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Mangement of RECURRENT IMPLANTATION FAILURE

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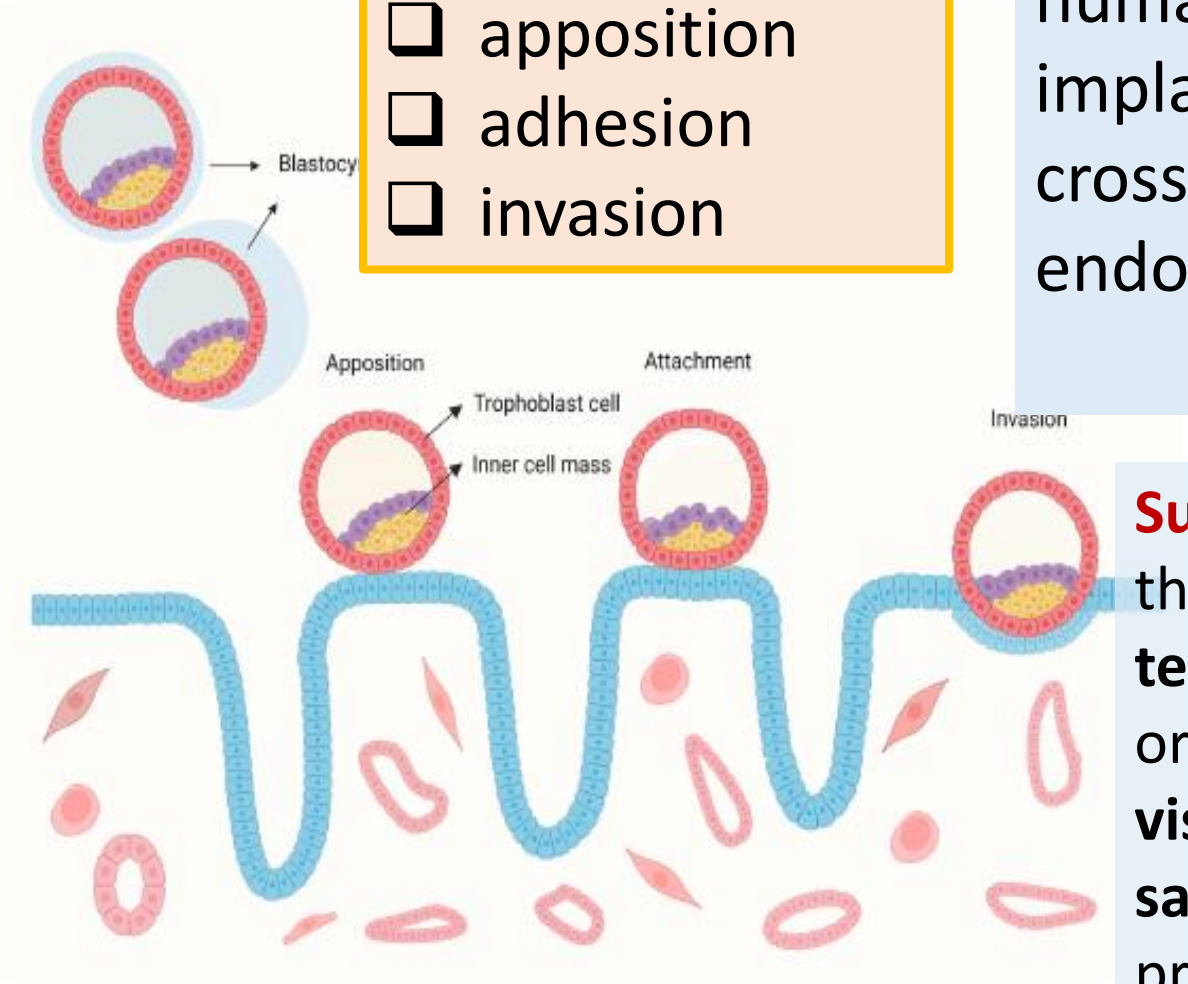
The implantation process includes:

- ❑ apposition
- ❑ adhesion
- ❑ invasion



Implantation is the first step in human reproduction. Successful implantation depends on the crosstalk between embryo and endometrium.

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** following an embryo transfer procedure.

- **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).

Recurrent implantation failure (RIF)
is a clinical phenomenon characterized by a lack of implantation after the transfer of several embryos and disturbs approximately 10% couples undergoing in vitro fertilization and embryo transfer.

- 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 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).

➢a good-quality embryo means day 3 embryo ≥ 8 cells, symmetric, with <10% fragmentation

➢blastocyst with a grade ≥ BBB

- Istanbul Consensus workshop on embryo assessment: proceedings of an expert meeting. Reprod BioMed Online(2011)
- Gardner DK, et al.,Fertil Steril (2004)

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.
- **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.**

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 **.**

- 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 &
- 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.

- **Such a model is currently not available**.
- Such models should at least consider **maternal age**, **euploidy rate** (if screened), and **the number of embryos** or **blastocysts** transferred.

- use existing prediction models :
- 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)
- the '**IVF predict**' **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

- To limit complexity, the likelihood of **implantation (or pregnancy) following a defined number of embryo transfers (n)**
- can be approximated by the following formula [likelihood of implantation]

$$[\text{likelihood of implantation}]_n = 1 - [(1- \quad PR)]^n$$

- **where PR is pregnancy rate (or live birth rate *1.16** (Kolibianak,2006)
- LBR from IVF predictor tool
- **PR =LBR⊗1.16**

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 27,6%	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**	$1- [(1-0.276) \times (1-0.276)] = 0.47$
• **62% over the course of 3 ET attempts**	$1- [(1-0.276)^3] = 0.62$
• **72% over the course of 4 ET attempts**	$1- [(1-0.276)^4] = 0.72$
• **80% over the course of 5 ET attempts**	$1- [(1-0.276)^5] = 0.80$

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

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

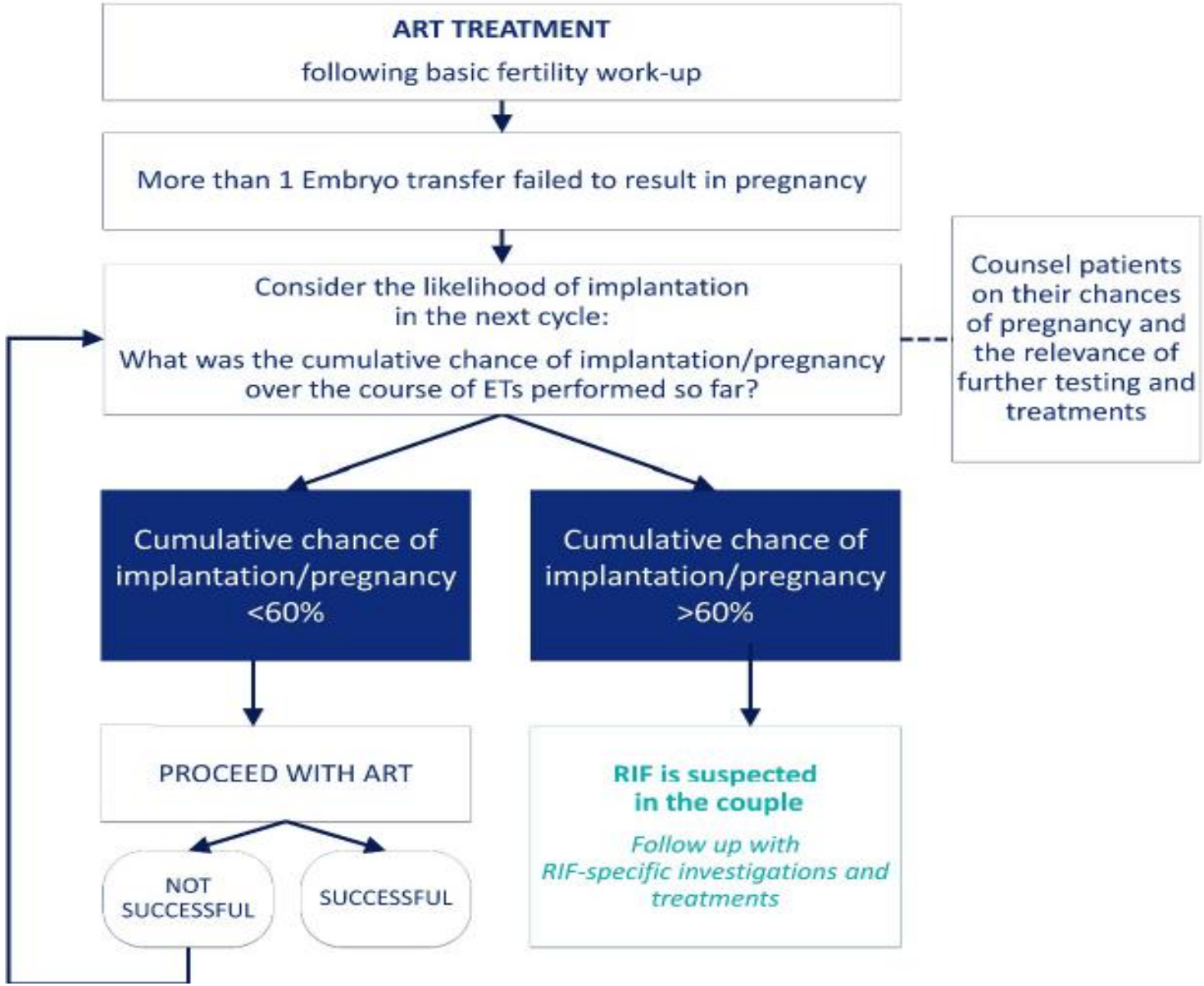
- The threshold will guide the clinical decision on whether the patient should simply proceed to a further embryo transfer or whether investigations for factors contributing to RIF should be explored .

