after IVF (*Liang et al., 2015*).

The endometrial immune profile was also studied in patients with RIF (*Ledee et al., 2016*; *Mariee et al., 2012*). While there

is much investigation into the biological plausibility of an immunological aetiology in RIF, there is limited evidence to justify translation to clinical practice.

Recommendation:

3. Serological or endometrial immune testing in RIF patients should be limited to research settings.

Strength: strong.

Quality of evidence: low.

Justification: observational studies with multiple immunological profiles were tested, with inconsistent results. Studies are heterogeneous and not yet applicable to clinical practice.

Parental karyotype analysis

Couples with balanced translocations often produce gametes with chromosomal aberrations, which may in turn result in various forms of reproductive failures, notably RPL (*Tharapel et al., 1985*). Stern and colleagues tested 514 patients with at least 10 failed embryo transfers and detected chromosomal anomalies in 2.5% of them (*Stern et al., 1999*). In comparison, 4.7% of patients with a history of recurrent miscarriage (319 patients with at least three consecutive first-trimester spontaneous abortions) had chromosomal anomalies (*Stern et al., 1999*). During that same period, 1.3% of 1000 infertile couples undergoing their first IVF had chromosomal anomalies, and 0.3% of 94,465 normal neonates had chromosomal anomalies (baseline rate).

Among the RIF population, the most common anomalies were translocations (reciprocal and Robertsonian), found in 7/514 (1.4%) of individuals and 7/219 of couples (3.2%), which was significantly higher than the rate of translocations in infertile controls (0.3%) and normal neonates (0.2%) (*Stern et al., 1999*). In 2013, Coughlan and colleagues published an observational study of the clinical characteristics and investigations of 111 couples with RIF (≥4 embryos in ≥3 IVF cycles). Chromosomal anomalies were found in 3% of females and 0% of males (*Coughlan et al., 2013*). De Sutter and colleagues in 2012 analysed the karyotypes of 317 women and 298 men after three sequential failed IVF/intracytoplasmic sperm injection cycles (*De Sutter et al., 2012*). Chromosomal anomalies were diagnosed in 2.1% (13/615) of patients (2.5% of females [8/317] and 1.7% of males [5/298]), which is significantly higher than in women with normal ovulatory function (0.6%) (*Raziel et al., 2002*). The most common type of anomaly was the autosomal reciprocal translocation, which applies to the general population as well (*De Sutter et al., 2012*). Non-karyotypic genetic polymorphisms linked to RIF are under investigation, but none is currently clinically applicable.

In summary, the published data are limited by small sample sizes with a large number of failed embryo transfers.

Recommendation:

4. Karyotype testing may be offered to couples with RIF.

Strength: weak.

Quality of evidence: low.

Justification: observational studies suggest that couples with RIF may have a slightly higher rate of chromosomal anomalies than fertile couples.

Sperm DNA fragmentation testing

Sperm DNA damage is associated with poor embryo development, and there is recent interest in the use of sperm DNA integrity testing in the evaluation of reproductive failure (*Caseiro et al., 2015*). DNA fragmentation may be associated with increased risk of miscarriage (*Simon et al., 2017*) but its association with RIF has not been established. In 2013, the American Society for Reproductive Medicine guideline on the clinical utility of sperm DNA integrity testing stated that there was insufficient evidence to recommend its routine use before IVF. Bronet and colleagues published a small prospective study on 30 patients with RPL and eight patients with RIF (three failed IVF cycles) but there was no correlation between a high DNA fragmentation index (DFI) and aneuploidy in the embryos tested (*Bronet et al., 2012*). Coughlan and co-workers in 2015 evaluated in a prospective study the spermatozoa of 35 RIF male patients (three failed IVF cycles) compared with seven recent fathers, and again there was no correlation between high DFI and RIF (*Coughlan et al., 2015*). These prospective studies involved a small number of patients, employed differing methodology, produced different results and showed no correlation between high DFI and RIF.

Recommendation:

5. Sperm DFI testing should not be routinely offered in RIF.

Strength: weak.

Quality of evidence: low.

Justification: small observational studies showed that high sperm DFI was not correlated to RIF.

