ESHRE GOOD PRACTICE RECOMMENDATIONS ON RECURRENT IMPLANTATION FAILURE.

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Introduction

Assisted Reproductive Technology (ART) provides treatment options for couples having difficulties conceiving naturally. For single women or same-sex couples ART represents the only option for achieving reproductive life plans. Despite advances in treatment approaches and laboratory technologies, many people fail to conceive with these technologies. When failure arises after serial attempts at IVF, the term 'recurrent implantation failure' (RIF) is often used. However, while this broadly descriptive term is often employed to focus discussions of clinical therapeutic options, it is evident that providing a name to unexplained IVF failure has not led to significant advances in its effective management. In contrast, RIF has become associated with widely publicised examples of poor and sometimes exploitative practices, leading to the so-called 'Add-on' debate. The field would appear to be at an impasse to which the very term 'RIF' may have contributed.

Implantation failure is a term commonly used to describe the situation in which a good quality embryo has been transferred into the uterine cavity but has failed to establish a pregnancy evidenced by ultrasound visualisation of an intrauterine gestational sac (Zegers-Hochschild, et al., 2017). Since this may happen more than once in women, the word 'recurrent' has been appended, leading to the emergence of a term akin to that used for women who experience more than one miscarriage. As with recurrent pregnancy loss (RPL), there is a lack of consistency in the clinical definition of RIF. Most definitions in current use are based on the number of embryos transferred with no pregnancy. However, with changing practices in embryo transfer, namely, from multiple to single embryo, from cleavage to blastocyst stage, from untested to chromosomally tested embryos, the implications of a single failed embryo transfer procedure have changed. A recent comprehensive survey of the definitions in use that employ this paradigm have suggested that a consensus is emerging that regards RIF as the failure to achieve a clinical pregnancy after two to three IVF cycles with one to four good quality embryos and that maternal age should also be taken into account (Cimadomo, et al., 2021). However, several problems arise with such a fixed and precise definition of RIF. Firstly, it does not take into account variables that affect the individual prognosis for successful treatment based on both patient and ART clinic-related factors. Secondly, the concept of RIF as a syndrome or disease that can be diagnosed and treated is open to challenge. This is illustrated by the difficulties faced by those seeking to provide clinical guidelines in this area, since the evidence base available does not permit robust conclusions to be drawn.

The ESHRE Working Group on RIF recognized that there is a need to look afresh at how RIF should be identified, defined, and managed. While there is an evidence base to scrutinise, it is the view of the RIF Working Group that the available literature has not generated clinical data of sufficient quality or clarity to permit a traditional guideline to be distilled. However, there is still a need for an evidence-supported document describing what represents 'Good Practice' in this challenging area of

reproductive medicine. This document aims to meet that need through a systematic search for and synthesis of published studies on the topic, a survey among stakeholders to support the threshold for RIF investigations, and clinical expertise of selected clinicians and embryologists.

Methods

The current good practice recommendations for RIF terminology, investigations and treatments have been developed according to the manual for development of ESHRE good practice recommendations (Vermeulen, et al., 2019).

A working group tasked with drafting a document for review was composed with representatives of the relevant ESHRE special interest groups (SIGs), notably the SIGs Implantation and Early pregnancy, Reproductive Endocrinology, and Embryology, and further completed with an independent chair (NM), an expert in statistics (DML) and support in literature searches and project management. In the first meetings, the working group discussed the topics to be covered and divided to work in subgroups with defined tasks. Progress with the different tasks and issues arising were discussed in regular online meetings.

A literature search through PUBMED and Cochrane was performed using the key terms “recurrent reproductive failure" OR "recurrent implantation failure" OR "repeated implantation failure“. All titles and abstracts were screened to identify relevant studies, for which full text papers were collected and summarized.

Recommendations for clinical practice were stated based on studies collected through the systematic search of the literature, recommendations in other guidelines (Coughlan, et al., 2014a, Mascarenhas, et al., 2021, Shaulov, et al., 2020, Sociedad Española de Fertilidad; Grupo de Trabajo de Fracaso Reproductivo), a previously performed survey providing details on current clinical practice (Cimadomo, et al., 2021) and the expert opinion of the working group.

The first draft of recommendations was shared among the different ESHRE SIGs for feedback and suggestions. Feedback was collected on the diagnosis and treatment options for RIF, as well as on the proposed threshold to determine RIF as a clinical situation warranting further clinical investigation or intervention. Feedback was received from 9 out of 14 SIGs. The feedback was discussed in an in-person working group meeting and addressed where relevant into a final draft of the paper which published on the ESHRE website between 1 November and 1 December 2022 for stakeholder review among the ESHRE membership. [TO BE COMPLETED IN THE FINAL VERSION] comments were received and incorporated where relevant. The report of the stakeholder review is available on www.eshre.eu/guidelines. The list of experts that contributed to the stakeholder review is included in **Supplementary data 1**.

The current document adheres to the previously published definitions for ART, in vitro fertilization (IVF), infertility, pregnancy, and live birth (Zegers-Hochschild, et al., 2017). Implantation rate is defined as the number of gestational sacs observed divided by the number of embryos transferred (usually expressed as a percentage), and is preferably calculated per ET procedure (Griesinger, 2016). Implantation is taken to describe the attachment and subsequent penetration by a zona-free blastocyst into the endometrium, resulting in the formation of a gestation sac (Zegers-Hochschild, et al., 2017). For the purposes of this document, successful implantation is taken to be the achievement of a positive pregnancy test (i.e. detection of beta hCG in serum or urine, or ultrasonographic

visualization of one or more gestational sacs with foetal heartbeat) following an embryo transfer procedure.

It is acknowledged that many studies investigating RIF and RIF interventions have primarily looked at pregnancy rates (PR) and live birth rates (LBR). Since these outcomes depend on many other factors that can arise after successful implantation, the focus of this document is on determinants of implantation, defined as having taken place when urinary or blood test is positive for hCG, rather than live birth. For consideration of factors causing recurrent pregnancy loss, the reader is referred to the ESHRE Guideline on Recurrent Pregnancy loss (ESHRE Guideline Group on RPL, et al., 2018).

Results

Defining RIF: from population to individual

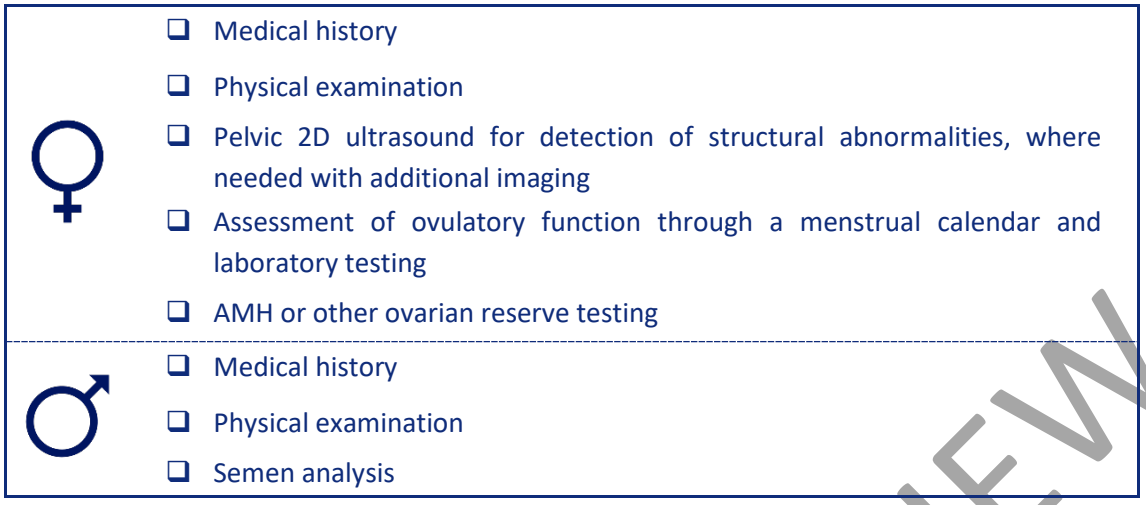
The ESHRE RIF Working Group recommends considering RIF as a secondary phenomenon of infertility or ART as it can only be observed in couples undergoing ART. In order to address a number of ambiguities in the definition to date, it is recommended that the following description of RIF is adopted:

RIF describes the scenario in which the transfer of embryos presumably viable has failed to result in a positive pregnancy test sufficiently often in a specific patient to warrant consideration of further investigations and/or interventions.

Considering RIF as a secondary phenomenon permits an individualized approach that is not dependent on a generic and 'one size fits all' criterion (e.g., fixed number of embryos transferred) but accounts for factors known to impact on the individual patient's chance of conception. Key to this concept is the need to identify how many embryos/embryo transfers would be expected to be necessary in a specific patient to provide an acceptable cumulative chance of successful implantation.

Another consequence of considering RIF as a secondary phenomenon of ART, is that it by definition can only occur in patients undergoing ART, and more specifically patients that would be able to achieve a pregnancy through ART. ART patients represent an heterogenous cohort with respect to the indication for treatment and the individual chances of achieving pregnancy. Infertile patients range from subfertile couples -who would be expected to conceive without treatment if they continue trying long enough - to couples who will not conceive without ART. Similarly, among those undergoing ART, some might be expected to succeed if sufficient cycles are undertaken while others will fail regardless of the number and types of treatments. In the latter group, a specific pathology or advanced ovarian age may account for the poor prognosis. Focussing on couples that would be able to achieve a pregnancy through ART implies that a standardised range of investigations (the 'fertility workup') will have already been completed before the treatment process starts and that patients are deemed suitable for ART and for carrying a pregnancy. The components of the fertility workup have been previously described by ESHRE (Vlaisavljevic, et al., 2021) (**Figure 1**). These recommendations for good practice in RIF assume that this baseline fertility workup will already have been carried out prior to commencing ART, but acknowledge that in different regions and jurisdictions other and/or additional tests and assessments are recommended (2019, National Institute for Health and Care Excellence, 2013, Toth, et al., 2019a, Toth, et al., 2019b) (see **Supplementary data 2**).

Figure 1. Standard fertility workup in female and male patients (Vlaisavljevic, et al., 2021).



Defining RIF in the individual couple or patient

Among ART patients, the chance of successful implantation will differ significantly. For the purposes of identifying RIF indicating further actions in specific patient, it is necessary to determine their residual chance of success should they simply carry on trying. If this is estimated to be less than an agreed cumulative threshold, then action may be indicated **(see figure 2)**. Patients whose history indicates that their chance of conceiving in a further cycle - given their specific clinical context - remains acceptable (i.e., their chance of implantation at the next cycle is higher than the threshold), should be advised to proceed to another ART cycle. However, in couples whose failure to conceive thus far indicates a relatively poor chance of success in the next cycle, the term RIF may be applied, and investigations of underlying contributing factors should be considered.

Two factors are essential for the individual approach for RIF: the model used to estimate the chance of implantation/pregnancy and the level at which the threshold to act is set.

Estimating the chance of implantation

The likelihood of successful implantation after ART is determined by a multitude of factors including, but not limited to, female-related factors such as age, hormonal levels, endometrial and uterine status and underlying conditions, embryo-related factors such as embryonic cleavage speed, euploidy, and previous implantations of sibling embryos, male factors like genetic disorders and external factors such as the performance of the laboratory and clinic, transfer policies and legal restrictions.

Ideally, a prediction model including all these factors should be used to provide estimates of the cumulative likelihood of successful implantation over a number of embryo transfers. Such a model is currently not available. However, published data from observational studies, the European IVF monitoring data collection, or the ART centre's own data can be used to derive a model that can provide guidance. Such models should at least consider maternal age, euploidy rate (if screened), and the number of embryos or blastocysts transferred.