- 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.**

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



Summary: Applying an individualised RIF definition in clinical practice

Recurrent implantation failure: reality or a statistical mirage?

Consensus statement from the July 1, 2022 Lugano Workshop on recurrent implantation failure

(The writing group) for the participants to the 2022 Lugano RIF Workshop, Paul Pirtea, M.D.,[a] Marcelle I. Cedars, M.D.,[b] Kate Devine, M.D.,[c] Baris Ata, M.D., M.Sc.,[d,e] Jason Franasiak, M.D.,[f] Catherine Racowsky, Ph.D.,[a] Jim Toner, M.D., Ph.D.,[g] Richard T. Scott, M.D.,[f] Dominique de Ziegler, M.D.,[a] and Kurt T. Barnhart, M.D., M.S.C.E.[h]

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A systematic review without meta-analysis of studies published in English from January 2015 to May 2022.
Findings: Data indicated that RIF has been largely overevaluated, overdiagnosed, and overtreated without sufficient critical assessment of its true nature.
. Our analyses show that true RIF is extremely uncommon—occurring in **<5%** of couples with infertility—and that reassurance and continued conventional therapies are warranted in most cases of assisted reproductive technology (ART) failure.

- these findings indicate that studying the outcome of euploid ET is most likely the best method for analyzing the prevalence of RIF.
- Cimadomo D, Capalbo A, Dovere L, Tacconi L, Soscia D, Giancani A, et al. Leave the past behind: women's reproductive history shows no associationwith blastocysts' euploidy and limited association with live birth rates after euploid embryo transfers. Hum Reprod 2021;

Estimation model for of the number of unscreened good-quality embryos needed to be equivalent to 3 successive euploid embryo transfers and achieve a 95% chance of sustained implantation on the basis of the observed aneuploidy rate (20).

Age (y)	Observed aneuploidy rate	No. of untested blastocysts to achieve a 95% chance of sustained implantation
<35	20%	4
35–37	30%	5
38–40	50%	7
41–42	70%	13
≥43	85%	27

Recurrent implantation failure. Fertil Steril 2023.

Risk factors

Known risk factors for RIF include

- **body mass index (BMI):**

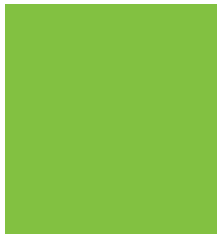
In IVF-ET, obese patients tend to have a **lower PR** than normal-weight Orvieto R, Int J Gynaecol Obstet(2009)

when BMI was ≥ 30 kg/m2 IVF-ET had significantly decreased odds of implantation(Moragianni VA, Fertil Steril (2012)). obesity can alter the markers of uterine receptivity and decidualization, which may contribute to a decrease in the implantation rateSchulte MM, Reprod Sci(2015)

Although most studies indicate that obesity does not significantly affect embryo quality (Bellver et al., 2021) , the role of BMI on oocyte quality cannot be completely ruled out (Bellver et al., 2010; Comstock et al., 2015)

- **smoking,** for patients who smoked for > 5 years, smoking was associated with fewer oocytes retrieved, a higher cycle cancellation rate, and a lower implantation rate .
- for male partners, smoking negatively affects sperm motility and counts and increases sperm DNA damage,Klonoff-CohenHum Reprod, (2001)
- **alcohol consumption**
- **stress.**
- Certain lifestyle behaviours, such as cigarette smoking, alcohol consumption or **caffeine**, have been associated with lower ART success rates (Kinney et al., 2007; Hornstein, 2016; Ozbakir and Tulay, 2021).

- Maternal stress, measured by the level of cortisol, increased the risk of miscarriage by 2.7-fold .
- that stress did not affect the outcomes of patients undergoing the first cycle. Failure of the last IVF cycle leads to a high risk of stress .
- Ma J, Gao W, Li D. Recurrent implantation failure: A comprehensive summary from etiology to treatment. Front Endocrinol (Lausanne). 2023



While lifestyle factors have been investigated during the fertility workup, patient behaviours can change so it is recommended to review these and their optimization when RIF is encountered.

There are insufficient data to recommend the routine measurement of vitamin D levels or treatment of vitamin D deficiency.

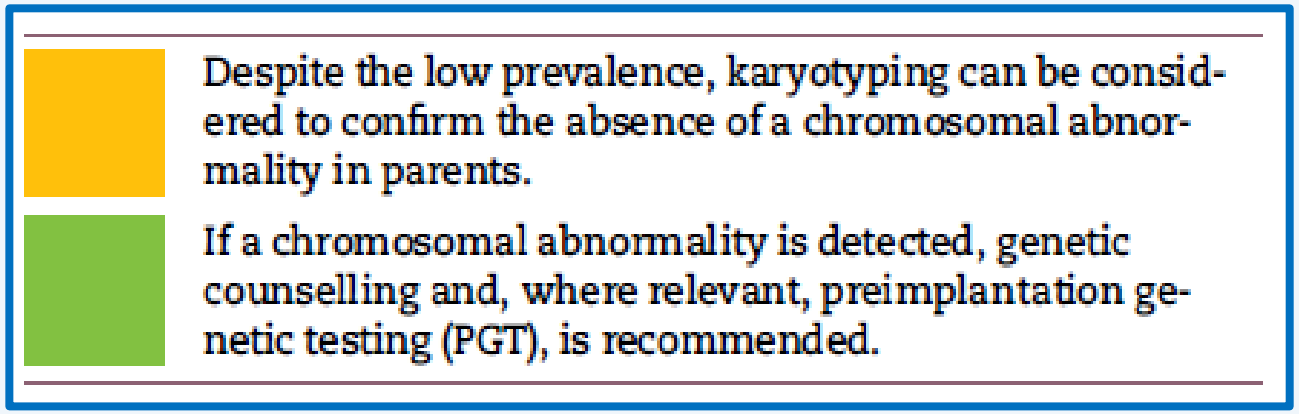
Screening for genetic factors: karyotyping of the female and male partner

In line with these observations, case–control studies have shown that karyotype anomalies are more frequent in patients with RIF, even if the absolute prevalence (2.1%) is low (Stern et al., 1999; Raziel et al., 2002; De Sutter et al., 2012).

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).

- Some of the most common Genetic anomalies suspected in **RIF** are translocations found in (1.4% and 3.2%) of individuals, **significantly higher** than the rate of translocations reported in couples with infertility (0.3%) .

- Stern, C.; Pertile, M.; Norris, H.; Hale, L.; Baker, H. Chromosome translocations in couples with in-vitro fertilization implantationfailure. Hum. Reprod.