Chronic endometritis in women with RIF

There is no consensus on the precise definition or prevalence of chronic endometritis (*Liu et al., 2018*). Historically, the diagnosis of chronic endometritis was based on the presence of plasma cells on endometrial sampling. Haematoxylin–eosin (H&E) staining has recently fallen out of favour and most studies rely on immunohistochemical identification of CD138 cells as this has been shown to be a more sensitive diagnostic method (*Kitaya and Yasuo, 2013*). However, a consensus cut-off of CD138 cells used to diagnose chronic endometritis has yet to be established. Hysteroscopic diagnosis has also been described, but has not been validated. In unexplained infertility, retrospective studies report a prevalence of chronic endometritis diagnosed by histology of 30% and 56.8% (*Cicinelli et al., 2005*; *Cicinelli et al., 2018*), but prospective studies in this population subgroup are lacking. In the largest prospective cohort study (n = 421) of patients regarding chronic endometritis and RIF, a prevalence of 33.7% for chronic endometritis was reported based on CD138 immunohistochemical staining (*Kitaya et al., 2017*), where chronic endometritis was defined as a ratio of the sum of stromal CD138+ cells per high-power field of 0.25 or more; this percentage ranges from 14% to 57.6% in other studies of RIF based on heterogeneous criteria (*Bouet et al., 2016*; *Cicinelli et al., 2015*; *Johnston-MacAnanny et al., 2010*; *Yang et al., 2014*; *Zargar et al., 2019*; *Zhang et al., 2019*). Only one recent study compared the prevalence of chronic endometritis between fertile patients (n = 40) and those with RPL (n = 93), RIF (n = 39) or infertility (n = 48) using CD138 immunohistochemical staining, and did

not find a statistically significant difference between these groups (5%, 10.8%, 7.7% and 10.4%, respectively) (*Liu et al., 2018*).

There are no RCT comparing clinical outcomes between patients with treated chronic endometritis, presence of untreated chronic endometritis and absence of chronic endometritis. A recent meta-analysis evaluating chronic endometritis and RIF performed different comparisons based on the published data on clinical outcomes to date (five observational studies, including one on oocyte recipients) and concluded that treatment of chronic endometritis may improve IVF outcomes in women with RIF (*Vitagliano et al., 2018b*). However, the four studies concerning RIF in autologous cycles were observational, had serious risk of bias, and were heterogeneous in their diagnostic criteria for chronic endometritis, method of treating chronic endometritis, test of cure for chronic endometritis or clinical outcomes assessed (*Cicinelli et al., 2015*; *Johnston-MacAnanny et al., 2010*; *Kitaya et al., 2017*; *Yang et al., 2014*). For these reasons, firm conclusions from each of these studies or their combined results cannot be drawn.

Recommendation:

6. Screening for chronic endometritis should not be routinely offered in RIF.

Strength: weak.

Quality of evidence: low (TABLE 2).

Justification: this decision was based on small, low-quality heterogeneous observational studies and a lack of consensus diagnostic criteria for CE.

Endometrial receptivity array in women with RIF

One of the critical elements involved in implantation is the synchronization between the endometrium and the implanting embryo. The transfer of blastocysts after IVF is routinely performed on day 5 after egg retrieval, or day 5–6 of progesterone therapy, based on classic studies of the timing of implantation (*Wilcox et al., 1999*). Failure to transfer a euploid embryo to a receptive endometrium during the appropriate window of implantation has been suggested as one of the causes of RIF (*Valdes et al., 2017*). The endometrial receptivity array (ERA) is a tool used to detect a receptive endometrium by means of a specific transcriptomic gene signature and is a reproducible and more accurate method than receptivity assessed by histological evaluation (*Diaz-Gimeno et al., 2013*). There are no RCT comparing clinical outcomes between patients with RIF undergoing ERA prior to their next embryo transfer and those not undergoing the procedure.

The literature on ERA and RIF is limited to four observational studies (three retrospective and one prospective non-randomized trial) showing that that the rate of presence of a receptive endometrium may be slightly lower in those with RIF compared with those without RIF in women having all undergone ERA; women with RIF have possibly similar pregnancy rates to women without RIF when endometrium is receptive on ERA; and within the RIF population, personalized embryo transfer may yield similar clinical and ongoing pregnancy rates in women with a displaced window of implantation on ERA as in those with receptive endometrium (*Hashimoto et al., 2017*; *Mahajan, 2015*; *Patel et al., 2019*; *Ruiz-Alonso et al., 2013*). None of these trials had an appropriate control group to be able to draw conclusions regarding efficacy.

Recommendation:

7. The use of endometrial receptivity assay in RIF patients should be limited to research settings.

Strength: strong.

Quality of evidence: very low (TABLE 3).

Justification: there is currently no evidence that endometrial receptivity assay improves clinical outcomes in RIF.

Preimplantation genetic testing for aneuploidies in couples with RIF

While much can be postulated regarding endometrial factors in RIF, embryonic considerations are also relevant. Aneuploidy leads to the majority of preclinical pregnancy losses (less than 6 weeks gestational age) (*Ohno et al., 1991*) and is therefore a possible cause of RIF, especially in women of advanced maternal age.

The use of fluorescence in-situ hybridization on cleavage-stage embryos to assess aneuploidy was associated with a high false-positive and false-negative rate, and therefore has created conflicting results with respect to the contribution of aneuploidy to implantation failure.