Another approach is to use existing prediction models developed to predict the chance of live birth following the first fresh embryo transfer (ET) (Ata, et al., 2021, Ratna, et al., 2020). Typically, such models use a validated set of factors shown to impact on the chance of live birth and consider the

weight or importance of the distinct factors. Such prediction models can provide more precise and personalised estimates. Examples also include the "Dhillon Model," which accounts for female age, BMI, cause of infertility, ethnicity, previous live birth, previous miscarriage, antral-follicle count, and duration of infertility (Dhillon, et al., 2016) and the 'IVFpredict' tool derived from female age, duration of infertility, own versus donor oocytes, cause of infertility, previous IVF attempts, pregnancy history, medication, and IVF vs ICSI. (Nelson and Lawlor, 2011). The IVFpredict tool has been subject to external validation with varying outcomes (Saha, et al., 2015, Smith, et al., 2015, te Velde, et al., 2014).

With respect to RIF, the chosen model would be used to estimate the chance of pregnancy after each subsequent ET, which implies that a different calculation would be required. However, to limit complexity, the likelihood of implantation (or pregnancy) following a defined number of embryo transfers (n) can be approximated by the following formula $[\text{likelihood of implantation}]_n = 1 - [(1-PR)]^n$ where PR is pregnancy rate (or live birth rate *1.16 (Kolibianakis, et al., 2006)).

Setting a threshold for the cumulative chance of successful implantation to signal action.

Irrespective of the model used, a threshold needs to be defined to determine whether failure of a patient to achieve successful implantation indicates an issue or simply 'bad luck.' The threshold will guide the clinical decision on whether the patient should simply proceed to a further embryo transfer or whether investigations for factors contributing to RIF should be explored **(Figure 2)**.

To establish a threshold, input from a focus group of relevant professionals was gathered through the online survey. Focus group members were presented with 3 RIF cases and the implications of three different thresholds for cumulative success of implantation leading to pregnancy (70%, 60% and 50%). The focus group considered a threshold of 60% was considered the most relevant to guide clinical practice.

The recommended threshold for RIF is 60%, meaning that couples who have not had a successful implantation despite an estimated cumulative chance of implantation to date of at least 60% should be counselled on further investigation and/or treatment options.

Individual ART centres can apply other thresholds but should consider that the defined threshold will affect the proportion of women identified with RIF in whom further investigation or treatment alternatives will be considered.

Figure 3 summarises how the individualised definition of RIF should be integrated in clinical pathways.

Figure 2. Applied Example (Reig, et al., 2020, Wyns, et al., 2021)

36-year-old woman who has been trying to conceive for 3 years, has damaged tubes, never been pregnant and never had IVF before. She uses her own eggs.

Estimation based on the IVFPredict calculator

With the use of the IVFPredict calculator from the Nelson and Lawlor model (ivfpredict.com), the following calculations can be made for this specific patient:

Her chance of live birth per IVF attempt is 23.8%	according to the IVFPredict tool
Her chance of pregnancy per IVF attempt is 25.0%	calculated by multiplying the LBR by 1.16 to obtain chance of pregnancy i.e., 23.8 X 1.16 = 27.6%
The chance of pregnancy is	calculated by $NP_n = (1\text{-}PR)^n$
• **47% over the course of 2 ET attempts**	1- [(1-0.25) X (1-0.276)] = 0.47
• **62% over the course of 3 ET attempts**	1- $[(1\text{-}0.276)^3]$ = 0.62
• **72% over the course of 4 ET attempts**	1- $[(1\text{-}0.276)^4]$ = 0.72
• **80% over the course of 5 ET attempts**	1- $[(1\text{-}0.276)^5]$ = 0.80

According to the threshold for RIF of >60%,
if the woman is not pregnant after 3 ETs we intervene.

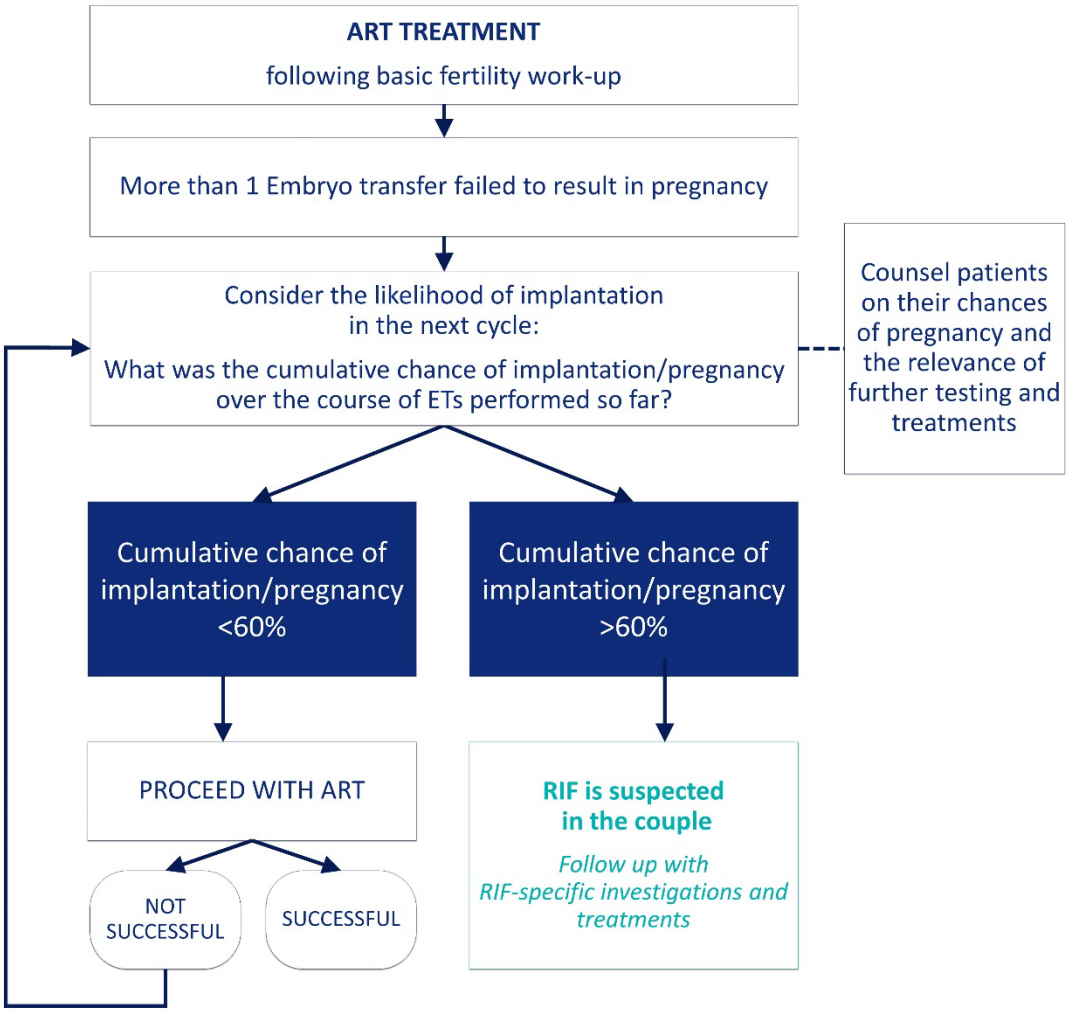
Crude estimation (without using a model) for maternal age and euploidy

It is recognized that carrying our individual calculations may not always be feasible in certain clinical contexts. In order to assist the concise identification of patients with RIF for whom further investigations/treatment are indicated, the following table provides an example of how individual clinic data can be used to guide management for embryos of unknown euploidy and embryos of known euploidy, respectively.

	Maternal age	Implantation rate / pregnancy rate[1]	Cumulative likelihood of implantation for each embryo transfer (embryos of unknown euploidy)						RIF THRESHOLD of >60%
			FIRST ET (n=1)	SECOND ET (n=2)	THIRD ET (n=3)	FOURTH ET (n=4)	FIFTH ET (n=5)	SIXTH ET (n=6)	
Embryos of unknown euploidy	**<34**	31,5	31,5	53,1	67,9	78,0	84,9	89,7	Intervene after 3 ETs
	35-39	25,9	25,9	45,1	59,3	69,9	77,7	83,4	Intervene after 4 ETs
	≥40	15	15,0	27,8	38,6	47,8	55,6	62,3	Intervene after 6 ETs
Euploid embryos	**<35**	68,4	68,4	90,0	96,8	99,0	99,7	99,9	Intervene after 1 ET
	35-40	64,1	64,1	87,1	95,4	98,3	99,4	99,8	Intervene after 1 ET
	>40	58,0	58,0	82,4	92,6	96,9	98,7	99,5	Intervene after 2 ETs

[1] For embryos of unknown euploidy, pregnancy rates for patients using own oocytes were used from the EIM data (Wyns C, *et al.*, 2021); for euploid embryos, pregnancy rates were used from published date (Reig A, *et al.,* 2020). For the sake of simplicity and because of a lack of positive hCG incidence data in the existing studies/registries, implantation and pregnancy were used exchangeable.

Figure 3. Summary: Applying an individualised RIF definition in clinical practice



Investigations and treatments for RIF

A myriad of different investigations and treatment procedures for RIF have been described in studies or applied in clinical practice. Systematic searches of the literature reveal most study populations to be small, often without inclusion of a control group and hampered by the lack of a standardised definition for RIF. Randomised controlled trials (RCTs) of tests or treatments for RIF are scarce. In order to derive recommendations for good practice when high quality evidence is sparse, it is necessary to look beyond published studies and consider additional information from sources such as published guidelines (Coughlan, 2018, Mascarenhas, et al., 2021, Shaulov, et al., 2020), reports of current practice (Cimadomo, et al., 2021), assessment of biological rationale and expert clinical opinion. Recognizing the limitations imposed by the current evidence base, this section aims to provide a framework to assist clinicians and couples in decision-making regarding RIF investigations and associated treatments.

In the context of RIF, investigations aim to identify contributing or causing factors for RIF. As previously stated, it is assumed that a complete pre-ART fertility workup as already been carried out and that the results are available for consideration. Similarly, the patient's age and past medical history - and

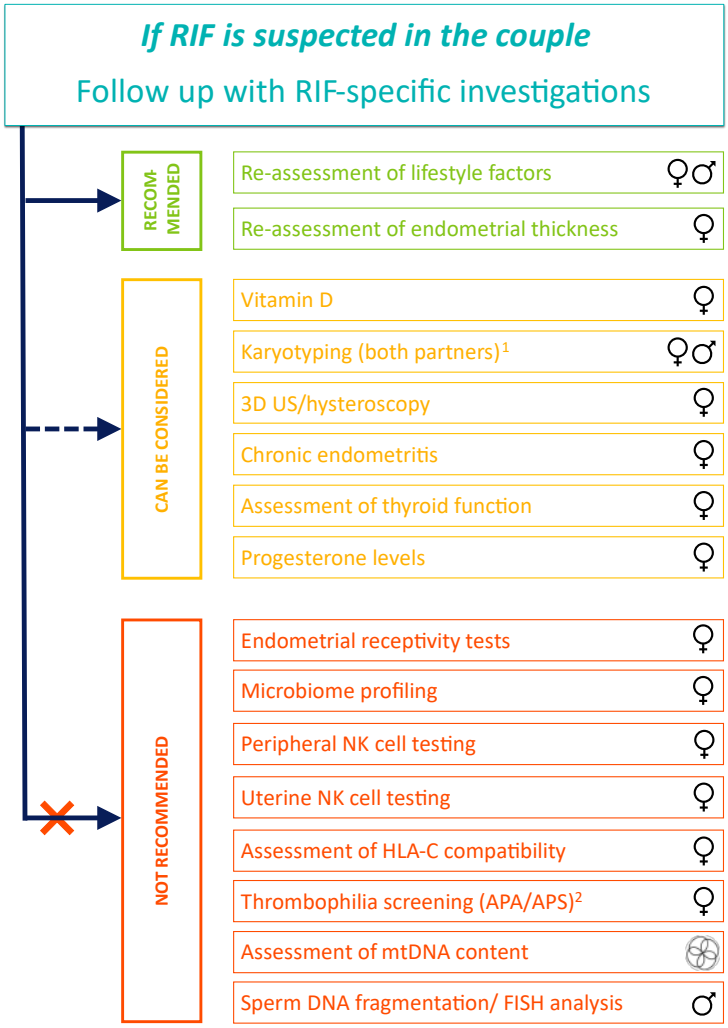
treatment (e.g., for malignant disease) are assumed to have been accounted for prior to embarking on treatment.