Anatomical abnormalities

- **Uterine Factors**
- Uterine/endometrial factors can certainly cause infertility by impairing embryo implantation. these possible causes of ART failure ought to be ruled out before undertaking ART, not after an unspecified number of ART failures.

➢Fibroids

➢ Polyps

➢ Intrauterine adhesions

➢Mullerian abnormalities

➢adenomyosis

➢hydrosalpinges

- **Polyps**
- The most frequent uterine lesions in patients with RIF
- Interfere with embryo implantation
- *Franasiak JM, Alecsandru D, Forman EJ, Gemmell LC, Goldberg JM, Llarena N, et al. A review of the pathophysiology of recurrent implantation failure. Fertil Steril (2021)*

➢the deformation of the uterine cavity

➢altering cytokines secreted by the endometrium, such as IGF-1 BP and TNF-a

- Kodaman PH. Hysteroscopic polypectomy for women undergoing IVFtreatment: when is it necessary? Curr Opin Obstet Gynecol 2016

- **Submucosal fibroids** can decrease implantation and pregnancy rates in patients undergoing IVF.
- The mechanism hindering implantation includes
  - increased uterine myometrial contractions
  - abnormal vascularization
  - disordered cytokine profile
  - A systematic review concluded that patients with submucosal fibroids had lower implantation and live birth rates than the control group.
- the removal of submucosal fibroids before IVF-ET seems to confer benefits
- Darici E, Blockeel C, Mackens S. Should we stop screening for chronic endometritis? Reprod Biomed Online 2023

- A study of 210 patients with RIF who underwent hysteroscopic evaluation showed that the frequency of **intrauterine adhesions** was 8.5% (*Demirol AReprod BioMed Online (2004)*
- **Mullerian abnormalities**, such as septate and bicornuateuteri, should be considered in patients with RIF.
- **Hydrosalpinges:**lower implantation rates, lower pregnancy rates, increased spontaneous abortion rates *Strandell AHum Reprod (1994)*

➢ physically flushing the embryo out

➢ Less expression of avb3 integrin, HOXA 10,& (LIF) during WOI

- Communicating hydrosalpinges significantly reduce the odds of sustained IRs .
- Practice Committee of the American Society for Reproductive Medicine. Role of tubal surgery in the era of assisted reproductive technology: a committeeopinion. Fertil Steril 2022
- Salpingectomy significantly increased implantation rates when patients with ultrasound-visible hydrosalpinges .*Strandell A, Hum Reprod (1999)*

Anatomical investigations

- The proportion of **unidentified intrauterine abnormalities** in patients with RIF varied between 14% and 51% .Cicinelli E, Hum Reprod2015
- Hysteroscopy is the most widely used technique for anatomical investigations, followed by 3D and2D transvaginal ultrasound (Cimadomo et al., 2021).
- A meta-analysis focussing on patients with RIF reported a significantly higher LBR after hysteroscopy compared to those that did not have hysteroscopy (Risk Ratio (RR) 1.29; 95%CI 1.03–1.62; 4 studies; n=2247; P=0.046)
- Cao H, You D, Yuan M, Xi M. Hysteroscopy after repeated implantation failure of assisted reproductive technology: a meta-analysisJ Obstet Gynaecol Res 2018
- Sonohysterography is another technique to diagnose uterine pathologies, but it is less well studied in RIF.
- (Negm et al., 2012; Reda et al., 2016).

There is a lack of studies evaluating hysterosalpingography (HSG) in the context of RIF, but HSG or other means of imaging of the fallopian tubes can be considered if there is a doubt about hydrosalpinx after ultrasound.

Hysteroscopy can be considered, especially when there is a suspicion of a uterine anomaly visualized on trans-vaginal ultrasound.

Endometrial function and receptivity tests

One test entails the analysis of a panel of genes

associated with endometrial receptivity from an endometrial biopsy

taken during the putative WOI{ window of receptivity (WOR)}.
Transcription of these genes is quantified and interpreted to report the endometrium as either **pre-receptive**, **receptive**, or **post-receptive**.

- Information relating to the response of the endometrium to **progesterone exposure** can be provided by **histological assessment** of **Noyes' criteria**, but this has been shown to be too subjective for clinical use.

A meta-analysis from 2022 included 11 studies and reported that the prevalence of **displaced WOI**, as detected through endometrial receptivity tests, was **34%** (95% CI 24–43%) in RIF/poor prognosis patients .

In patients with RIF, comparable ongoing pregnancy rates (**OPR)/LBR** were found between those with diagnosed non-receptive endometrium undergoing personalized ET (p-ET) and those with receptive endometrium undergoing routine ET (40.7% versus 49.6%; odds ratio (OR) 0.94; 95% CI 0.70–1.26; 6 studies; n=2552)

Liu Z, Liu X, Wang M, Zhao H, He S, Lai S, Qu Q, Wang X, Zhao D, BaoH. The clinical efficacy of personalized embryo transfer guided bythe endometrial receptivity array/analysis on IVF/ICSI outcomes:a systematic review and meta-analysis. Front Physiol 2022b

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. Only in a per-protocol analysis, were higher cumulative LBRs in the p-ET reported.

Simo´n C, Go´mez C, Cabanillas S, Vladimirov I, Castillo´n G, Giles J, Boynukalin K, Findikli N, Bahc¸eci M, Ortega I et al.; ERA-RCTStudy Consortium Group. A 5-year multicentre randomized controlled trial comparing personalized, frozen and fresh blastocysttransfer in IVF. Reprod Biomed Online 2020

While there are insufficient data to recommend the routine use of any commercially available test of endometrial receptivity to diagnose the cause of RIF, assessment of specific aspects of endometrial function by testing can be considered.

Investigating chronic endometritis

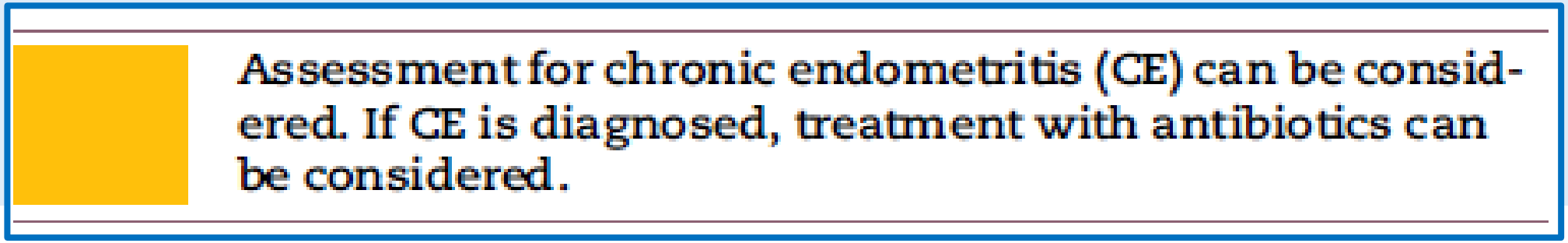
Chronic endometritis (CE) has been described in patients with RIF with bacterial colonization, but also in women without clinical signs of infection and can lower the pregnancy rate (Johnston-MacAnanny et al., 2010; Kitaya et al., 2014, 2019; Cicinelli et al., 2015; Bouet et al., 2016; Kushnir et al., 2016; Song et al., 2018; Li et al., 2020; Saxtorph et al., 2020; Zargar et al., 2020).

It can be diagnosed by hysteroscopy, haematoxylin and eosin staining, and CD138-labelling (Kitaya et al., 2014, 2019).

- CE (and vaginal infection) seems to be routinely investigated in clinical practice (85% of clinicians)
- (Cimadomo et al., 2021)

one systematic review reported significantly higher LBR/OPR (OR 5.33; 95% CI 2.41–11.79; I2=0%) in patients with cured CE (treated with antibiotics) compared to those with persistent CE .

- Vitagliano A, et al, Chronicendometritis in infertile women: impact of untreated disease, plasma cell count and antibiotic therapy on IVF outcome—a systematicreview and meta-analysis. Diagnostics (Basel) 2022



Re-assessment of endometrial thickness

A systematic review and meta-analysis investigating

the association between endometrial thickness and LBR in **fresh**

cycles reported that women with a thin endometrium

(EMT<7mm) had a significantly lower LBR compared to women with EMT >7mm (OR 0.47; 95% CI 0.37–0.61) (Liao et al., 2021).