A prospective study (*Greco, 2014*) was designed as a pilot cohort study to assess the clinical pregnancy rate with transfer of a single euploid blastocyst in a group of 76 patients less than 36 years of age with a history of RIF (3–6 failed embryo transfer cycles) compared with 45 couples undergoing their first IVF cycle. Participants with RIF were subsequently divided into two subgroups: those that consented to array comparative genomic hybridization (CGH) and those that did not. In the group of RIF with PGT-A, 98 blastocysts were aneuploid, therefore giving an aneuploidy rate of 53.8%. In the non-RIF group, the aneuploidy rate was found to be 48.2%. The clinical pregnancy rate in the RIF with preimplantation genetic testing for aneuploidies (PGT-A) group was 68.3% compared with 22% in those with RIF without PGT-A ($P < 0.001$).

Next-generation sequencing (NGS) is the latest technological advancement in PGT, enabling whole-genome analysis with greater accuracy. There has been one isolated case report describing the application of NGS in otherwise unexplained RIF and recurrent early miscarriage in two couples having undergone IVF with normal routine CGH-microarray results (*Ou et al., 2015*). While further prospective data are needed, NGS technology may provide insight into previously unexplained cases of preimplantation failure in terms of the detection of segmental polymorphisms.

Recommendation:

8. There are insufficient data to recommend for, or against, PGT-A for RIF.

Strength: strong.

Quality of evidence: low.

Justification: the studies are few in number and small with respect to sample size. There are no RCT data available.

TREATMENTS

Endometrial injury in women with RIF

Endometrial injury, also referred to as 'endometrial scratching', has been

TABLE 2 SUMMARY OF FINDINGS FOR ENDOMETRIAL BIOPSY AND TREATMENT OF CE COMPARED WITH NO CE, NO BIOPSY, NO TREATMENT OR PERSISTENT CE FOR RIF

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Endometrial biopsy and treatment of CE	No CE, no biopsy, no treatment or persistent CE	Relative (95% CI)	Absolute (95% CI)		
CPR in treated CE versus persistent or untreated CE												
2	Observational studies[1,2]	Serious[a]	Not serious	Not serious	Serious[b]	None	54/114 (47.4%)	8/35 (22.9%)	OR 3.04 (1.27–7.25)	245 more per 1000 (from 45 more to 454 more)	⊕OOO Very low	Important
LBR in treated CE versus persistent CE												
1	Observational studies[1]	Serious[c]	Not serious	Not serious	Very serious[b]	None	28/46 (60.9%)	2/15 (13.3%)	OR 10.11 (2.04–50.19)	475 more per 1000 (from 106 more to 752 more)	⊕OOO Very low	Important
Clinical pregnancy rate in CE + treatment versus no CE												
1	Observational studies [3]	Serious[d]	Not serious	Not serious	Not serious	None	43/116 (37.1%)	61/226 (27.0%)	RR 1.37 (0.99–1.90)	100 more per 1000 (from 3 fewer to 243 more)	⊕OOO Very low	Important
Live birth rate in CE + treatment versus no CE												
1	Observational studies[3]	Serious[d]	Not serious	Not serious	Not serious	None	38/116 (32.8%)	50/226 (22.1%)	RR 1.48 (1.03–2.12)	106 more per 1000 (from 7 more to 248 more)	⊕OOO Very low	Important
CPR in CE+antibiotics versus no endometrial biospy + no ATB												
1	Observational studies[4]	Very serious[e]	Not serious	Not serious	Serious[b]	None	2/10 (20.0%)	197/485 (40.6%)	OR 0.37 (0.08–1.74)	204 fewer per 1000 (from 354 fewer to 137 more)	⊕OOO Very low	Important

[a] Small sample, especially the control group. Both studies are retrospective. In Yang and colleagues' study,[2] baseline characteristics were not recorded, and there was a major risk of confounding.

[b] Wide confidence interval.

[c] Small sample, especially the control group. Retrospective study.

[d] Not randomized. All patients underwent biopsy; 23 patients had CE and refused treatment so were excluded.

[e] Retrospective study with a very small sample in the treatment group.

ATB, antibiotics; CE, chronic endometritis; CI, confidence interval; OR, odds ratio; LBR, live birth rate; RIF, recurrent implantation failure; RR, risk ratio.

1. Cicinelli, E., Matteo, M., Tinelli, R., Lepera, A., Alfonso, R., Indraccolo, U., Marrocchella, S., Greco, P., Resta, L., 2015. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod. 30 (2), 323-30.

2. Yang, R., Du, X., Wang, Y., Song, X., Yang, Y., Qiao, J., 2014. The hysteroscopy and histological diagnosis and treatment value of chronic endometritis in recurrent implantation failure patients. Arch Gynecol Obstet. 289(6), 1363-9.

3. Kitaya, K., Matsubayashi, H., Takaya, Y., Nishiyama, R., Yamaguchi, K., Takeuchi, T., Ishikawa., T., 2017. Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. Am J Reprod Immunol. 78(5).