In order to place each test or treatment into context, data is provided (where available) on the reported prevalence of their use in clinical practice and the biological rationale underpinning their use.

This GPR document has been drafted and the statements hereby made have been agreed upon from the working group based on the current level of evidence on RIF. The group recognizes limitations to rely upon hard data in this regard, mainly due to the lack of standardization across the literature in the definition of RIF in the first place. Therefore, we suggest to re-assess in the context of academic and/or clinical research, especially not recommended diagnostic and treatment strategies, adopting the reviewed definition of RIF outlined in this GRP document.

A summary of all investigations and whether they are recommended, to be considered or not recommended is provided in **figure 4**.

Figure 4. Summary of RIF investigations



[1] to confirm the absence of a chromosomal abnormality; [2] in absence of additional risk factors

APA, antiphospholipid antibodies; APS, antiphospholipid antibody syndrome; mtDNA, mitochondrial DNA; NK, natural Killer; RIF, recurrent implantation failure; US, ultrasound

Investigating female factors

Lifestyle factors

In a large survey among 735 clinicians and 300 embryologists, more than two-thirds of clinicians reported taking female lifestyle factors into account, mainly drugs, smoking and BMI when managing RIF (Cimadomo, et al., 2021). Diet, stress, and caffeine intake were evaluated by about 50% of clinicians (Cimadomo, et al., 2021). Certain lifestyle behaviours such as cigarette smoking, alcohol consumption or caffeine have been associated with lower ART success rates (Hornstein, 2016, Kinney, et al., 2007, Ozbakir and Tulay, 2021). However while association studies abound, evidence from well designed intervention studies demonstrating an improvement in ART outcomes following short and/or long-term lifestyle changes remains scarce (Freour, et al., 2018, Kermack, et al., 2020, Wang, et al., 2021).

BMI is considered to be a relevant risk factor for ART failure (Moragianni, et al., 2012). Although most studies indicate that obesity does not significantly affect embryo quality (Bellver, et al., 2021a), the role of BMI on oocyte quality cannot be completely ruled out (Bellver, et al., 2010, Comstock, et al., 2015). Moreover, obesity may affect endometrial receptivity by displacing the window of implantation (WOI), the effect of which has been reported to be more pronounced in patients with class II-III obesity (Bellver, et al., 2021b).

While vitamin D assessment and supplementation is widely offered (Cimadomo, et al., 2021), its role in ART remains controversial: some studies found an association of serum and intrafollicular levels of vitamin D with pregnancy rates (Baldini, et al., 2021, Ozkan, et al., 2010) while others did not (Franasiak, et al., 2015). Recent data question the accuracy of vitamin D measurement (Franasiak, et al., 2021) and consequently the ability to determine vitamin D deficiency and potentially the susceptibility to poor ART outcome. Despite that , Vitamin D measurement and supplementation is considered a relevant RIF intervention by published guidelines and is widely applied in clinical practice (Cimadomo, et al., 2021).

	While lifestyle factors have been investigated during the fertility workup, patient behaviours can change so it is recommended to review these and their optimisation when RIF is encountered.
	Measuring vitamin D levels and treating deficiency can be considered.

Screening for genetic factors : karyotyping of the female partner

Embryonic chromosomal disorders represent the major cause of (early) pregnancy loss in humans (Papas and Kutteh, 2021). Aneuploid blastocysts have a significantly reduced developmental capacity during the preimplantation stage (Martín, et al., 2021, Rubio, et al., 2007) and reduced sustained implantation potential (Grati, et al., 2018). However, most of the embryonic chromosomal aneuploidies are of maternal meiotic origin.

In a survey of clinical practice, 67% of clinicians reported taking chromosomal disorders into consideration as potential risk factor for RIF and most clinicians assess both the female and male karyotype (Cimadomo, et al., 2021).

In line with these observations, case control studies have shown that karyotype anomalies are more frequent in RIF patients, even if the absolute prevalence is low (2.1%) (De Sutter, et al., 2012, Raziel, et al., 2002, Stern, et al., 1999). In fact, these figures are within the prevalence range of chromosomal abnormalities described in infertile couples undergoing ART, ranging from 2.8% to 12% in males and from 3.0% to 15% in females (Meschede, et al., 1998). With regards to the type of karyotype abnormalities in RIF couples (8 females and 5 males), autosomal abnormalities, sex chromosome aberrations and chromosomal mosaicism were found in 6, 2 and 1 females and 4, 0 and 1 males, respectively (De Sutter, et al., 2012).

The contribution of abnormal parental karyotype to predispose to chromosomal embryonic errors is plausible (Insogna, et al., 2021, Yuan, et al., 2021).

	Despite the low prevalence, karyotyping can be considered to confirm the absence of a chromosomal abnormality.
	If a chromosomal abnormality is detected, genetic counselling and, where relevant preimplantation genetic testing (PGT), is recommended.

Anatomical investigations

Eighty-five percent of clinicians have been reported to take anatomical and gynaecological investigations into account in diagnosing the cause of RIF (Cimadomo, et al., 2021). Asherman's syndrome, hydrosalpinx, endometriosis/adenomyosis, uterine malformations, endometrial atrophy, endometrial thickness, endometritis, and vaginal infections, as well as uterine fibroids are widely considered relevant. The endometrial microbiome, WOI and ovarian cysts were considered relevant by only 47%, 59% and 23% of clinicians, respectively. Hysteroscopy is the most widely used technique for anatomical investigations, followed by 3D and 2D transvaginal ultrasound (Cimadomo, et al., 2021).

Assessment of the uterine cavity

Transvaginal ultrasound is considered to be performed as part of the fertility workup.

Given the general diagnostic accuracy attributed to 3D transvaginal ultrasound, it has been proposed as an alternative non-invasive procedure for diagnosis of uterine anomalies (Grimbizis, et al., 2016) and a good practice approach. Currently, there are no studies evaluating whether 3D transvaginal ultrasound improves the outcomes in RIF patients. Given the limited cost and non-invasiveness, it can be considered as a routine diagnostic tool during fertility work up, when available. If not performed at the start of the ART treatment, it may be of benefit when assessing the patient presenting with RIF.

	If 3D ultrasound has not been performed at fertility workup, it can be considered.

The use of hysteroscopy is often proposed when uterine pathology has been detected by transvaginal ultrasound and further diagnostics are indicated (e.g., submucous fibroids, uterine adhesions). However, a large RCT (the TROPHY study) reported similar live birth rates (LBRs) after ART in RIF

patients (two to four failed IVF cycles) without a previous recognized pathology (n=702) when comparing those undergoing hysteroscopy versus those proceeding to ART without hysteroscopy (29% versus 29%, RR 1.0; 95% CI 0.79 to 1.25; p=0.96) (El-Toukhy, et al., 2016).

A meta-analysis focusing on patients with RIF, reported a significantly higher LBR after hysteroscopy compared to RIF patients that did not have hysteroscopy (RR 1.29; 95% CI 1.03 to 1.62; 4 studies; n=2247; p =0.046) (Cao, et al., 2018, Moffett and Shreeve, 2015).

Uterine cavity anomalies can be treated by established interventions including endometrial polypectomy, surgical removal of submucous fibroids, salpingectomy, uterine septum resection, or removal of intrauterine adhesions. While the interventions are established for treatment of symptoms, their impact on pregnancy or LBRs have, to our knowledge, not been evaluated in patients with RIF. Similarly, the effect of treatment of adenomyosis on pregnancy or live birth rates in women with RIF has not been evaluated.

Hysteroscopy can be considered, especially when there is a suspicion for a uterine anomaly visualised on transvaginal ultrasound.

There is a lack of studies evaluating hysterosalpingography (HSG) in the context of RIF.

Endometrial receptivity tests

The principal mechanisms underlying human endometrium receptivity are complex and still unclear. Given the numerous endometrial functions that can collectively be considered to represent 'receptivity' , it is unlikely that a single test would provide sufficient insight for clinical use. However, tests have emerged that focus on specific aspects of endometrial function. One such test entails the analysis of a panel of genes associated with endometrial receptivity from an endometrial biopsy taken during the putative WOI. Transcription of these genes is quantified and interpreted to report the endometrium as either pre-receptive, receptive, or post-receptive. Similar information can be provided by histological assessment of Noyes' criteria, but this has been shown to be too subjective for clinical use. Since then, several other endometrial receptivity tests similarly focusing on measuring maturation have been marketed. Recently, a comprehensive in-depth analysis of all the transcriptomic panels investigated for their association with an impaired endometrial receptivity have supported the hypothesis that RIF might be due to both displacement and disruption of the WOI (Koot, et al., 2016). This implies that a test aimed at assessing only one aspect will be of limited utility (Sebastian-Leon, et al., 2018).

A meta-analysis from 2022 included 11 studies and reported the prevalence of displaced WOI, as detected through endometrial receptivity tests was 34% (95% CI 24 to 43%) in RIF/poor prognosis patients (Liu, et al., 2022). In this patient population, comparable ongoing pregnancy rate (OPR)/LBR was found between patients undergoing personalised embryo transfer (p-ET) with endometrial receptivity testing and those with routine ET (40.7% vs. 49.6%; OR 0.94; 95% CI 0.70 to 1.26; 6 studies; n=2552) (Liu, et al., 2022).

A propensity score matching approach adopted to limit the effect of putative confounders showed no significant improvement in clinical outcomes after using an endometrial receptivity test for p-ET (Bergin, et al., 2021). A recent 5-year multicentre RCT comparing p-ET after endometrial receptivity

testing to fresh and frozen ET without the test showed comparable outcomes per transfer, but higher cumulative LBRs in the p-ET, particularly in a per-protocol analysis (Simón, et al., 2020).

There is insufficient evidence to support the routine use of endometrial receptivity testing in ART and more studies are required to discern its value in identifying and enabling the treatment of endometrial maturation defects in women presenting with RIF.

Tests of endometrial receptivity increasingly assess other aspects. One example is a test for 'uterine immunological disruption' based on RT-PCR analysis of a range of factors considered to be involved in differentiation of the secretory endometrium to the receptive state (Lédée, et al., 2017). While this test remains to be subject to assessment in RCTs, cohort studies (Lédée, et al., 2020). have suggested that it may have a role in the diagnostic work up of the endometrium in RIF, as indeed may other emerging tests.

There is insufficient data to recommend the routine use of any commercially available test of endometrial receptivity to diagnose the cause of RIF.