- An association between EMT and clinical outcomes has also been reported in **frozen ETs** and stimulated cycles
- (Nishihara et al., 2020; Shalom-Paz et al., 2021)

- A large retrospective study concluded that EMT at the time of ET does not seem to predict the chance of implantation in case of euploid frozen blastocyst transfer.
- Ata B,et al, Effect of the endometrial thickness on thelive birth rate: insights from 959 single euploid frozen embryotransfers without a cutoff for thickness [published online aheadof print]. Fertil Steril 2023
- EMT may still be a contributor in the context of RIF, but it may be particularly relevant for noneuploid embryos.

If EMT 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 EMT, and some studies have suggested it can be effective in improving endometrial proliferation .

(Mouanness et al., 2021)

Re-assessment of endometrial thickness is recommended. A review of the estradiol treatment regimen is recommended if the endometrium is noted to remain thin. Hysteroscopy to rule out Asherman's syndrome can be considered.

Microbiome

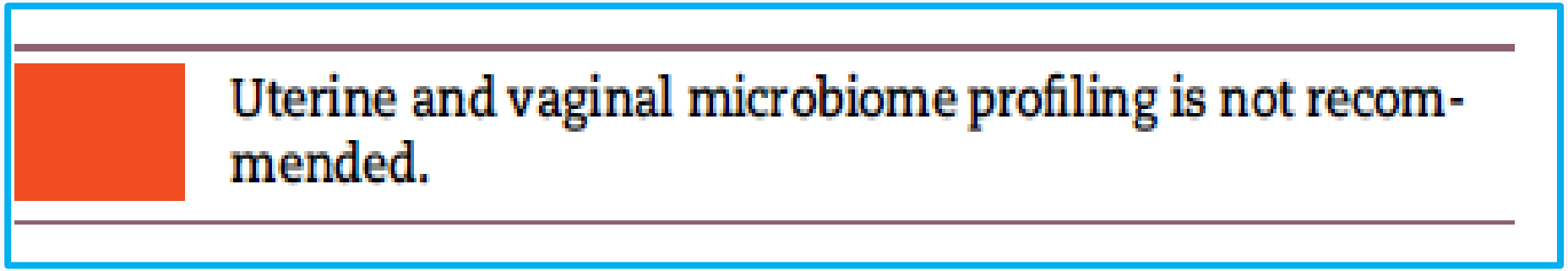
- The human microbiome, called “the other human genome,”
- Involved in normal physiology and homeostasis
- Associated with states of health and disease Maranduba CM, J Immunol Res(2015)-Belkaid Y, Immunity (2017)
- Continuous microbiota changing from the vagina to the ovaries
- Microbiota might be involved in several steps of IVF-ET, including gametogenesis, implantation, and delivery.
- Vagina is dominated by the Lactobacillus genus(probiotic), inhibit the invasion of bacteria by producing high concentrations of lactic acid and short-chain fatty acids.

- Vaginal microbiota in patients with unexplained RIF indicated that **vaginal Lactobacillus** was significantly decreased compared to patients who became pregnant in the first FET cycle.
- Vaginal Lactobacillus in patients with RIF was significantly decreased compared with healthy women, and the vaginal microbiota profiles in patients with RIF had significantly higher levels of five bacterial genera than in healthy women
- **The number of vaginal Lactobacillus spp. Is assumed to be a predictive biomarker of implantation**

- Fu M, mBio 11 (2020)--Schoenmakers S, Curr Opin Obstet Gynecol (2020)

- **the gut microbiota**
- may also be involved in *embryo implantation* by affecting
  - The immune system Alexander KL, Immunol Rev (2014)
  - The coagulation system
  - the endometriosis pathology Baker JM, Maturitas (2017)
- Patients with RIF display abnormal gut microbiota Patel N, BMC Womens Health (2022) ,but the relationship between gut microbiota and implantation failure needs to be further investigated.

- High numbers of Lactobacillus spp. in the **endometrium** during the implantation window were associated with higher successful implantation rates, whereas non-Lactobacillus-dominated microbiota, such as Streptococcus, during the implantation window resulted in negative pregnancy outcomes .Moreno I, Am J Obstet Gynecol (2016)



Thyroid function

Recent guidance fromthe European Thyroid Association suggested that in the context of ART, serum thyroid stimulating hormone **levels >4 mIU/l**

(subclinical hypothyroidism) or **<0.4 mIU/l** (subclinical hyperthyroidism)

may be considered **thyroid dysfunction** and require further follow-up and treatment (Biondi et al., 2015; Poppe et al., 2021).

Assessment of thyroid function **can be considered** during

the ART fertility workup or when RIF is detected, but as no specific

association with implantation failure has been reported,

assessment is not generally recommended as an investigation

for RIF.

Progesterone

There has been growing interest in the reported association between **premature progesterone rises**, measured around the **time of triggering** oocyte maturation, and clinical outcomesafter **fresh ET** (Venetis et al., 2013). there is a widespread view that this can lead to endometrial/ embryo asynchrony, meriting delaying ET to a subsequent freeze-thaw cycle (Bosch et al., 2010; Venetis et al., 2013)

- Deferred ET in cases of premature progesterone elevation has been shown to restore implantation rates in a cohort study (Lawrenz et al., 2018).

- Another topic is the **assessment of mid-luteal progesterone** levels to evaluate exogenous progesterone therapy. A Cochrane meta-analysis reported a **higher LBR/OPR** with progesterone comparedto placebo/no treatment for luteal phase support in women undergoing ART (OR 1.77; 95% CI 1.09–2.86; I=5%; 5 RCTs; N=642) (van der Linden et al., 2015).
- Consistent with the possibility that **absorption from the vagina** may be variable between women, there is increasing evidence linking low blood progesterone levels on the day of ET to poorer outcomes after fresh ET and after frozen ET.
  - (Thomsen et al., 2018) (Alsbjerg et al., 2018; Lawrenz et al., 2018; Labarta et al., 2021).

A matched cohort study showed low **mid-luteal progesterone** levels to be more prevalent in women with a history of **RIF** versus controls

(Saxtorph et al., 2020).

Individualized progesterone administration has been shown to restore implantation rates in cohort studies

Labarta E, Individualized luteal phase support normalizes live birth rate in women with low progesterone levels on the day of embryo transfer in artificial endometrial preparation cycles. Fertil Steril 2022

- A lvarez M, Individualised luteal phase support in artificially prepared frozen embryo transfer cycles based on serum progesterone levels: a prospective cohort study. Hum Reprod 2021

- Local validation of cut-off progesterone levels is recommended

Immunological screening

The notion that an excessive maternal immune response to the implanting embryo is disruptive to implantation has become widely accepted.

Uterine and peripheral natural killer cells

Both NK cell types act as immunomodulators but demonstrate a different profile of secreted cytokines and receptor/gene expression.(Vomstein et al., 2020).

Besides functional differences, measured numbers of pNK and uNK cells do not correlate in an individual and therefore should be regarded as two individual markers . (Kuon et al., 2017a; Woon et al., 2022).

uNK cell concentrations undergo tremendous changes **during the menstrual cycle**,

showing hormone dependent changes in phenotype and high levels in the luteal

- phase, underlining the need for defining strict criteria when analyzing uNK cell counts and functions (Fraser and Zenclussen, 2022).

A systematic review, including eight studies with patients with **RIF**, a significant difference in total CD56+ uNK cells was shown in women with RIF compared with controls (standardized mean difference 0.49; 95% CI 0.01–0.98; P=0.046; 604 women) .