4. Johnston-MacAnanny, E.B., Hartnett, J., Engmann, L.L., Nulsen, J.C., Sanders, M.M., Benadiva, C.A., 2010. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil Steril. 93(2), 437-41.

TABLE 3 SUMMARY OF FINDINGS FOR ERA COMPARED WITH NO ERA FOR RIF

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**ERA**	**No ERA**	**Relative (95% CI)**	**Absolute (95% CI)**		
Ongoing pregnancy rate												
1	Observational studies[1]	Serious[a]	Not serious	Not serious[b]	Not serious	All plausible residual confounding would suggest a spurious effect, while no effect was observed	28/66 (42.4%)	38/68 (55.9%)	OR 0.58 (0.29–1.15)	135 fewer per 1000 (from 290 fewer to 34 more)	⊕⊕OO Low	Important
Implantation rate												
2	Observational studies[1,2]	Serious[c]	Not serious	Not serious	Not serious	All plausible residual confounding would suggest a spurious effect, while no effect was observed	61/185 (33.0%)	64/155 (41.3%)	OR 0.70 (0.45–1.09)	83 fewer per 1000 (from 173 fewer to 21 more)	⊕⊕OO Low	Important
Pregnancy rate (definition?)												
1	Observational studies	Serious[d,e]	Not serious	Not serious	Serious[f]	All plausible residual confounding would suggest a spurious effect, while no effect was observed	15/29 (51.7%)	9/11 (81.8%)	OR 0.24 (0.04–1.30)	299 fewer per 1000 (from 666 fewer to 36 more)	⊕OOO Very low	Important

[a] Retrospective study. No control group without ERA. Not specified whether pregnancy rates were reported only for receptive endometrium but this is likely considering the denominator.

[b] No control group without ERA. Both groups had implantation failure (control group had one).

[c] Both were observational studies. No control group without ERA. Ruiz-Alonso and colleagues' study included oocyte donor patients.[2]

[d] Not randomized. No control group without ERA.

[e] Very small sample; includes oocyte donor patients.

[f] Wide confidence interval

CI, confidence interval; ERA, endometrial receptivity array, OR, odds ratio; RIF, recurrent implantation failure.

1. Mahajan, N., 2015. Endometrial receptivity array: Clinical application. J Hum Reprod Sci. 8(3), 121-9.

2. Ruiz-Alonso, M., Blesa, D., Diaz-Gimeno, P., Gomez, E., Fernandez-Sanchez, M., Carranza, F., Carrera, J., Vilella, F., Pellicer, A., Simon, C., 2013. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. Fertil Steril. 100(3), 818-24.

defined as intentional damage to the endometrium, performed most commonly with a biopsy pipelle, in order to improve its receptivity and in turn improve reproductive outcomes in ART (*Nastri et al., 2015*; *Simon and Bellver, 2014*). Proposed mechanisms to explain the improved endometrial receptivity include: release of inflammatory factors favourable to implantation (cytokines, interleukins, growth factors, macrophages and dendritic cells) (*Gnainsky et al., 2015*); delay in endometrial maturation caused by ovarian stimulation, which may lead to better synchronization between the embryo and the endometrium; and induction of endometrial decidualization, which is also favourable to implantation (*Nastri et al., 2015*). A number of meta-analyses and a recent well-designed RCT show no benefit of endometrial injury on LBR in unselected women undergoing IVF (*Nastri et al., 2015*, *Lensen et al., 2019*) or in women undergoing their first embryo transfer (*Vitagliano et al., 2019*).

Several studies have evaluated this technique specifically in the context of RIF. Four recently published meta-analyses on endometrial injury performed sub-analyses in patients with RIF, defined as either two or more failed full IVF cycles (all fresh and frozen embryo transfers following one stimulation cycle) or two or more failed embryo transfer cycles. Two of these meta-analyses (*Nastri et al., 2015*; *Vitagliano et al., 2018a*) demonstrated a benefit in terms of clinical pregnancy rate (CPR) (RR 1.63, 95% CI 1.12–2.38 and RR 1.57, 95 % CI 1.22–2.03, respectively) and LBR (RR 1.96, 95% CI 1.21–3.16, and RR 1.64, 95% CI 1.21–2.21, respectively). On the other hand, one of the meta-analyses (*van Hoogenhuijze et al., 2019*) demonstrated an improvement in CPR when excluding studies with unintentional endometrial injury in the control group (RR 2.03, 95% CI 1.20–3.43), but not in LBR (RR 1.15, 95% CI 0.52–2.55). In addition, Sar-Shalom and colleagues demonstrated no improvement in CPR (RR 1.53, 95% CI 0.93–2.51) or LBR (RR 1.22, 95% CI 0.52–2.82) (*Sar-Shalom Nahshon et al., 2019*). These reviews included a combination of 13 RCTs (up to eight studies and up to 817 patients in each meta-analysis), all heterogeneous for quality, technique used for endometrial injury, number of injuries performed, timing of endometrial injury and inclusion and exclusion criteria; most were also not specifically designed for RIF patients but only performed subgroup analyses.