Investigating chronic endometritis

Chronic endometritis (CE) has been described in RIF patients with bacterial colonisation, but also in women without clinical signs of infection and can lower the pregnancy rate (Bouet, et al., 2016, Cicinelli, et al., 2015, Johnston-MacAnanny, et al., 2010, Kitaya, et al., 2019, Kitaya, et al., 2014, Kushnir, et al., 2016, Li, et al., 2020, Saxtorph, et al., 2020, Song, et al., 2018, Zargar, et al., 2020). It can be diagnosed by hysteroscopy, haematoxylin, and eosins (H&E) staining as well as CD138-labelling (Kitaya, 2019 #206;Kitaya, 2014 #207). Nowadays, chronic endometritis seems to be routinely investigated in clinical practice (85% of clinicians) (Cimadomo, et al., 2021), even if there is a lack of standardisation with regard to the concentration of plasma cells that should be regarded as a threshold (e.g. >1 or >5 plasma cells per high power field) and available studies often include only small numbers of patients, or lack controls.

Antibiotics (e.g., doxycycline) can be considered for the treatment of CE. A systematic review and meta-analysis, including 3 prospective and 2 retrospective studies, compared patients with cured chronic endometritis (treated with antibiotics) versus persistent chronic endometritis and reported significantly higher LBR/ongoing pregnancy rates (OR 6.81, 95% CI 2.08 to 22.24) in patients with cured chronic endometritis (Vitagliano, et al., 2018).

Assessment for chronic endometritis can be considered. A standardised diagnostic procedure for detection of CE in RIF is needed. If CE is diagnosed, treatment with antibiotics can be considered.

Re-assessment of endometrial thickness

Thin endometrium (≤ 7mm) in the late follicular phase may be associated with failed implantation. Despite the fact that endometrial thickness (EMT) is usually assessed before and monitored during IVF cycles, review of endometrial thickness and laminar pattern can be considered when facing RIF.

A recent systematic review and meta-analysis investigating the association between endometrial thickness and live birth rates in fresh cycles, reported that women with thin endometrium (EMT<7 mm) had significantly lower LBR compared to women with EMT>7 mm (OR 0.47, 95% CI 0.37-0.61) (Liao, et al., 2021). There was significant heterogeneity observed in the results, however, sensitivity analysis did not change the direction of the effect. An association between endometrial thickness/pattern and PRs has also been reported in frozen embryo transfers and stimulated cycles (Nishihara, et al., 2020, Shalom-Paz, et al., 2021). In a univariate aggregated data meta-analysis, the probability of clinical pregnancy in a next cycle in women with thin endometrium was found to be significantly lower compared to those with endometrial thickness > 7mm, with a positive and negative predictive value of 77% and 48%, respectively (Kasius, et al., 2014). After controlling for confounders, the potential independent association of endometrial thickness with ART treatment outcome has been reported as weak (Griesinger, et al., 2018, Yuan, et al., 2016).

If endometrial thickness is assessed and thin endometrium documented, ensuring sufficient exposure to estradiol by augmenting oral therapy with patches or vaginal treatment remains the mainstay of management (Vartanyan, et al., 2020). Intrauterine platelet-rich plasma (PRP) infusion has been investigated as a therapy to increase endometrial thickness, and some studies have suggested it can be effective in improving endometrial proliferation (Mouanness, et al., 2021), none to date have been conducted to evaluate its relevance for RIF patients with thin endometrium. Similarly, intrauterine G-CSF infusion for ART patients with thin endometrium has been proposed, and the few published studies show conflicting results (Rocha, et al., 2020). Further studies should elucidate the value of these and other interventions following the detection of thin endometrium in RIF patients.

If the endometrium remains thin despite adjustment of the endometrial preparation regimen, hysterosccopy should be considered to rule out adhesions or Asherman syndrome.

Re-assessment of endometrial thickness is recommended. Review of estradiol treatment regimen is recommended if the endometrium is noted to remain thin and hysteroscopy to rule out Asherman syndrome can be considered.

Microbiome profiling

Almost 10% of the bacterial population present in the body resides in the female genital tract and Lactobacillus species are part of the physiologic flora (Moreno and Simon, 2019). Whether microbial dysbiosis is among the explanatory factors of implantation failure is under study, but in clinical practice, about 50% of clinician considers this a relevant factor (Cimadomo, et al., 2021). Microbiome testing in the context of fertility treatment is attracting much attention and a number of studies have indicated it to offer promise as a potentially treatable factor to assist embryo implantation. A recent meta-analysis of cohort studies reviewed the outcomes in 1095 women, including 893 with a normal and 202 with disturbed vaginal microbiota. This indicated that dysbiotic vaginal microbiota lowered the chance of becoming pregnant after ART (Koedooder, et al., 2019, Singer, et al., 2019). Other studies have failed to demonstrate a correlation between the presence of Lactobacillus strains and pregnancy after ART (Franasiak, et al., 2016). With respect to RIF, a case-control study comparing the vaginal and endometrial microbial configuration through 16S rRNA gene sequencing in 145 RIF and

21 healthy women with male factor infertility showed lower levels of Lactobacillus only at the vaginal level but not in the endometrium of RIF patients (Ichiyama, et al., 2021).

While this is a dynamic area of research, a number of questions remain to be addressed before the proper place of microbiome testing in the context of RIF can be ascertained. These include the rate of spontaneous resolution of an unfavourable microbiome, changes that can occur during IVF treatment, and the efficacy of interventions aims at improving the microbiome. Finally, it remains unclear whether a suboptimal microbiome can itself disrupt implantation, or whether it is a marker for some other causative factor.

Uterine and vaginal microbiome profiling is not recommended.

Metabolic and endocrinologic factors

In a survey of clinical practice, endocrine aspects were considered relevant in RIF by 82% of clinicians, with the focus being mostly on thyroid function (98%), hyperprolactinemia (84%), diabetes (82%), and PCOS (Cimadomo, et al., 2021).

Whereas thyroid function may be considered as a diagnostic test, other endocrine factors such as thyroid autoimmunity, prolactin, free androgen levels or diabetes (HBA1C) are either not addressed or considered not to be relevant in RIF by other guidelines. However, as can be seen from the survey, the use of thyroid function in the diagnosis of RIF is well established in clinical practice (Cimadomo, et al., 2021). With regards to ART, serum thyroid stimulating hormone (TSH) levels >4 mIU/L (subclinical hypothyroidism) or <0.4 mIU/l (subclinical hyperthyroidism) may be considered as thyroid dysfunction and require further follow-up and treatment (Biondi, et al., 2015, Poppe, et al., 2021).

Assessment of thyroid function can be considered.

In recent years there has been growing interest in the link between late follicular and luteal phase blood progesterone (P4) levels and clinical outcomes. Initially the focus of attention was primarily on the reported association between premature progesterone rises, measured around the time of triggering oocyte maturation and outcomes after fresh embryo transfer. While still a topic of debate, there is a widespread view that this can lead to endometrial/embryo asynchrony, meriting delaying embryo transfer to a subsequent freeze thaw cycle (Bosch, et al., 2010, Venetis, et al., 2013). In many clinical contexts, vaginal progesterone represents the first line luteal support in frozen thaw cycles. Consistent with the possibility that absorption from the vagina may be variable between women, there is increasing evidence linking low blood P4 levels on the day of embryo transfer to poorer outcomes after fresh embryo transfer (Thomsen, et al., 2018) and after frozen embryo transfer (Alsbjerg, et al., 2018) (Labarta, et al., 2021, Lawrenz, et al., 2018). Deferred embryo transfer in cases of premature P4 elevation (Lawrenz, et al., 2018) and individualized P4 administration for the latter scenario (Álvarez, et al., 2021, Labarta, et al., 2021), have been shown to restore implantation rates in cohort studies. However, questions remain about the validity of published cut-off levels for individual centres as assays can vary. Local validation of cut-off P4 levels is recommended.

Assessment of late follicular and mid-luteal progesterone levels can be considered.

Immunological screening

The concept that an excessive maternal immune response to the implanting embryo is disruptive to implantation has obtained considerable traction. In clinical practice, immunological screening of some kind was applied by 69% of clinicians when managing RIF. The most cited tests were antithyroid antibodies (80%) and anti-neutrophil autoantibodies (ANA) (>60%) (Cimadomo, et al., 2021). However, a review published in 2017 did conclude that there is a lack of evidence to support ANA screening in RIF and supportive data for this practice remain scarce.

A full assessment of the clinical basis and utility of immunological screening in RIF is beyond the scope of this GPR, but the more common approaches used are addressed below.

Uterine and peripheral natural killer cells

Uterine natural killer cells (uNK cells) are known to be key players at the feto-maternal interface, where they represent around 70% of immune cells (Lash and Bulmer, 2011, Lédée-Bataille, et al., 2004, Moffett and Colucci, 2014, Seshadri and Sunkara, 2014, Tuckerman, et al., 2010, Vomstein, et al., 2020). However, as compared to peripheral NK cells (pNK cells), uNKs are less cytotoxic and demonstrate a different profile of secreted cytokines and receptor/gene expression, while both act as immunomodulators (Seshadri and Sunkara, 2014, Tang, et al., 2011, Vomstein, et al., 2020). Some studies found higher than normal uNK levels, resulting in an unfavourable implantation milieu (Kuon, et al., 2017b, Odendaal and Quenby, 2021). However, recently a theory has emerged that inadequate activation of uNK cells might be the cause of RIF (Alecsandru, et al., 2020). Either way, standardisation regarding a threshold remains elusive, even the definition of what constitutes a normal uNK cell population has yet to be agreed on, despite the application of range of techniques (FACS analysis, immunohistochemistry). In part, this is likely to represent the highly dynamic nature of uNK cell populations during the menstrual cycle: in the non-pregnant endometrium, uNK cells are mostly inactive but can undergo differentiation during the menstrual cycle in preparation of pregnancy (Strunz, et al., 2021).

While a meta-analysis, including 6 studies, and several other studies identified a subgroup of RIF patients suffering from high uNK concentrations (Chen, et al., 2017, Harrity, et al., 2019, Kuon, et al., 2017a, Marron, et al., 2019, Vomstein, et al., 2020, Woon, et al., 2022), others did not (Donoghue, et al., 2019) and the same is true for pNK cells in RIF (Salazar, et al., 2022, Seshadri and Sunkara, 2014). More recently, attention has moved from simply counting uNK populations to measuring their activity (see endometrial receptivity investigations).

One study compared CPR in women with RIF having high and normal uNK levels and found no significant difference between groups (RR 1.09; CI 0.75, 1.59; total 369 women; P = 0.29; (Marron and Harrity, 2019, Woon, et al., 2022).

A number of treatment approaches for RIF patients with elevated uNK including lipid infusions as well as glucocorticoid administration have been proposed (Quenby, et al., 2005). However, adequately

powered RCTs of targeted interventions are still required, and at present the value of testing remains unclear.

Peripheral NK cell testing is not recommended.

Uterine NK cell testing is not recommended.

T lymphocytes

Imbalances in CD4+ T-helper lymphocytes, i.e., Th1, Th2, Th17 and Treg, have been implicated to contribute to RIF (Ali, et al., 2018). In a systematic review, including 8 studies with RIF patients, a significant difference in total CD56+ cells was shown in women with RIF compared with controls (SMD 0.49, CI -0.01, 0.98; p=0.046; 604 women) (Woon, et al., 2022).

In a small case-control study, RIF patients showed significant reductions of blood polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), Myeloid-derived suppressor cells (M-MDSCs), Tregs and NO production by PMN-MDSCs, whereas the expression of ζ chain on CD4+ T-cell receptor and CD8+ T-cell receptor displayed a remarkable upregulation in RIF patients (Jiang, et al., 2017). Furthermore, a retrospective study reported a reduced blocking efficiency of CD3, CD4 and CD8 in patients with RIF (Gao, et al., 2021). Huang *et al*. compared patients with RIF who were successful to conceive with patients who failed and found higher percentages of CD3+ lymphocytes in the failed group (Huang, et al., 2021). However, no differences were observed in CD4+ and CD8+ lymphocytes in RIF (Harrity, et al., 2019). In another study, no significant differences in circulating T-lymphocytes were observed, although the authors reported a higher production of Th1 and Th2 cytokines (Lashley, et al., 2015).