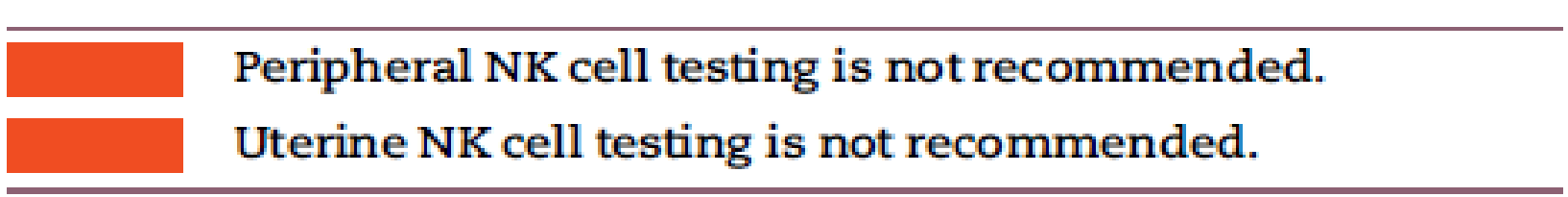
- Woon EV, et al; Numberand function of uterine natural killer cells in recurrent miscarriage
- and implantation failure: a systematic review and metaanalysis. Hum Reprod Update 2022

- Functional tests, including the constitution of receptors (e.g. killer immunoglobulin-like receptors: KIRs), may have more clinical value (Woon et al., 2022).

- It has also been proposed that inadequate activation of **uNK** cells might be acause of RIF (Donoghue et al., 2019; Alecsandru et al., 2020) and the same is true for **pNK** cells in RIF
- Salazar MD, et al. Post-hoc evaluation of peripheral blood natural killer cell cytotoxicity in predictingthe risk of recurrent pregnancy losses and repeated implantation failures. J Reprod Immunol 2022

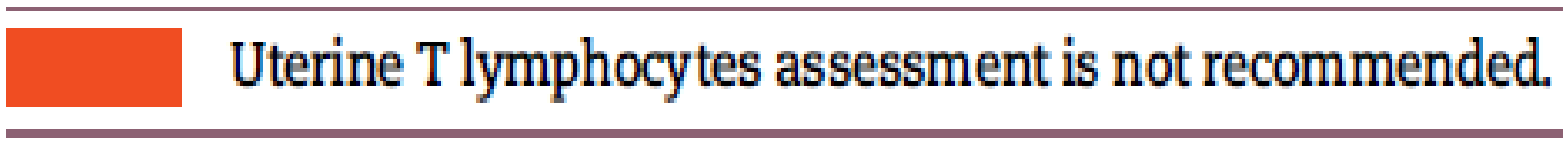
Treatment approaches have been proposed for patients with elevated uNK cells or evidence of disrupted function including **lipid infusions** and **glucocorticoid** administration.

While some cohort studies have suggested an impact of uNK cells on clinical outcomes, adequately **powered RCTs** of targeted interventions in RIF are still required.



T lymphocytes

- Imbalances in CD4+ T-helper lymphocytes, i.e. Th1, Th2, Th17,
- and regulatory T cells (Treg), have been suggested as contributing
- to RIF (Ali et al., 2018).



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).

some studies with small study populations showed that a proinflammatory -state persist in women with RIF.

(Inagakiet al., 2003; Liang et al., 2015a,b; Marron and Harrity, 2019).

- time-consuming
- expensive

The assessment of blood cytokine levels is not recommended.

Inherited thrombophilias

Inherited thrombophilia comprises conditions in which a genetic mutation affects the amount or the function of a protein in the coagulation pathway. Mutations in several genes were involved:

- G1619A (factor V Leiden){ common forms of inherited thrombophilias}
- 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)
- 4G/5G (plasminogen activator inhibitor-1 (PAI-1))

- Neamt¸u 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 Qublan et al., 2006; Neamt¸u et al., 2021

- significantly more homozygous mutations in the **Factor V Leiden** and the **MTHR (C677T)** gene in women experiencing multiple IVF failures Qublan et al., 2006
- higher prevalence of **PAI-1** 4G/5G mutations in RIF Azem et al. (2004
- Significantly increased incidence of inherited thrombophilia in women with a history of **four or more** IVF failures compared to healthy fertile (**44.4**% versus 18.2%; OR 3.6; 95% CI 1.25–10.6). Coulam et al. (2006)

Acquired thrombophilia

- Acquired thrombophilia includes:
  - acquired C protein, S protein deficiency
  - APS
  - antithrombin III deficiency
  - drug-induced thrombophilia
- Acquired thrombophilia has been associated with pregnancy morbidity, specifically RPL.

The RR for the presence of any type of APA was 3.06 (95% CI 1.97–4.77; I_2=15%; 5 studies; n=864) in women with **RIF** compared to women having at least one successful IVF-ET.

In women experiencing at least two implantation failures, the presence of **anti-cardiolipin antibodies** only or **lupus anticoagulant** was associated with a significant RR of, respectively, 5.06 and 5.81 for impaired implantation.

- Papadimitriou E,et al; Presence of antiphospholipidantibodies is associated with increased following in vitro fertilization technique and embryo transfer: aimplantation **failuresystematic review and meta-analysis.** PLoS One 2022

The role of testing is likely to be very limited in the context of RIF and should mainly focus on women with a clinical or family history of thromboembolic events.

Assessment of APA and APS is recommended in RIF women with additional risk factors for thrombophilia and can be considered in women without such risk factors.

Investigating male factors

Sperm FISH analysis and sperm DNA fragmentation (SDF) are not recommended.

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

Interventions for RIF

- The pressure on clinicians to intervene in cases with RIF is considerable.

Nearly 80% of clinicians offer treatments **preconception**
and 75% offer **additional treatments during the next ART cycle**.
Preconception treatments mainly focus on **lifestyle advice** (73–97%), **vitamin supplementation** (83%), **antioxidant therapy** (71%), and **treatments for endometritis** (90%) and **endometriosis** (80%), but **endometrial injury** (57%) and **immune-modulation therapy** (46%) are offered.

- Cimadomo D,. Definition, diagnostic and therapeutic options in recurrent failure: an international survey of clinicians and implantation embryologists. Hum Reprod 2021

Widely practised interventions during ART **include personalized luteal phase support** (83%), **cycle segmentation and freeze-all** (70%), and **p-ET** (62%).

Popular strategies employed in the ART lab include **PGT-A** (68%),

assisted hatching (61%), the **addition of growth factors to culture media**

(27%) and **time-lapse microscopy** (40%).

TESE is offered by 57% of clinicians, with fewer clinicians offering **physiological ICSI** (41%) .

Most interventions are applied empirically and without diagnostic rationale.

Sixty-nine per cent of the clinicians completing the survey consider **oocyte** or **sperm donation** a valuable option in RIF.

Intentional endometrial injury

- A meta-analysis based on three RCTs, there was **no significantly** increased chance of PR and LBR in women who underwent intentional endometrial injury.
- Busnelli A, Efficacyof therapies and interventions for **repeated embryo implantationfailure**: a systematic review and meta-analysis. Sci Rep 2021
- A consistent positive effect of endometrial injury on clinical PR (CPR) was reported in two observational studies. Raziel ,2007; Matsumoto ,2017
- RCT including211 women also reported **no** significant difference in CPR between patients with RIF who underwent hysteroscopy and intentional endometrial injury versus hysteroscopy only .Zahiri et al., 2021

Intentional endometrial injury is not recommended.

Granulocyte colony-stimulating factor 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

- When administere systemically, G-CSF has been reported to play a role in embryonic development, implantation, and trophoblastic growth(Wu¨ rfel, 2015), while local intrauterine administration could improve endometrial receptivity (Rahmati et al., 2014).

Two meta-analyses :Subcutaneous G-CSF administration

was associated with an **increased chance** of **clinical pregnancy**

compared with no treatment in both meta-analyses.

- Intrauterine administration had no impact on CPR Busnelli A
- An increased chance of clinical pregnancy with intrauterine G-CSF Hou

- Busnelli A, Efficacyof therapies and interventions for repeated embryo implantationfailure: a systematic review and meta-analysis. Sci Rep 2021
- Hou Z, What isthe impact of granulocyte colony-stimulating factor (G-CSF) insubcutaneous injection or intrauterine infusion and during boththe fresh and frozen embryo transfer cycles on recurrent implantationfailure: a systematic review and meta-analysis? Reprod BiolEndocrinol 2021

- conflicting evidence

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

Intravenous intralipid infusion

Immune modulation: through the **reduction of platelet aggregation**, a **decrease of IL-2**, **tumour necrosis factor-a**, and **IL-1b production** & **suppression of NK cell**

- **higher CPR** (172/417 versus 119/426; RR 1.55; 95% CI 1.16–2.07; 5 RCTs; I_2=44.2%) and **LBR** (132/417 versus 73/426; RR 1.83; 95% CI 1.42–2.35; 5 RCTs; I_2=0%) with the intervention but concluded there is limited evidence to support the use of intravenous intralipid at the time of ET in women with a history of RIF.
- Rimmer MP, Black N, Keay S, Quenby S, Al Wattar BH. Intralipid infusionat time of embryo transfer in women with history of recurrentimplantation failure: a **systematic review and metaanalysis**. J Obstet Gynaecol Res 2021

Intravenous intralipid infusion is not recommended.