Two RCT on endometrial injury, robust in terms of size and quality, were recently published (*Lensen et al., 2019*; *Olesen et al., 2019*) and performed sub-analyses on women with RIF. The first trial, by Lensen and co-workers randomized patients to either endometrial injury with a pipelle between day 3 of the previous cycle and the beginning of a fresh or frozen treatment cycle, or no endometrial injury (*Lensen et al., 2019*). A planned and adequately powered sub-group analysis of 337 patients with two or more implantation failures showed no benefit in terms of LBR (34/166 versus 46/171 in the treatment and control groups respectively; odds ratio [OR] 0.68, 95% CI 0.39–1.17). The second RCT, by Olesen and colleagues, studied the effect of endometrial injury versus no treatment prior to a fresh IVF cycle in women with at least one prior implantation failure, and randomized 151 and 153 women to the treatment and control groups, respectively (*Olsesen et al., 2019*). In analyses of subgroups allocated by number of previous implantation failures (one, two or ≥3), there was no difference between CPR or LBR with two prior failures in the intention-to-treat analysis. However, with three or more previous failures, there was an increased CPR with endometrial injury (30/56 versus 14/51; RR 1.72, 95% CI 1.01–2.78) but no difference in LBR. These subgroups were smaller and analyses may therefore have been underpowered.

Therefore, given the heterogeneity of previous studies and robust evidence from two recent RCT with mainly neutral results, endometrial injury to improve clinical outcomes in RIF patients is not recommended.

Recommendation:

9. Endometrial injury in the cycle preceding the embryo transfer should not be routinely offered in RIF.

Strength: strong.

Quality of evidence: moderate (TABLE 4).

Justification: results from two well-designed RCT and pooled results from RCT reporting on LBR show no benefit of endometrial injury.

Anticoagulants in the management of RIF

The role of low molecular weight heparin (LMWH) in implantation has been widely studied and it has been shown to modulate the expression of certain factors involved in endometrial receptivity and implantation (*Potdar et al., 2013*). It has been shown to decrease trophoblastic apoptosis, and promote angiogenesis and trophoblastic invasion (*Hills et al., 2012*; *Shomer et al., 2016*). With regards to its use in the context of RIF, two RCT have been performed but they included heterogeneous populations. One of these included patients who screened positive for one or more inherited or acquired thrombophilia defects (*Qublan et al., 2008*) while the other included patients who screened negative for any coagulative defect (*Urman et al., 2009*). Results from the two RCT produced conflicting conclusions, with the latter study, albeit underpowered, showing no difference between LMWH and no treatment with respect to CPR and LBR. The other RCT on the matter either were not properly randomized, did not evaluate heparin exclusively or did not evaluate outcomes that were clinically relevant (*Berker et al., 2011*; *Fawzy and El-Refaeey, 2014*; *Hamdi et al., 2015*; *Stern et al., 2003*).

Low-dose aspirin (acetylsalicylic acid [ASA]) is thought to have anti-inflammatory and antiplatelet properties that may enhance uterine perfusion and improve endometrial receptivity (*Siristatidis et al., 2016*). In unselected women undergoing IVF, one RCT has been performed showing no benefit with regards to LBR (*Pakkila et al., 2005*). Low-dose ASA as an adjuvant treatment in RIF has not been studied on its own in any randomized trial. In a randomized controlled crossover trial comparing unfractionated heparin and aspirin with placebo in RIF patients (≥10 embryos transferred), LBR was similar in the two groups (*Stern et al., 2003*). the sparsity of studies on low-dose ASA in patients with RIF make it impossible to form a recommendation.

Recommendations:

10. Empirical LMWH for RIF patients should be limited to research settings.

Strength: weak.