Peripheral and uterine T lymphocytes assessment is not recommended.

Cytokine levels

During implantation, cytokines in the peripheral blood have been described as changing from a proinflammatory (Th1 type) to an anti-inflammatory (Th2 type) profile (Zhao, et al., 2021). While this may represent an over-simplification, some studies with small study populations showed that a pro-inflammatory state persists in women with RIF which might disturb implantation (Inagaki, et al., 2003, Liang, et al., 2015a, Liang, et al., 2015b, Marron and Harrity, 2019). However, as the assessment of cytokine levels is time-consuming and expensive, it is not applied in clinical practice.

The assessment of cytokine levels is not recommended.

HLA-C compatibility

Due to their genetic variability and ability to bind to specific HLA class I allotypes, killer immunoglobulin-like receptors (KIRs) on uNK cells have been considered good candidates for balancing maternal leukocyte tolerance towards the embryo. It has been postulated that an adequate interaction between maternal KIRs and their ligands human leukocyte antigen (HLA) class I molecules,

expressed by the extravillous trophoblast cells, is crucial for a sustained implantation (Díaz-Hernández, et al., 2021).

An increased risk of RIF is observed in women carrying the HLA-C2 allotype and the HLA-G allele with a 14bp insertion (Lashley, et al., 2014). However, the fact that neither human blastocysts at the time of transfer nor the syncytiotrophoblast express HLA-C, and that HLA-C starts to be expressed later during placentation, when the endovascular trophoblast starts to replace the spiral arteries (Blaschitz, et al., 2001), raises the importance of further research on the role of HLA-C in RIF. Moreover, its analysis is not widely applied in practice.

Assessing HLA-C compatibility is not recommended.

Thrombophilia screening

Thrombophilia is defined as a predisposition to form clots inappropriately. The presence of thrombophilia are considered to induce local vascular impairment with consequent difficulty in embryo implantation.

In a survey of clinical practice, haemostatic aspects were considered worthy of investigation in RIF by respectively 74% of clinicians, of whom 96% reported performing investigations for antiphospholipid antibody syndrome (APS) and 75% perform hereditary thrombophilia screening tests (Cimadomo, et al., 2021).

Qublan *et al.* reported that 68.9% of women with RIF had at least one inherited or acquired thromophilic factor, compared to 25.6% in women with a successful first IVF cycle and 25% in healthy fertile controls (Qublan, et al., 2006).

Inherited thrombophilia

Inherited thrombophilia are conditions in which a genetic mutation affects the amount or the function of a protein in the coagulation pathway. Mutations in several genes have been shown to be involved: G1619A (Factor V Leiden), R2 H1299R (Factor V Leiden polymorphism), A1298C (Methylenetetrahydrofolate reductase (MTHFR) enzyme mutation), C677T (MTHFR polymorphism), V34L (Factor XIII polymorphism), G20210A (mutation of the prothrombin gene), a/b L33P (ribosomal polymorphism of MTHFR enzyme) and 4G/5G (plasminogen activator inhibitor-1 (PAI-1)) (Neamţu, et al., 2021).

Inherited thrombophilia has been implicated in early pregnancy loss and implantation failure, by impairment of the vascular changes, necessary for successful pregnancy (Neamţu, et al., 2021, Qublan, et al., 2006).

Qublan *et al.* reported significantly more homozygous mutations in the Factor V Leiden and the MTHR (C677T) gene in women experiencing multiple IVF failures compared to women with a successful first IVF cycle and 25% in healthy fertile controls (Qublan, et al., 2006). Coulam *et al.* reported a higher prevalence of PAI-1 4G/5G mutations than controls in women with a history of implantation failure after IVF-embryo transfer (Coulam, et al., 2006). Azem *et al.* reported a significantly increased incidence of inherited thrombophilia in women with a history of four or more IVF failures compared to healthy fertile women (44.4% vs. 18.2%; OR 3.6; 95% CI 1.25 to 10.6) (Azem, et al., 2004). However,

several studies have reported that the incidences of aforementioned inherited thrombophilic defects in RIF women were not different from those in control (Simur, et al., 2009, Vaquero, et al., 2006).

Acquired thrombophilia

Examples of acquired thrombophilic abnormalities include acquired C protein, S protein, antiphospholipid syndrome (APS), antithrombin III deficiency, drugs induced thrombophilia are a well-known cause of RPL (Neamţu, et al., 2021).

There are some studies indicating an association with APS. So far, only few studies focused on APA or APS in RIF patients with diverging results (Bellver, et al., 2008, Hornstein, et al., 2000, Qublan, et al., 2006, Sauer, et al., 2010, Vaquero, et al., 2006). Furthermore, a recent study evaluated the prevalence of APS (meeting all clinical and laboratory criteria) in RIF patients with only 5/138 (2,88%) being affected by APS and <5% having APA (Vomstein, et al., 2020). While the investigation and management of both inherited and acquired thrombophilia's has been mainstay of the clinical approach to RIF and recurrent pregnancy loss, their role in the aetiology of both of these conditions is being increasingly challenged. Consistent with the recent ESHRE guideline on the management of recurrent pregnancy loss, the role of testing is likely to be very limited in the context of RIF. However, given the severe implications that Antiphospholipid syndrome can have on perinatal outcomes, it should be excluded prior to ART when there is any clinical suspicion.

Assessment of APA and APS without any additional risk factors for thrombophilia is not recommended.

Investigating factors related to the embryo

Mitochondrial DNA (mtDNA) content

The mtDNA content of human embryos has been proposed as a possible indicator of embryo viability and implantation potential. Several studies have reached contradictory results on mtDNA content according to embryo developmental day, embryo quality, maternal age, and implantation capacity. Due to the novelty of the topic, it has not been addressed in the guidelines, nor in the survey. The most recent study did not focus on embryos, but on the endometrium, studying the relationship between endometrial mtDNA copy number in RIF patients (Eker, et al., 2021). Receiver operating characteristic (ROC) curves showed 74% correct diagnoses for RIF, however given the experimental nature of the test, the small sample size and the small number of studies, further studies are required to reach a conclusion.

Evaluation of mitochondrial DNA (mtDNA) content in the embryos is not recommended.

Embryo/blastocyst quality

Poor embryo/blastocyst quality and morphokinetic abnormalities are associated with reduced reproductive competence, also in the context of euploid embryo transfers (Bamford, et al., 2022, Shear, et al., 2020, Zhan, et al., 2020). Nevertheless, embryo grading is highly subject to limited

(especially inter-center) reproducibility (Cimadomo, et al., 2022, Fordham, et al., 2022, Khosravi, et al., 2019). Artificial intelligence -powered tools are currently under investigation, which may standardize embryo evaluation and improve its reliability in the coming years (Kragh and Karstoft, 2021, Riegler, et al., 2021). In particular, artificial intelligence may provide objective definitions of embryo quality and generalizable estimates of its impact on implantation failure/success, with evident implications also in the definition of RIF.

Similarly, IVF spent media omic analyses are currently subject to intense academic, pre-clinical and clinical investigations. Nevertheless, the data to date are still preliminary and they have not been studied in the context of RIF, therefore they cannot be considered for the time being.

Investigating male factors

Investigating factors that can contribute to RIF in the male partner is widely applied and considered important by almost 80% of the participants. Such investigation includes questioning about lifestyle (e.g., smoking, drugs), semen analysis and sperm DNA fragmentation test (Cimadomo, et al., 2021).

Semen analysis; spermiogram, sperm fluorescence in situ hybridization (FISH), and sperm DNA-fragmentation

Semen analysis is part of the routine fertility workup prior to ART (2015). Deviations in sperm concentration, motility and morphology seem to be associated with lower conception rates (Jouannet, et al., 1988, WHO, 2021), but also low fertilisation and poor embryo development. In a study comparing RIF patients to controls, significantly better sperm motility and morphology were detected in the RIF couples, indicating a lack of robustness of sperm parameters as a contributing factor to RIF (Ocal, et al., 2012).

Sperm FISH is a cytogenetic clinical diagnostic assay that assesses the frequencies of chromosomal abnormalities, considered useful in counselling RPL patients with previously failed ART (WHO, 2021). A retrospective case control study showed no correlation of FISH analysis with RIF (Rodrigo, et al., 2019) and others reported aberrant FISH results in only 14.8% (4/27) of RIF patients without impact on implantation or pregnancy rates (Sarrate, et al., 2019).

There are a number of different sperm DNA-fragmentation test, and currently there is no standardisation on the methodologies and threshold for normal values. In addition, there are conflicting results regarding sperm DNA fragmentation testing and clinical pregnancy following ART (Cissen, et al., 2016, Evenson and Wixon, 2006, Simon, et al., 2017). A recent large retrospective cohort study including 1339 undergoing 2759 IVF/ICSI cycles reported that there was no significant difference in live birth rate per first embryo transfer between ≤15% and >15% SDF groups: 38.2% (95% CI 34.5 to 41.9; n = 665) versus 41.9% (95% CI 34.2 to 49.7; n = 155; OR 1.2, 95% CI 0.8 to 1.7; p = 0.4). Similarly, cumulative LBR was not significantly different between groups with high or low SDF (Hervás, et al., 2022). While sperm DNA fragmentation is suggested to be a contributing factor to RPL and unexplained infertility, data specifically in RIF patients are scarce. Furthermore, there is no consensus on the cost-effectiveness of the test in general or in couples with RIF (Hervás, et al., 2022, Minhas, et al., 2021).

Sperm DNA fragmentation and Sperm FISH analysis are not recommended .

Different treatments have been suggested as viable options for male partners of RIF patients. These include improving semen quality, such as antioxidant use, and techniques to select functional sperm, such as Magnetic-Activated Cell Sorting (MACS), Intracytoplasmic morphologically selected sperm injection (IMSI) and other sperm selection techniques, and surgical sperm retrieval (e.g., testicular sperm extraction). However, so far there are no studies that have evaluated these interventions in couples with RIF which were of sufficient quality to support any recommendations.

Lifestyle factors

Obesity, especially when is accompanied by metabolic syndrome, correlates with poor semen quality (Ma, et al., 2019, McPherson and Tremellen, 2020, Tremellen and Pearce, 2020). Likewise, lifestyle habits in men, such as smoking, high caffeine intake or alcohol consumption and drug abuse seem to negatively alter conventional semen parameters, but also other molecular aspects such as sperm DNA integrity or redox status (Rahban and Nef, 2020).

Lifestyle interventions in men can help to improve certain sperm parameters as well as embryo quality (Velotti, et al., 2021), but such interventions have not been evaluated with regards to their impact on RIF.

While lifestyle factors have been investigated during the fertility workup, it is recommended to revise lifestyle factors and their optimisation at the time of RIF, especially since lifestyle factors may have changed in the course of the ART treatment.

Screening for genetic factors – karyotyping of the male partner

Despite the low prevalence, karyotyping can be considered to confirm the absence of a chromosomal abnormality.

If a chromosomal abnormality is detected, genetic counselling and, where relevant preimplantation genetic testing (PGT), is recommended.