Intravenous immunoglobulin

The intravenous injection of IgG (IVIG) is suggested to have immunomodulatory actions by neutralizing autoantibodies, down regulationof B-cell and T-cell function.

- significant difference in the IVIG group compared to controls in LBR
- Abdolmohammadi-Vahid S, The effectivenessof IVIG therapy in pregnancy and live birth rate of womenwith recurrent implantation failure (**RIF**): a **systematic reviewand meta-analysis**. J Reprod Immunol 2019
- study populations are small and RCTs are lacking
- Side effects or adverse events of IVIG include aseptic meningitis, renal failure, thromboembolism, haemolytic reactions, anaphylactic reactions, lung disease, enteritis, dermatologic disorders, and infectious diseases.

Intravenous immunoglobulin (IVIG) is not recommended.

Low molecular weight heparin

The anticoagulation effect of heparin
prevents placental thrombosis and infarction, and promotes the 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 patients undergoing ART, even in the absence of acquired or inherited thrombophilia.

A meta-analysis investigated the use of LMWH in patients with RIF (3 failed ET) but failed to show an effect of LMWH on LBR (RR 1.38; 95% CI 0.64–2.96; 2 RCTs; n=71) and CPR (RR1.39;

- 95% CI 0.87–2.23; 2 RCTs; n=218).small study populations including a
- mix of patients with RIF

Busnelli A, Efficacyof therapies and interventions for repeated embryo implantationfailure: a systematic review and meta-analysis. Sci Rep 2021

Low molecular weight heparin (LMWH) is not recommended to increase the chance of pregnancy or live birth in women with RIF.

GnRH agonist and aromatase inhibitor pre-treatment

Considering endometriosis may be an underlying and undiagnosed cause of RIF, it was hypothesized that empirical GnRH agonist and aromatase inhibitor treatment before ET may improve pregnancy outcomes .Steiner et al., 2019

Prior to the third ET, 143 women received **2 months of GnRH agonist** (3.75mg intramuscular leuprolideacetate monthly) **only**,
176 received **GnRH agonist** and **aromatase inhibitor** (5mg oral letrozole daily for 60 days),
204 received **no pre-treatment**.
CPR and LBR were higher amongwomen who received GnRH agonist plus letrozole compared withwomen who received GnRH agonist-only or women without pretreatment
(CPR: **63%**, 42%, and 40%, respectively; P<0.0001;
LBR:56%, 36%, and 34%, respectively; P<0.0001

GnRH agonist and aromatase inhibitor pre-treatment isnot recommended.

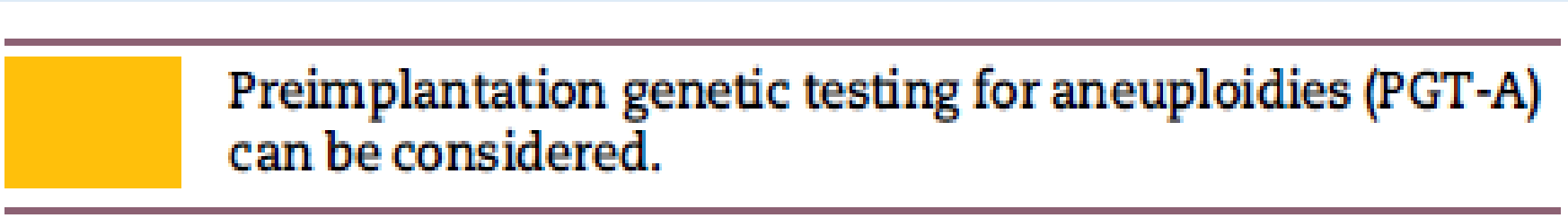
Preimplantation genetic testing for aneuploidies

- **PGT for aneuploidies (PGT-A) is offered to RIF couples in general**.

The meta-analysis of RCTs failed to show an improvement in both clinical pregnancy and LBR (random effects model: RR 1.07; 95% CI 0.36–3.15; P=0.90; I2=89% and RR 0.98; 95% CI 0.32–2.94; P=0.97; I2=87%) in women who underwent PGT-A. two RCTs, 3 observational

Busnelli A, Efficacyof therapies and interventions for repeated embryo implantationfailure: a systematic review and meta-analysis. Sci Rep 2021

the retrospective studies where embryo testing was conducted by either **array comparative genomic hybridization** 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 ETs were required to achieve pregnancy and live birth Cozzolino et al., 2020; Ni et al., 2020; Tong et al., 2021.



Blastocyst-stage ET

- Blastocyst-stage embryos may have :

➢a better chance of implantation

➢owing to a lower risk of embryo aneuploidy

➢ better synchronization with the endometrium

➢fewer uterine contractions at the time of transfer.

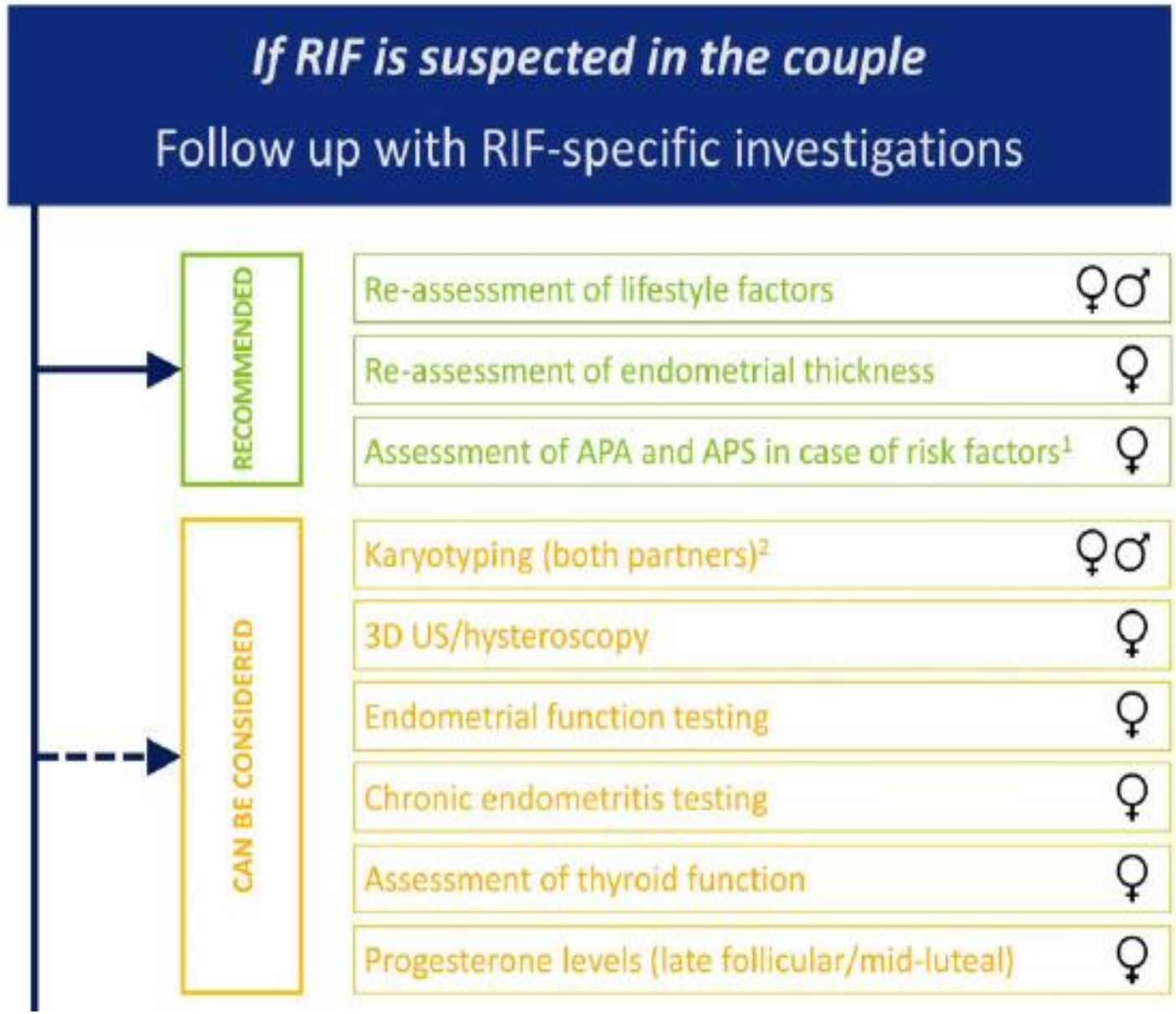
A systematic review of 27 studies in ART patients showed, with a low quality of evidence, that **LBR** after a **fresh transfer** was higher in the **blastocyst** transfer group compared to the **cleavage-stage** ET group (OR 1.27; 95% CI 1.06–1.51; I2=53%; 15 studies; n=2219 women)