TABLE 4 SUMMARY OF FINDINGS FOR ENDOMETRIAL INJURY COMPARED WITH NO ENDOMETRIAL INJURY IN RIF

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Endometrial injury	No endometrial injury	Relative (95% CI)	Absolute (95% CI)		
Clinical pregnancy rate (RCT): ≥1 failure												
6	Randomized trials[1,2,3,4,5,6]	Serious[a]	Not serious	Serious[b,c]	Not serious	None	212/557 (38.1%)	164/561 (29.2%)	RR 1.30 (1.10–1.54)	88 more per 1000 (from 29 more to 158 more)	⊕⊕OO Low	Important
Clinical pregnancy rate (observational studies)												
3	Observational studies[7,8,9]	Serious[a,d]	Not serious	Serious[c]	Not serious	Strong association All plausible residual confounding would reduce the demonstrated effect	54/149 (36.2%)	39/199 (19.6%)	OR 2.33 (1.44–3.78)	166 more per 1000 (from 64 more to 284 more)	⊕⊕OO Low	Important
Live birth rate (RCT): limited to ≥2 failures												
6	Randomized trials[3,4,5,6,10,11]	Serious	Not serious	Not serious[c,e]	Not serious	None	118/480 (24.6%)	104/482 (21.6%)	RR 1.14 (0.90–1.44)	30 more per 1000 (from 22 fewer to 95 more)	⊕⊕⊕O Moderate	Critical
Live birth rate (observational studies)												
1	Observational studies[7]	Serious[f]	Not serious	Not serious	Serious[g]	All plausible residual confounding would reduce the demonstrated effect	18/51 (35.3%)	8/52 (15.4%)	OR 2.82 (1.04–7.61)	185 more per 1000 (from 5 more to 427 more)	⊕OOO Very low	Critical
Miscarriage rate (RCT)												
2	Randomized trials[3,5]	Serious	Not serious	Serious[c]	Not serious	None	8/127 (6.3%)	10/98 (10.2%)	RR 0.62 (0.25–1.51)	39 fewer per 1000 (from 77 fewer to 52 more)	⊕⊕OO Low	Important
Miscarriage rate (observational studies)												
2	Observational studies[7,8]	Serious[c]	Not serious	Serious[c]	Not serious	All plausible residual confounding would suggest spurious effect, while no effect was observed	13/69 (18.8%)	12/59 (20.3%)	RR 0.93 (0.46–1.87)	14 fewer per 1000 (from 110 fewer to 177 more)	⊕OOO Very low	Important

[a] Not blinded.
[b] Three out of six studies had some intervention in the control group (either HSC alone or pipelle or uterine sound up to the cervix).[3,4,5] This might dilute the final effect, as it might not be the same as no intervention.
[c] Heterogeneous definitions of RIF, but all had ≥2 failures; the number of embryos varied.
[d] Two prospective and one retrospective study, with a risk of selection bias. Not randomized, risk of confounding factors that were not all evaluated in TABLE 1.
[e] Three out of studies had some intervention in the control group (HSC or pipelle up to the cervix).
[f] Selection bias. HSC and injury were performed so there may have been an added effect. Prospective non-randomized trial.
[g] Wide confidence interval, with OR adjusted from logistic regression.
CI, confidence interval; HSC, hysteroscopy; OR, odds ratio; RCT, randomized controlled trial; RIF, recurrent implantation failure; RR, risk ratio.

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TABLE 5 SUMMARY OF FINDINGS FOR ANTICOAGULANTS COMPARED WITH NO ANTICOAGULANTS IN WOMEN WITH RIF

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Anticoagulants**	**No anticoagulants**	**Relative (95% CI)**	**Absolute (95% CI)**		
Live birth rate (RCT)												
2	Randomized trials[1,2]	Not serious	Not serious	Serious[a,b]	Serious[c]	None	36/117 (30.8%)	21/116 (18.1%)	RR 1.79 (1.06–2.73)	143 more per 1000 (from 11 more to 313 more)	⊕⊕OO Low	Critical
Live birth rate (RCT): restricted to ≥3 failures												
2	Randomized trials[1,2]	Not serious	Not serious [d]	Serious[a]	Serious[c]	None	22/79 (27.8%)	9/75 (12.0%)	RR 2.32 (1.14–4.71)	158 more per 1000 (from 17 more to 445 more)	⊕⊕OO Low	Critical
Clinical pregnancy rate (RCT)												
1	Randomized trials[2]	Not serious	Not serious	not serious[f]	Serious[e]	None	34/75 (45.3%)	29/75 (38.7%)	RR 1.17 (0.80–1.71)	66 more per 1000 (from 77 fewer to 275 more)	⊕⊕⊕O Moderate	Important
Implantation rate (RCT): restricted to ≥3 failures												
2	Randomized trials[1,2]	Not serious	Not serious	Serious[a]	Not serious	None	52/235 (22.1%)	26/229 (11.4%)	RR 1.95 (1.26–3.00)	108 more per 1000 (from 30 more to 227 more)	⊕⊕⊕O Moderate	Important
Miscarriage rate (RCT): restricted to ≥3 failures												
2	Randomized trials[1,2]	Not serious	Not serious	Serious[a]	Serious[g]	Strong association	2/29 (6.9%)	6/17 (35.3%)	RR 0.20 (0.04–0.86)	282 fewer per 1000 (from 339 fewer to 49 fewer)	⊕⊕⊕O Moderate	Important

[a] Qublan and colleages: patients were included only if they had ≥1 thrombophilic defect.[1] Urman and co-workers: patients were included if they screened negative for a coagulative defect.[2]

[b] Heterogeneous definition of RIF: Qublan and colleagues: ≥3 failures; Urman and co-workers: ≥2 failures but subanalysis for ≥3 failures.