Interventions for RIF

Nearly 80% of clinicians offer treatments preconceptionally, 75% offer additional treatment during next ART, and 69% consider oocyte or sperm donation a treatment option in RIF (Cimadomo, et al., 2021). Preconception treatments mainly focus on lifestyle advice, vitamin supplementation, antioxidant therapy and treatments for endometritis and endometriosis are widely prescribed. In addition, endometrial scratch and immune-modulation therapy are also applied, usually empirically and without any diagnostic rationale. Other widely practised interventions include luteal phase adjuvant therapies after ET and the transfer of frozen thawed embryos. Popular strategies employed in the ART lab include PGT-A (68%), assisted hatching (61%), addition of growth factors to culture

media (27%) and time-lapse microscopy (40%). TESE is offered by 57% of clinicians, with fewer clinicians offering PICSI of MACS.

The considerable range of interventions employed does not reflect the evidence base, but the perceived need to act. Given this challenging landscape, this good practice document aims to support clinical practice by summarizing studies evaluating interventions aimed at improving the chance of successful implantation ad indicating when the evidence base suggests that an intervention is recommended, can be considered, or is not recommended. The results of these studies should be interpreted with caution for several reasons. Firstly, the definition of RIF applied varies, and the study cohort of one study may differ significantly from that of another. Variations in what constituted the fertility workup prior to ART also leads to heterogeneity, as does embryo transfer strategy. Moreover, sample sizes tend to be small, and, in most cases, interventions are tested without any attempt to diagnose the cause of RIF.

A summary of all interventions and whether they are recommended, to be considered or not recommended is provided in **figure 5**.

Treatments independent of RIF investigations

Most studies focusing on treatment options in RIF evaluated interventions independent of any diagnostic investigation.

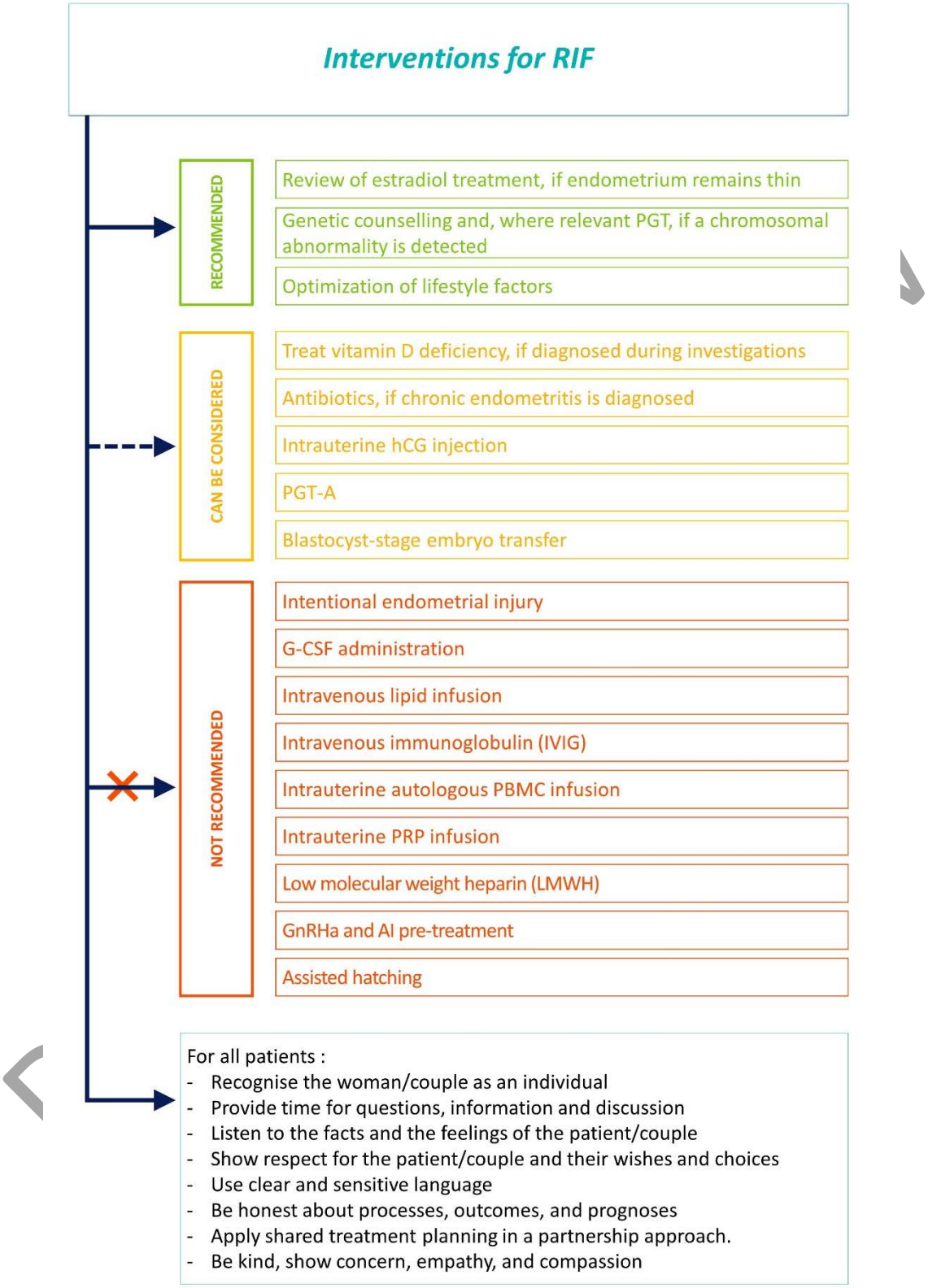
Intentional endometrial injury

Endometrial injury or scratch is performed to improve the receptivity of the endometrium towards the transferred embryo. The biological mechanism of action is not fully understood.

A meta-analysis by Busnelli *et al*. reported that, based on 3 RCTs, there was no significantly increased chances of pregnancy and LBR in women who underwent intentional endometrial injury (random effects model, RR 1.43; 95% CI 0.79 to 2.61; p=0.24; I^2=52% and random effects model, RR 1.55; 95% CI 0.81 to 2.94; p=0.18; I^2=46%, respectively) (Busnelli, et al., 2021). Consistent conclusions on CPR were reported from two included observational studies. A more recent RCT, including 211 women also reported no significant increase in foetal heartbeat, abortion or multiple pregnancy rate in women who underwent intentional endometrial injury (Zahiri, et al., 2021). A Cochrane review by Lensen and colleagues reported similar data from a sub-analysis on RIF (Lensen, et al., 2021).

Intentional endometrial injury is not recommended.

Figure 5. Summary of RIF interventions



AI, aromatase inhibitor; GnRHa, GnRH agonist; G-CSF, Granulocyte colony-stimulating factor; mtDNA, mitochondrial DNA; PBMC, peripheral blood mononuclear cells; PGT, preimplantation genetic testing; PRP, platelet-rich plasma, RIF, recurrent implantation failure.

Granulocyte colony-stimulating factor (G-CSF) administration

G-CSF plays a role in embryo implantation and the continuation of pregnancy by temporarily suppressing immune response through its effects on lymphocytes, macrophages and T helper-2 cells (Moldenhauer, et al., 2010). Its use may be associated with recruiting dendritic cells, promoting Th-2 cytokine secretion, and activating T-regulatory cells, favouring the local immune responses, vascular remodelling of the endometrium, and cellular adhesion pathways (Rahmati, et al., 2014). When administered systemically, G-CSF has been reported to play a role in embryonic development, implantation and trophoblastic growth (Würfel, 2015), while local intrauterine administration could improve endometrial receptivity (Rahmati, et al., 2014).

Few studies evaluated the effect of subcutaneous or intrauterine G-CSF administration in RIF. A meta-analysis investigated the impact of intrauterine and subcutaneous G-CSF infusion in patients with RIF (Busnelli, et al., 2021). Subcutaneous G-CSF administration was associated with an increased chance of clinical pregnancy (RR 2.29; 95% CI 1.58 to 3.31, 4 RCT, n=333) compared with no treatment. Intrauterine administration had no impact on CPR (RR 1.53, 95% CI 1.00 to 2.33, 2 RCT, n=257). The only RCT reporting live birth rates failed to show a benefit (RR 0.84; 95% CI 0.41 to 1.73, n=157). Two more recent RCTs on intrauterine G-CSF administration in patients with RIF confirmed these findings (Karimi A., et al., 2020, Torky, et al., 2021b).

Side-effects or adverse events for G-CSF administration include mucositis, splenic enlargement, hepatomegaly, transient hypotension, epistaxis, urinary abnormalities, osteoporosis, exacerbation of rheumatoid arthritis, anaemia, pseudogout (Moffett and Shreeve, 2015).

G-CSF administration (either intrauterine or subcutaneous) is not recommended.

Intravenous lipid infusion

Intravenous lipid infusion may have a role in immune modulation including reduction of platelet aggregation, decrease of IL-2, TNF-α, and IL-1β production as well as suppression of natural killer cell levels and activity.

Few RCTs evaluated the effectiveness of lipid infusions during ART in RIF patients. A systematic review and meta-analysis, including 5 RCTs totalling 843 patients, reported a higher clinical pregnancy (172 vs. 119; RR 1.55; 95% CI 1.16 to 2.07; I^2=44.2%) and LBR (132 vs. 73; RR 1.83; 95% CI 1.42 to 2.35; I^2=0%) with intervention (Rimmer, et al., 2021).

In a multicentre study evaluating lipid infusions and prednisone in 64 RIF patients higher CPR were found in treated patients (44% vs. 9%; p<0.001) with odds ratio at 8.13 (95% CI 4.49 to 14.72; p<0.0001) (Kolanska, et al., 2021). Another study evaluated lipid infusions in 94 RIF patients with an immune profile of endometrial over-immune activation and reported a LBR of 54% following the next ET (Lédée, et al., 2018).

Side-effects or adverse events for intralipid therapy include hepatomegaly, jaundice, cholestasis, splenomegaly, thrombocytopenia, leukopenia and fat overload syndrome (Moffett and Shreeve, 2015).

Intravenous lipid infusion is not recommended.

Intravenous immunoglobulin (IVIG)

The intravenous injection of IgG is suggested to have immunomodulatory actions by neutralizing autoantibodies, downregulation of B-cell and T-cell function and blockage of Fc Receptors.

The review of Abdolmohammadi-Vahid *et al.* included 2 cohort studies and 2 cross-sectional studies focusing on IVIG in RIF and showed a significant difference in the pregnancy rate (cohort studies: OR 1.82; 95% CI 1.14 to 2.89; p=0.01 and cross-sectional studies: OR 11.12; 95% CI 6.43 to 19.23; p<0.00001) and LBR (cohort studies: OR 2.17; 95% CI 1.30 to 3.61; p=0.003 and cross-sectional studies: OR 7.57; 95% CI 4.53 to 12.64; p<0.00001) in the IVIG group compared to controls (Abdolmohammadi-Vahid, et al., 2019). One more recent observational study reported significantly increased CPR and LBR in treated women (OR 2.08; 95% CI 1.28 to 3.36; p=0.003 and OR 1.76; 95% CI 1.08 to 2.89; p=0.02, respectively) (Busnelli, et al., 2021, Ho, et al., 2019). However, study populations are small.

Side-effects or adverse events for IVIG include aseptic meningitis, renal failure, thromboembolism, haemolytic reactions, anaphylactic reactions, lung disease, enteritis, dermatologic disorders and infectious diseases. An additional ethical concern is the diversion of IVIG from patients with serious conditions necessitating strict allocation of the limited supplies available (Moffett and Shreeve, 2015).

Intravenous immunoglobulin (IVIG) is not recommended.

Intrauterine autologous peripheral blood mononuclear cells (PBMC) infusion

The rationale supporting this treatment is the local production of cytokines by such stimulated peripheral blood mononuclear cells which could improve blastocyst invasion to the endometrium. However, this hypothetic mechanism of actions has not been substantiated in *in vivo* studies.