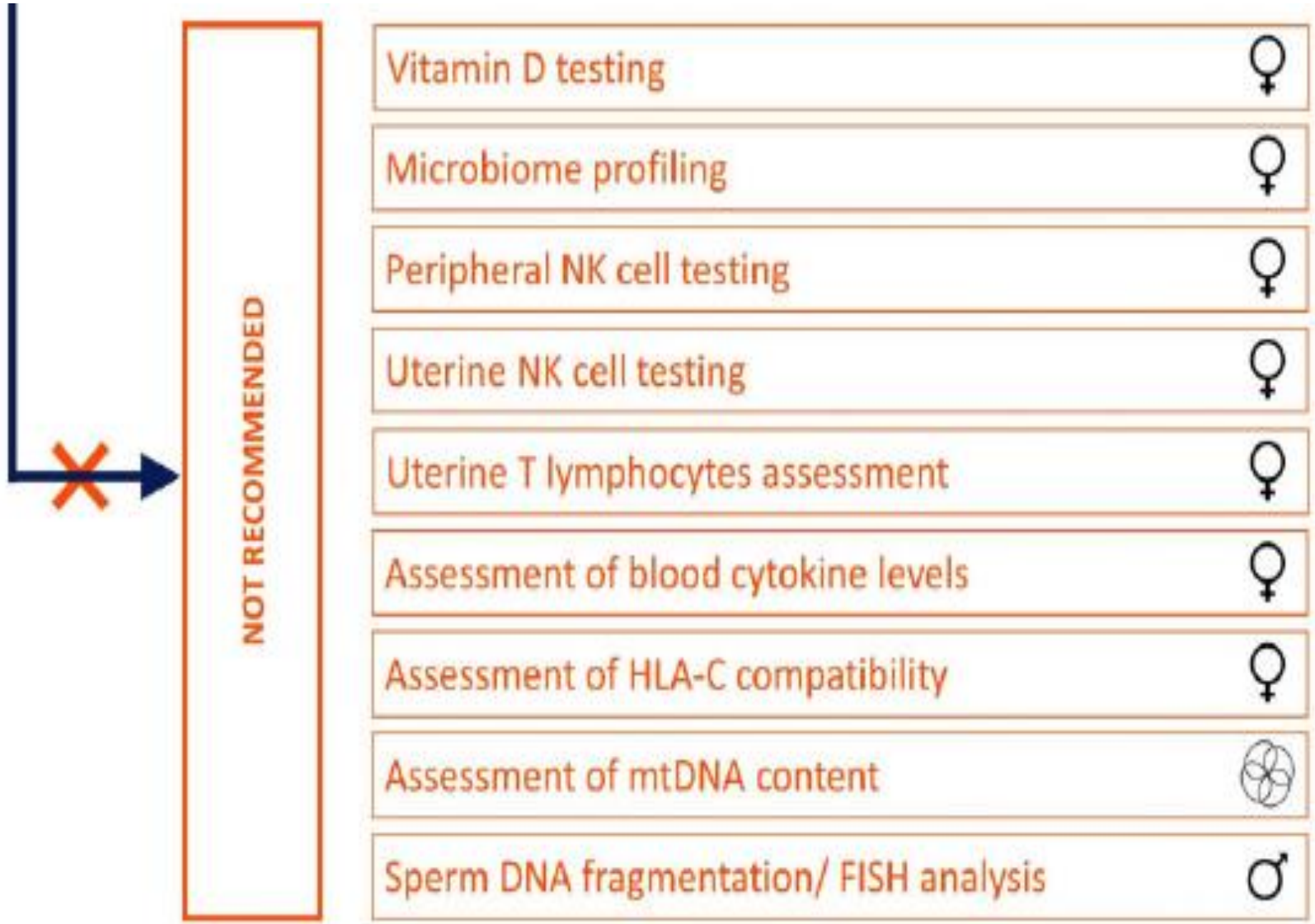
Glujovsky et al., 2022

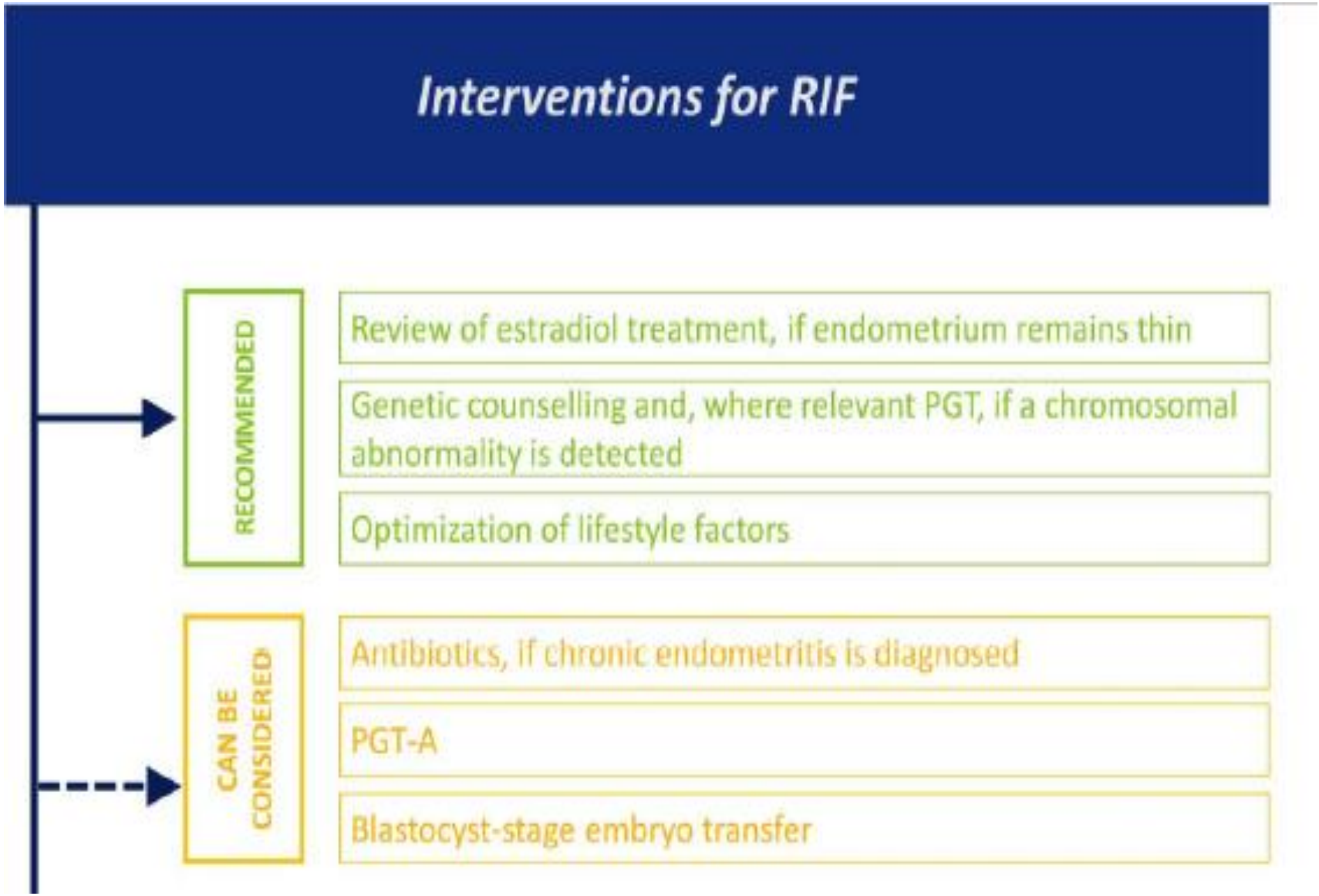
Blastocyst-stage embryo transfer can be considered.