[c] CI are wide (Qublan et al.) or cross a value of 1 (Urman et al.).

[d] Qublan and colleagues showed a much higher effect size (RR 9.76) than Urman and colleagues (RR 1.38), which could be related to patients having a thrombophilia defect in Qublan and colleagues' study.

[e] CI are wide and crosses a value of 1.

[f] Urman and co-workers: ≥2 failures but subanalysis for ≥3 failures.

[g] CI are wide and cross a value of 1. The number included was very small in both studies.

CI, confidence interval; RCT, randomized controlled trial; RIF, recurrent implantation failure; RR, risk ratio.

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Quality of evidence: low (TABLE 5).

Justification: studies are few, populations studied are heterogeneous, and study results are inconsistent.

11. Aspirin should not be routinely offered in RIF.

Strength: weak.

Quality of evidence: low.

Justification: there are no RCT evaluating aspirin alone, and only one RCT evaluating aspirin as a combination treatment.

Immune therapy in women with RIF

Implantation is an intricate process necessitating that a high-quality embryo interacts with and breaches the luminal surface of the endometrium before embedding in the decidualized endometrium. With embryo differentiation comes angiogenesis and remodelling of the spiral arteries that enable placentation and the maternal–fetal circulation. Immune modulation allows for this to occur, specifically a balance between T-helper 1 (Th1) and T-helper 2 (Th2) cytokines. Shifts toward Th1 lead to the production of pro-inflammatory cytokines (IFN-γ, IL-2 and tumour necrosis factor-alpha) that mediate a cytotoxic cell-mediated immune response and increase phagocytosis and inflammation. Th2 cells produce an anti-inflammatory response (via production of interleukins that inhibit phagocytosis). The role of peripheral natural killer cells is questionable in implantation and early pregnancy; however, uterine natural killer cells are the dominant immune cells in the decidualized endometrium after ovulation, with increasing accumulation around the trophoblast cells, therefore playing an important role in the regulation of placentation and normal invasion of the trophoblast (*Hviid, 2017*).

Intravenous immunoglobulin (IVIG) has been considered to enhance the action of regulatory T cells and reduce Th1 cytotoxic reactions (*Moraru et al., 2012*; *Ramos-Medina et al., 2014*). A single RCT included 54 women with repeated unexplained IVF failure completing 51 treatment cycles, with 26 women in the IVIG group and 25 in the placebo group. There was no statistically significant difference in any of the clinical outcomes between those receiving IVIG and those receiving saline (*Stephenson, 2011*).

Another area of interest in clinical therapy for RIF has been the role of intravenous Intralipid on pregnancy outcomes. Intralipid, a 20% fat emulsion, typically comprises 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerine and water. The typical role of Intralipid is in total parental nutrition; however, it is also proposed to have some role in immune modulation. Specifically, Intralipid has been thought to decrease natural killer cell activation and the production of pro-inflammatory cytokines (*Makrigiannakis, 2011*).

A recent single-blinded RCT investigated the effect of administration of intravenous Intralipid on pregnancy outcome in women with previous implantation failure (*Singh, 2019*). While some benefit was found, the study population was not specific for RIF. This trial enrolled 105 consecutive women with a history of one or more previous failed embryo transfers. Fifty-three women were assigned to Intralipid (4 ml Intralipid 20%; first dose on the day of oocyte retrieval, and second dose 1 h prior to embryo transfer). A statistically significant increase was found in LBR in the study group (18/52) compared with the control group (7/50), who were given a normal saline infusion ($P = 0.023$; RR 2.5, 95% CI 1.13–5.40; number needed to treat 4.9). When the results were analysed by number of prior failed IVF cycles, the beneficial effect was found in the women with only one previous failed IVF. Therefore, while there is an attempt to study the effect of Intralipid, conclusions regarding its benefit in a population of RIF cannot be made.

In recent years, infusion of peripheral blood mononuclear cells (PBMC) has been considered in the treatment of RIF. This therapeutic intervention is based on the theory that local immune cells at the implantation site actively contribute to embryo implantation. There are several prospective studies that report on the treatment of women with RIF using intrauterine infusion of treated PBMC (*Li et al., 2013*; *Madkour et al., 2016*; *Makrigiannakis et al., 2015*; *Okitsu et al., 2011*; *Yu et al., 2016*). Although most studies report an increase in LBR in the group receiving treatment compared with the placebo or control groups, these studies are inconsistent in terms of study population, exclusion criteria, PBMC dosage, preparation of PBMC and treatment protocol (i.e. fresh versus frozen and day of embryo transfer). Importantly, the cost and side effect profile of this treatment have not been documented.

The use of immune therapy in RIF is widespread yet not based on robust clinical evidence. It is therefore difficult to justify the use of limited resources such as human blood products, and potentially harmful therapies.