A meta-analysis, including studies with RIF patients experiencing ≥3 failed embryo transfers, showed a beneficial effect of intrauterine PBMC infusion with regard to PR and LBR (RR 1.92; 95% CI 1.48 to 2.49; p<0.001 and RR 1.93; 95% CI 1.35 to 2.76; p<0.001; 1 RCTs + 3 studies) (Maleki-Hajiagha, et al., 2019). A more recent systematic review, RCT and study confirmed the findings of the meta-analysis (Busnelli, et al., 2021, Chakrabarti, et al., 2019, Pourmoghadam, et al., 2020). However, the study populations are small and the definitions for RIF inconsistent. Furthermore, techniques to prepare PBMC differed substantially between studies (co-cultured in the presence of HCG, CRH, HMG, a mixture of fresh and co-cultured PBMC).

Comprehensive data regarding side effects, complications, and adverse pregnancy outcomes were not available (Maleki-Hajiagha, et al., 2019).

Intrauterine autologous peripheral blood mononuclear cells (PBMC) infusion is not recommended.

Intrauterine platelet-rich plasma (PRP) infusion

Platelet-rich plasma (PRP) is an autologous concentrate of platelets in plasma. Cytokines and growth factors present in PRP are considered to exert a regenerative effect on tissues and cells, including the endometrial lining (Mouanness, et al., 2021).

Busnelli *et al.* reported, based on 2 RCTs and a total of 195 patients (Nazari, et al., 2019, Zamaniyan, et al., 2020), that administration of intrauterine PRP resulted in a significantly increased chance of clinical pregnancy (fixed effects model: RR 2.45; 95% CI 1.55 to 3.86; p=0.0001; I^2=0%) (Busnelli, et al., 2021). A more recent RCT confirmed findings of significantly higher pregnancy outcomes in women receiving PRP (Nazari 2022; PMID 34651260). Women included in the trials were not selected for thin endometrium.

A previous meta-analysis, which did not include the most recent RCT, and employed less stringent inclusion criteria, included 3 RCTs and 4 cohort studies and reported a significantly higher probability of clinical pregnancy in the PRP group (RR: 1.79; 95% CI 1.37 to 2.32; p<0.001; I^2=16%; n=625) (Maleki-Hajiagha, et al., 2020).

Aghajanzadeh *et al*. reported from a study of 30 RIF patients that there is no significant improvement in the implantation or OPR of frozen-thawed embryo recipients treated with PRP as compared to previous cycles without PRP (implantation rate 6.7% vs. 0.0%, with or without PRP) (Aghajanzadeh, et al., 2020). In another small retrospective cohort study, PRP in 15 patients with RIF and 39 with thin endometrium (< 8mm) resulted in significantly improved CPR (27.2% versus 9.6%, respectively), but no increase endometrial thickness in the PRP cycle compared to the previous ET cycle (Enatsu, et al., 2022). Comprehensive data regarding side effects, complications, and adverse pregnancy outcomes were not available. Furthermore, PRP is characterized by its absolute platelet concentration, which is any concentration above that of whole blood, causing wide variance between studies. Information regarding PRP preparation in individual studies is insufficiently reported (Maleki-Hajiagha, et al., 2020).

Intrauterine platelet-rich plasma (PRP) infusion is not recommended.

Intrauterine hCG injection

The infusion of hCG may help to initiate and control blastocyst invasion and improve immune tolerance from the mother (Zenclussen, et al., 2006).

Based on two observational studies, the effect of intrauterine hCG injection in women with RIF (≥3 failed ET) and normal endometrial thickness (8–16 mm) was reported to significantly increased CPR (fixed effects model: OR 1.81; 95% CI 1.23 to 2.65; n=482; p=0.002; I^2=0%) and LBR (OR 1.78; 95% CI 1.02 to 3.09; n=303; p=0.04) (Busnelli, et al., 2021, Huang, et al., 2018, Liu, et al., 2019). Liu *et al.* showed a beneficial effect of intrauterine hCG injection on implantation rate (OR 1.71; 95% CI 1.08 to 2.71; p=0.02) (Liu, et al., 2019).

An older, less stringent systematic review on intrauterine hCG administration in RIF patients (≥2 failed ET) also showed increased live birth rates of 27.8 vs. 18.0% in controls (RR 1.52; 95% CI 1.18 to 1.96; 3 studies, n=870) and increased CPR in the treatment group versus controls (41.8 vs. 31.2%; RR 1.30; 95% CI 1.14 to 1.50; 6 studies; n=1432) (Xie, et al., 2019). A more recent RCT, including 98 women also

compared intrauterine hCG injection with placebo and reported significantly higher CPR (23/49 (46.9%) vs. 11/48 (22.9%)) and implantation rates (28/120 (23.3%) vs 16/118 (13.6%)) with hCG treatment (Torky, et al., 2021b).

There is significant heterogeneity between trials concerning hCG dosage and timing of administration, volume of perfusion fluid and type of transfer cycle (fresh or frozen).

Intrauterine hCG injection can be considered.

Low molecular weight heparin (LMWH)

Low molecular weight heparin (LMWH) was found to have a significant impact on LBR in women with acquired thrombophilia. It has been postulated that the anticoagulation effect of heparin prevents placental thrombosis and infarction and promotes establishment and continuation of pregnancy (Nelson and Greer, 2008). Considering a possible association of thrombophilia with RPL and RIF, the use of LMWH has been expanded to these ART patients, even in the absence of acquired or inherited thrombophilia.

A systematic review and investigated the use of LMWH in patients with RIF (≥3 failed ET). Meta-analysis of the two included RCTs failed to show an effect of LMWH on both LBR (RR 1.38; 95% CI 0.64 to 2.96, n=71) and CPR (RR1.39; 95% CI 0.87 to 2.23, n=218) (Busnelli, et al., 2021). The observational study by Berker *et al.* also failed to show a difference in live birth or pregnancy rates (Berker, et al., 2011, Busnelli, et al., 2021).

Included studies had small study populations and focusing on RIF patients without thrombophilia or including patients with thrombophilia (Busnelli, et al., 2021, Potdar, et al., 2013, Siristatidis, et al., 2018). LMW heparin has a good safety profile in pregnancy, however, it may cause bruising and bleeding.

Low molecular weight heparin (LMWH) is not recommended.

GnRH agonist and aromatase inhibitor pre-treatment

Considering endometriosis may be an underlying and undiagnosed cause of RIF, it was hypothesised that empirical treatment prior to ET may improve pregnancy outcomes (Steiner, et al., 2019).

In an RCT, 67 women with at least two implantation failures were randomised to receive GnRH agonist (0.1 mg/day) from day 21 of the cycle preceding FET. The dose was reduced to 0.05 mg/day from cycle day 2. Control group received no GnRH agonist. No significant differences were found in CPR (25.8% vs. 19.4%) or implantation rate (13.55% vs. 10.52%) in study versus control group (Davar, et al., 2020).

In a retrospective cohort study, older infertile patients (36-43 years of age) undergoing their third or more embryo transfer after autologous IVF or ICSI were included. The study group received a single injection of 3.75 mg long acting triptorelin acetate on day 2 of the preceding cycle, followed by hormone replacement therapy (HRT). The control group received HRT only. CPR (124/290 (48.97%) vs. 68/194 (35.05%), OPR 109/290 (37.59%) vs. 44/194 (22.68%), and LBR (106/290 (36.55%) vs. 43/194

(22.16%)) were significantly higher in the study group compared to controls. Miscarriage rates did not differ between groups (Pan, et al., 2022).

In a retrospective cohort study, infertile women who failed two blastocyst transfers underwent a third frozen blastocyst transfer (Steiner, et al., 2019). Prior to the third ET, 143 received 2 months of GnRH agonist (3.75 mg intramuscular leuprolide acetate monthly) only, and 176 received GnRH agonist and aromatase inhibitor (5 mg oral letrozole daily for 60 days), and 204 received no pre-treatment. CPR and LBR were higher among women who received GnRH agonist plus letrozole compared with women who received GnRH agonist only or women without pre-treatment (CPR: 63%, 42%, and 40%, respectively; p<0.0001; LBR: 56%, 36%, and 34%; p<0.0001). However, there was no difference between no pre-treatment and GnRH agonist only pre-treatment.

GnRH agonist and aromatase inhibitor pre-treatment is not recommended.

Preimplantation genetic testing for aneuploidy (PGT-A)

While the rationale for offering PGT for structural rearrangements (PGT-SR) for RIF couples with a diagnosed chromosomal disorder seems clear, PGT-A is also offered to RIF couples in general**.** Treatment benefit is suggested from the deselection of embryos diagnosed with uniform whole-chromosome aneuploidies, namely the main embryonic cause of pregnancy loss and implantation failure in humans. Specifically, aneuploid blastocysts transferred in the context of blinded non-selection or unblinded cohort studies resulted in an overall 98% lethality rate per transfer and >86% miscarriage rate per clinical pregnancy (Capalbo, et al., 2022), thus supporting the use of PGT-A in populations of patients subject to higher embryo aneuploidy rates, such advanced maternal age women.

Busnelli *et al.* included 2 RCTs (Blockeel, et al., 2008, Rubio, et al., 2013) and three observational studies (Greco, et al., 2014, Sato, et al., 2020, Yakin, et al., 2008) investigating the potential role of PGT-A in improving IVF outcomes in women with RIF. The meta-analysis of RCTs failed to show an improvement in both clinical pregnancy and RIF (random effects model: RR 1.07; 95% CI 0.36 to 3.15; p=0.90; I^2=89% and RR 0.98; 95% CI 0.32 to 2.94; p=0.97; I^2=87%) in women who underwent PGT-A.

Comparable results were obtained in Yakin *et al*, however, they all used the old-fashioned FISH approach analysing a limited number of chromosomes in conjunction with the Day 3-biopsy (Yakin, et al., 2008).

In contrast, the two retrospective studies where embryo testing was conducted by either array CGH or NGS approaches on blastocyst biopsies, concluded that PGT-A could be considered a good strategy for women with RIF as a reduced number of embryo transfers were required to achieve pregnancy and live birth.

Preimplantation genetic testing for aneuploidy (PGT-A) can be considered.

Blastocyst-stage ET

Blastocyst stage embryos may have a better chance of implantation due to a lower risk of embryo aneuploidy, better synchronisation with the endometrium and fewer uterine contractions at the time of transfer. A systematic review of 27 studies showed, with a low level of evidence, that BR after fresh transfer was higher in the blastocyst transfer group compared to the cleavage group (OR 1.48; 95% CI 1.20 to 1.82) (Glujovsky, et al., 2016).

A more recent RCT found no difference in CPR or LBR between Day 3 double ET (DET) and Day 5 DET (Torky, et al., 2021a).

Another prospective cohort study with 575 RIF patients, compared single frozen/thawed blastocyst-stage transfer with frozen/thawed double-cleavage-stage embryo transfer and reported higher clinical pregnancy (OR 1.27; 95% CI 1.11 to 1.47); implantation (OR 1.51; 95% CI 1.21 to 1.89) and OPR (OR 1.43; 95% CI 1.19 to 1.73) in the patients undergoing single blastocyst transfer (Zhang, et al., 2019).

Blastocyst-stage embryo transfer can be considered.

Assisted Hatching

The inability of the blastocyst to escape from its zona pellucida is considered one of the pathways leading to unsuccessful ART, including implantation failure. Assisted blastocyst hatching could in that respect be an option to facilitate implantation.