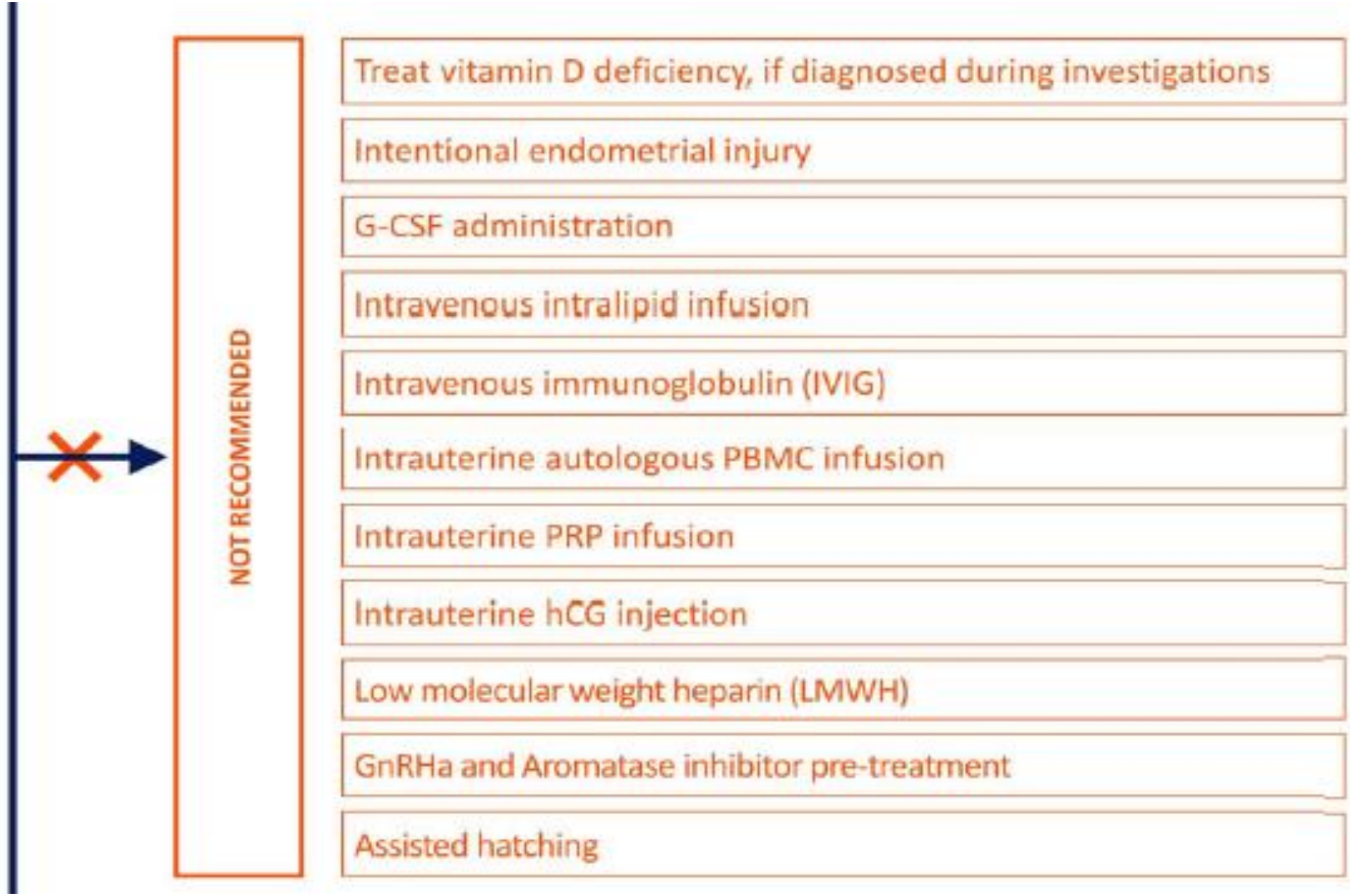
- time-lapse imaging
- ultrasound-guided ET, performing a trial ET,
- ensuring the catheter tip is >15mm from the fundus
- recommending a full bladder at ET
- cervical dilatation, cervical mucus removal
- use of fibrin sealant, use of antibiotics,
- there are no studies evaluating the effect of these interventions on the chances of LBR in patients with RIF.

- Recognize 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,
- avoid false reassurance.
- Apply shared treatment planning in a partnership approach.


If RIF is suspected in the couple
Follow up with RIF-specific investigations
RECOMMENDED
Re-assessment of lifestyle factors ♀♂
Re-assessment of endometrial thickness ♀
Assessment of APA and APS in case of risk factors[1] ♀
CAN BE CONSIDERED
Karyotyping (both partners)[2] ♀♂
3D US/hysteroscopy ♀
Endometrial function testing ♀
Chronic endometritis testing ♀
Assessment of thyroid function ♀
Progesterone levels (late follicular/mid-luteal) ♀


NOT RECOMMENDED
Vitamin D testing
Microbiome profiling
Peripheral NK cell testing
Uterine NK cell testing
Uterine T lymphocytes assessment
Assessment of blood cytokine levels
Assessment of HLA-C compatibility
Assessment of mtDNA content
Sperm DNA fragmentation/ FISH analysis


Interventions for RIF
RECOMMENDED
Review of estradiol treatment, if endometrium remains thin
Genetic counselling and, where relevant PGT, if a chromosomal abnormality is detected
Optimization of lifestyle factors
CAN BE CONSIDERED
Antibiotics, if chronic endometritis is diagnosed
PGT-A
Blastocyst-stage embryo transfer


NOT RECOMMENDED
Treat vitamin D deficiency, if diagnosed during investigations
Intentional endometrial injury
G-CSF administration
Intravenous intralipid infusion
Intravenous immunoglobulin (IVIG)
Intrauterine autologous PBMC infusion
Intrauterine PRP infusion
Intrauterine hCG injection
Low molecular weight heparin (LMWH)
GnRHa and Aromatase inhibitor pre-treatment
Assisted hatching

frontiers | Frontiers in Medicine

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Analysis of predictors of clinical pregnancy and live birth in patients with RIF treated with IVF-ET technology: a cohort study based on a propensity score approach

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retrospective cohort study
2019 and August 31, 2022

FET at day 5 or 6 blastocyst stage

ERA testing as the **study group** and those who underwent FET only as the control group.

The success rate of **clinical pregnancy in RIF patients** was **50.74%** and the **live birth rate was 33.09%.**

Patients in the **FET** group were less likely to achieve clinical pregnancy compared to the **ERA** group (*HR* = 0.788, 95%*CI* 0.593–0.978, $p < 0.05$). Patients **with >3 previous implantation failures** had a lower probability of achieving a clinical pregnancy (*HR* = 0.058, 95%*CI* 0.026– 0.128, $p < 0.05$) and a lower likelihood of a live birth (*HR* = 0.055, 95%*CI* 0.019– 0.160, $p < 0.05$), compared to **patients with ≤3** previous implantation failures .

two embryos transferred were **more** likely to achieve a **clinical pregnancy** (*HR* = 1.357, 95%*CI* 1.079–1.889, $p < 0.05$) and a higher likelihood of a live birth (*HR* = 1.845, 95%*CI* 1.170–2.910, $p < 0.05$) than patients who had **a single embryo transfer**.

Not receiving an ERA, having >3 previous implantation failures, using single embryo transfer and not transferring high quality embryos are predictors for clinical pregnancy in patients with RIF.


THANKS FOR YOUR
ATTENTIONS