Other adjuvant therapies in the management of RIF

Adjuvant therapy is a treatment given in addition to the primary or initial therapy to maximize its effectiveness. The frustrations of both clinicians and patients in dealing with RIF has often led to the empirical use of additional therapies postulated to enhance implantation, but with very little evidence and biological plausibility to support their application.

Glucocorticoids have immunoregulatory properties and have been demonstrated to alter uterine natural killer cell activity (*Polanski et al., 2014a*); however, no prospective data exist evaluating their application in RIF.

Granulocyte colony stimulating factor (G-CSF) is a haematopoiesis-specific cytokine produced by bone marrow cells, stromal cells, fibroblasts and macrophages. It plays a role is in the promotion of neutrophilic granulocyte proliferation and differentiation, and has had clinical application in the treatment of myelosuppressive states such as aplastic anaemia and neutropenia in its recombinant form. It is expressed and produced by decidual cells and therefore its role in implantation has been considered.

In women with RIF there are two randomized datasets to consider in the evaluation of G-CSF as a therapeutic option. Davari-Tanha and colleagues published a randomized double-blinded placebo-controlled trial in which 40 patients with RIF were assigned to intrauterine infusion via transcervical catheterization of G-CSF, and 60 patients were assigned to placebo (40 saline and 20 placebo) (*Davari-Tanha et al., 2016*). There were no statistically significant differences in clinical pregnancy rates

TABLE 6 SUMMARY OF RECOMMENDATIONS

May be offered	Limited to research settings only	Not recommended
Karyotype testing	Serological and endometrial immune testing	Hysteroscopy if baseline ultrasound is normal
Preimplantation genetic testing for aneuploidies	Endometrial receptivity assays	Acquired and congenital thrombophilia workup
	Empirical low molecular weight heparin	Sperm DNA fragmentation index
	Immunotherapy, Intralipid, glucocorticoids, granulocyte colony-stimulating factor	Screening for chronic endometritis
		Endometrial injury in the preceding menstrual cycle
		Aspirin

or implantation rates. These data were, however, limited by the small sample size. A second trial included 90 patients, with 45 women in the treatment group who received a uterine infusion of 0.5 ml of human G-CSF at the time of oocyte retrieval (*Eftekhar et al., 2016*), and 45 women in the control group who received standard care; both groups received progesterone suppositories (400 mg twice a day) for luteal phase support. Results demonstrated an increased pregnancy rate in the G-CSF group of 29.5% (13/44) compared with 13.3% (6/45) in the control group ($P = 0.043$). While this study showed some benefit, the numbers are small and subsequent live birth or miscarriage rates were not reported. An further important consideration is that neither of these RCT reported on the side effect profile of their study group. In the literature, side effects include localized reaction and some cases of elevated leukocyte count (*Sbracia et al., 2014*; *Scarpellini and Sbracia, 2012*).

Recommendation:

12. The use of immunotherapy, Intralipid, glucocorticoids and G-CSF in RIF patients should be limited to research settings.

Strength: strong.

Quality of evidence: low.

Justification: the studies are few in number and small with respect to sample size. There are limited RCT data available, and the side effect profile is questionable and not documented, as well as there being a questionable use of resources.

CONCLUSION

RIF is a devastating reality for some patients employing assisted reproduction, and a perplexing problem for clinicians. There is heterogeneity in the definition itself, which has confounded the process of consistent study design and data collection, and overall has led to a lack of evidence to effectively guide investigation and management, as demonstrated in this GRADE-based guideline (TABLE 6). RIF can therefore be considered as an evolving field with a significant need for well-designed research. In the interim, patients need to be counselled on a case by case basis, taking into careful consideration their history, number of transfers to date and prognosis. Of note, many of the studies reviewed above include in their cohort of 'RIF' those that have failed only one or two embryo transfers.

In the current generation of ART recipients and practitioners, the temptation to intervene after two or more failed embryo transfers is significant. In doing so, we may just not be allowing for time and repetition to solve this clinical problem without the addition of unproven, costly and potential harmful treatments. A live birth can be achieved even in patients with RIF. For example, when cumulative LBR are examined over consecutive cycles, there is reason to maintain hope for most couples. Published Society for Assisted Reproductive Technology data (*Luke, 2012*) on cumulative LBR over seven embryo transfers show a conservative LBR of 56.8% with an optimal LBR of 87%. A more recent retrospective analysis (*Koot et al., 2019*) showed a cumulative LBR of 49% (95% CI 39–59%) with a median time to pregnancy of 9 months after diagnosis among women with RIF. These data suggest a reasonable LBR over time where patients and couples persevere and continue to try. For this issue where scientific data is lacking, a focus on more qualitative analysis and therapeutic attempts to support resilience in this group of vulnerable patients may be of more benefit, in comparison to harmful, ineffective therapeutics.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2020.08.007.

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