A systematic review, including one RCT and one observational study, evaluated assisted hatching on ART outcomes in RIF patients after at least three failed ETs and exclusion of probable causes of RIF (Busnelli, et al., 2021). Assisted hatching did not increase CPR (RCT data: RR 0.78; 95% CI 0.48 to 1.27; p=0.31; observational data: OR 1.42; 95% CI 0.45 to 4.48; p=0.55) or LBR (observational data: OR 1.92; 95% CI 0.48 to 7.67; p=0.36) (Busnelli, et al., 2021, Primi, et al., 2004, Rufas-Sapir, et al., 2004).

Other studies, excluded in the review based on their definition of RIF, reported similar outcomes for CPR. Two studies additionally reported that the contribution of assisted hatching by partial zona dissection to successful implantation was related to the patient's age: patients older than 38 years showed a markedly higher PR after assisted hatching (Kanyo, et al., 2016, Stein, et al., 1995). Valojerdi *et al.* commented that a benefit of assisted hatching was found in the patients with frozen-thawed embryos, the rates were statistically significantly higher in the test group as compared with those of the control group (31.2% and 12.8%, respectively) (Valojerdi, et al., 2008). Yet another study compared the benefit of assisted hatching in patients with optimal versus suboptimal embryo quality and reported better results in patients with optimal embryo quality (Grace, et al., 2007)

Assisted hatching is not recommended.

Other treatments

Other treatments, that have been suggested for RIF, including additional interventions in the lab (e.g., time-lapse imaging), medical treatments (sildenafil), adaptations in the embryo transfer procedure (e.g., ultrasound-guided ET, performing a trial ET, ensuring the catheter tip is >15mm from the fundus,

recommending a full bladder at ET, cervical dilatation, cervical mucus removal, use of fibrin sealant, use of antibiotics, using hyaluronic acid supplemented ET medium, bed rest following the procedure), and adaptations in the ET strategy (e.g., frozen ET). To our knowledge, there are no studies evaluating the effect of these interventions on the chances of LBR in RIF patients.

It should be added that couples diagnosed with RIF may benefit from moving to third-party donation for further ART cycles. While third-party donation brings a new set of challenges, and requires support and stringent provision of information, it could bypass an underlying (unidentified) issue with the sperm, oocyte, or embryo. Studies are needed to confirm that resorting to ART with donated sperm or oocytes indeed improves the chances of a pregnancy after RIF.

Treatment based on diagnostic findings

Few studies have evaluated interventions for RIF with an established underlying factor, including antibiotics for treatment of CE or operative hysteroscopy for uterine disorders.

Within the OPTIMUM trial, RIF patients (n=116) were treated according to an identified possible risk factor (e.g., CE with antibiotics, aberrant high Th1/Th2 cell ratios with vitamin D and/or tacrolimus, overt/subclinical hypothyroidism with levothyroxine, and thrombophilia with low-dose aspirin) (Kuroda, et al., 2020). In the patients aged <40 years and ≥40 years, the ongoing pregnancy rate in the OPTIMUM group was significantly higher than that in the control group (57.4% and 30.3% versus 21.4% and 0% per ET, respectively; $p < 0.01$).

Patient care and counselling

The fertility treatment journey, from the fertility work-up to the actual treatments and pregnancy, has an effect on the mental health of patients, and the effect is significantly higher in patients with unsuccessful treatments (Boivin, et al., 2022). Women with RIF have been reported to have significantly higher levels of stress as compared to fertile healthy controls and admitted to feelings of social isolation, sensitivity to comments, a need for parenthood, diminished sexual enjoyment, and rejection of a childfree lifestyle (Coughlan, et al., 2014b). “Low levels of hope” is another factor closely related to mental health and emotional state. The study by Ni *et al.* showed that the levels of hope were significantly lower in patients after repeated IVF cycles as compared to those undergoing a first cycle (Ni, et al., 2021). No information was available for the male partners in RIF couples.

It has been suggested that the stress level experienced by RIF women may fluctuate in response to the amount of supportive care that they receive from the clinical staff, the results of investigative procedures (which influence the prognosis), and the experience and outcome of any subsequent treatment, but this has not been studied (Coughlan, et al., 2014b). Still, as psychosocial care is considered an essential part of the fertility treatment and should be provided before, during and after ART treatments (Gameiro, et al., 2015), efforts should be made to provide supportive care to couples with RIF.

There is no “one-size-fits-all” model for supportive care for couples with RIF, but based on guidance on RPL (ESHRE Guideline Group on RPL, et al., 2018), the following approach can be applied:

- Recognise the woman/couple as an individual

- Provide time for questions, information, repetition, and discussion, especially when the patient/couple is distressed or anxious.
- Listen to the facts and the feelings of the patient/couple
- Show respect for the patient/couple and their wishes and choices
- Use clear and sensitive language: explain terminology, avoid insensitive terms, and mirror the patient's preferred terms
- Be honest about processes, likely outcomes, and prognoses, and avoid false reassurance. This includes being honest on the evidence and benefit (or lack of benefit) for the investigations and treatments that have been proposed for RIF and are being applied in clinical practice without a solid ground. Patients/couples can further be reassured based on their individual estimation of the likelihood of implantation in a next cycle that simply continuing with ART treatment is a good option for them. Further support on this can be derived from a study showing that half of patients with RIF achieve a live birth with ART within 5 years (Koot, et al., 2019).
- Apply shared treatment planning in a partnership approach. It was recently suggested that a multi-cycle approach could be beneficial in this respect as it would consider cycle failure and how to cope with it, from the start of the treatment process (Harrison, et al., 2022).
- Be kind, show concern, empathy, and compassion.

Discussion

In these recommendations for good clinical practice, the ESHRE Working group encourages the reconsideration of RIF from being a medical condition with fixed diagnostic criteria, to a clinical secondary phenomenon of ART that can arise at different moment in different patients, and which requires a degree of empathy and pragmatism to manage well. The recommendations provided are based on this approach, with a clear acknowledgement of that lack of a robust evidence base to support them. However, it is the nature and requirement of clinical medicine to advise what is best for a patient given their individual clinical context, even when hard data is scarce. It is to be hoped that that in the coming years, studies will be published that can provide a firmer basis to clinical recommendations and allow a clear consensus for the optimal management of RIF to emerge. Ideally, all investigations used in RIF patients will have proven clinical utility and relevance. Tests will be performed in order to detect an underlying problem or assess a contributing factor to the implantation failures and linked to a specific intervention that has been shown to improve the chances of a live birth in a next cycle. Additional tests that do not have a linked intervention can be considered for patient counselling and to estimate the relevance of continuing ART treatment or resort to other reproductive options.

The need for further research in RIF

The need for research into the causes of implantation failure has been identified as one of the top ten research priorities in MAR (Duffy, et al., 2021). This is indeed key to making progress the clinical management of RIF. Further studies of empirical interventions in patients with RIF of unknown cause are unlikely to be helpful and may be considered a waste of research resources. Ideally interventions should be tested in those with clear cause of RIF for which a biological rationale exists for the intervention. To date such studies have been few. Ideally, future clinical guidance in RIF would allow

a set of relevant investigations, each with a specific linked treatment options shown to be effective for resolve the specific and detected indication.

In this respect, the herein proposed definition of RIF should be applied in future research studies as it will reduce homogeneity both in the study population as well as across studies which should be helpful towards meaningful study outcomes and feasible meta-analysis.

With regards to specific investigations and treatments, the following topics should be priorities for researchers:

- The role of vitamin D determination and supplementation (in case of low levels) in RIF patients.
- The role of immunological factors as an underlying factor in RIF, methods to investigate these and efficacy of targeted treatments.
- The role of thin endometrium, as well as the relevance of specific treatments to increase the chance of a pregnancy in patients with RIF and detected thin endometrium.
- The clinical value of sperm DNA-fragmentation tests
- Possible genetic predispositions to extreme IVF outcomes, such as RIF (Capalbo, et al., 2021).
- The value of treatments such as intrauterine autologous PBMC infusion, intrauterine PRP infusion and intrauterine hCG injection to prevent implantation failure in a next cycle should be further evaluated.

Apart from the clinical aspect of RIF, more insight and data are needed on the impact of RIF on the stress, mental health, and wellbeing of patients, and on supportive treatment options that could minimize such impact and lead to better care.

While awaiting the results of further studies and trials, the ESHRE Working group recommends the approach summarised in Figures 3, 4 and 5, which is to individualise the diagnosis of RIF based on the chance of successful implantation for the individual patient or couple, and to restrict investigations and treatments to those supported by a clear rationale and data on their benefit.

Conflict of Interest

NM declared consulting fees from ArtPRED (The Netherlands) and Freya Biosciences (Denmark); Honoraria for lectures from Gedeon Richter, Merck, Abbott and IBSA; being co-founder of Verso Biosense. DC declared honoraria for lectures from Merck, Organon, IBSA and Fairtility; support for attending meetings from Cooper Surgical, Fujifilm Irvine Scientific. GG declared Grants from Ferring, Merck, Gedeon-Richter, and ObsEVA; Consulting fees from Ferring, Merck, Gedeon-Richter, PregLem, Abbott, Vifor; Honoraria for lectures from Ferring, Merck, Gedeon-Richter, PregLem, Abbott, Vifor, Cooper, Organon, ReprodWissen, ObsEVA; Payment for expert testimony from Abbott Saudi Arabia; Member of the Guideline Development Group on ART of the German Medical Association ("wissenschaftlicher Beirat der Bundesärztekammer", 2014-2022); Head of the PGD working group of the German Association of IVF Centres (BRZ) since 2017; Member of the Quality Control Group of the German Medical Association ("Lenkungsgremium QS Repromed der Bundesärztekammer",since 2013); Delegate for the federal state Schleswig-Holstein in the Northern German Quality Control Audit commission for ART practice ("Küstenanrainerkommission", since 2018); Editor at Journal RBMonline (since 2022); Editor at Journal Archives of Obstetrics and Gynceology (since 2015); Editor in Chief of Journal Gynäkologische Endokrinologie. DM declared being associate Editor for Human Reproduction Open and statistical Advisor for Reproductive Biomed Online. BT declared being shareholder of Reprognostics; support for attending meetings from Astropharm, Ferring. The other authors had nothing to disclose.

986 **Supplementary data 1 – List of experts participating in the stakeholder review**

987 [LIST TO BE ADDED IN THE FINAL VERSION]

988 **Supplementary data 2 - Basic fertility work-up**

TEST	*Detection of*	ESHRE (Vlaisavljevic, et al., 2021)	ASRM/ ACOG (2019)	DGGG, OEGGG and SGGG (Toth, et al., 2019a, Toth, et al., 2019b)	NICE (National Institute for Health and Care Excellence, 2013)
Female					
Medical history		V	V	V	
Physical examination		V	V[1]	V	
2D US (+extra imaging)	*structural abnormalities*	V	V	V	
Hysterosalpingography	*Tubal patency*			V	V
Menstrual calendar + laboratory testing	*ovulatory function*	V	V	V[2]	V
Serum progesterone	*ovulatory function*			V	V
AMH or other ovarian reserve testing	*ovarian reserve*	V	V	V	V
Chlamydial serology	*chronic chlamydia infection*			**Optional**	V
HIV, hepatitis B and hepatitis C				V	V
Further tests based on clinical suspicion				V	
Male					
Medical history		V	V	V	
Physical examination		V		V	
Semen analysis		V	V	V	V
Endocrine examination				V[3]	
Sperm DNA fragmentation				**optional**	

989

[1] focus on vital signs and include a thyroid, breast, and pelvic examination

[2] determination of LH, FSH, prolactin, testosterone, DHEAS, SHBG, free androgen index, estradiol

[3] FSH and testosterone

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