Approaches to Improve Endometrial Receptivity in Case of Repeated Implantation Failures

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Repeated implantation failures are a constant challenge in reproductive medicine with a significant impact both on health providers and on infertile couples. Several approaches have been proposed so far as effective; however, accumulative data have clarified that most of the treatment options do not have the evidence base for a generalized application to be suggested by the relevant societies. Implantation failures are attributed to either poor quality embryos or to defected endometrial receptivity. The current review aims to summarize in a systematic way all the new trends in managing RIF via interference with endometrial receptivity. The authors focus mainly, but not exclusively, on endometrial injury prior to embryo transfer and endometrial priming with autologous cells or biological agents. To this direction, a systematic search of the Pubmed database has been conducted taking into account the emerged evidence of the last two decades. All the suggested interventions are herein presented and analyzed in terms of reproductive outcomes. It is evident that properly powered and designed randomized trials are needed to support a new standard approach in RIF treatment that will safely be incorporated in national and international guidelines.

Keywords: repeated implantation failures, HCG, PBMC, PRP, microbiome, G-CSF, atosiban, growth hormone

INTRODUCTION

Repeated implantation failure (RIF) is one of the main challenges in human reproduction. Due to the fact that RIF was initially considered a rather heterogeneous entity, a definition was difficult to establish. It is however accepted that RIF is defined as “the failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years” (Coughlan et al., 2014a). This definition is further challenged upon the number and the type of embryos transferred (number of cleavage embryos vs. number of blastocysts), along with the definition of the primary endpoint for a cycle to be successful (biochemical vs. clinical pregnancy) (Cakiroglu and Tiras, 2020). Even so, the even existence of RIF as a clinical entity is under doubt (Ben Rafael, 2020). Due to the diversity of the RIF definitions, data on RIF incidence is rather restricted (Bashiri et al., 2018).

RIF is a burden both for the health providers and the couples. Health providers are required to proceed to assisted reproduction techniques with rather small success rates, while the couples are overloaded with psychological stress (Coughlan et al., 2014b; Stanhiser and Steiner, 2018), not to mention the financial pressure due to the repeated cycles. It is thus imperative for health providers to employ novel tools aiming to improve the reproductive outcome. So far, only hysteroscopy to treat endometrial pathology (Mao et al., 2019), and treatment of hydrosalpinges (Coughlan, 2018)
have been proven significantly beneficial and as such they have been incorporated in standard care. However, several approaches have emerged in the literature claiming to act like the “Holy Grail” in management of unexplained RIF.

Herein, we present a systematic effort to present the existing evidence on most of the novel approaches aiming to improve implantation and thus reproductive outcomes in case of unexplained RIF.

THE ENDOMETRIAL PATHOPHYSIOLOGY OF RIF

The etiology of RIF can be attributed to dysfunction of the two major players of implantation, namely the embryo and the endometrium. As far as the embryo is concerned, poor quality embryos or sperm along with parental chromosomal anomalies are the main causes of an embryo failing to implant (Coughlan et al., 2014a). Such issues of poor quality gametes can be easily diagnosed prior to IVF. On the other hand, deranged endometrial receptivity is more difficult to evaluate; apart from hysteroscopy to assess the endometrial cavity, only very recently molecular diagnostic arrays have been available in order to predict an IVF candidate as a RIF patient; however, the evidence is rather weak for such approach to be established in clinical practice (Bassil et al., 2018). The molecular signature of RIF is constantly under investigation; a recent report has shown that a molecular signature of 303 genes extracted from endometrial sampling could safely discriminate between normal and RIF individuals (Koot et al., 2016). Such approaches, although promising, need further validation in order to be released for clinical practice.

The Endometrial Pathophysiology of RIF: Well-Established RIF Causes

Anatomical Disorders

RIF may be attributed to anatomical disorders that distort the endometrial cavity, being undiagnosed before IVF treatment. In that context, fibroids have been reported as negative prognosticators to IVF success (Wang et al., 2018; Rikhraj et al., 2020), altering endometrial receptivity by modifying HOXA10 and LIF expression (Makker et al., 2017; Kara et al., 2019; Pier et al., 2020). Endometrial adhesions as a result of infection or prior surgical procedure may also be considered to be associated with thin endometrium and low receptivity potential (Wang et al., 2020). Finally, hydrosalpinges are well-accepted as a cause for RIF, since the inflammatory fluid may affect both the embryo and the endometrium (Volodarsky-Perel et al., 2019). Of note is the fact that patients with the above mentioned anatomical disorders may receive surgical treatment prior to IVF with significantly improved reproductive outcomes.

Unexplained RIF: Proposed Endometrial Pathophysiology

Immunological Disorders

The immunological profile of the receptive endometrium presents several characteristics that seem to be impaired in case of implantation failure. The first cellular population to be considered of interest was the natural killer (NK) cells, due to their ability to destroy allogenic cellular signals. It has been previously reported that increased numbers and activity of peripheral NK cells are associated with a negative pregnancy outcome (biochemical pregnancy or miscarriage) (Yamada et al., 2003). To the same direction, a parallel increase in peripheral and uterine NK cell numbers and NK activity was found in women diagnosed as RIF (Santillan et al., 2015). However, the role of the NK cells in human reproduction is quite complex; NK cells constitute a rather diverse cellular population making the discrimination between peripheral and uterine NK cells rather difficult. Interestingly, it was shown that even uterine NK cells may be divided into three subsets with different immunological properties (Vento-Tormo et al., 2018). Perhaps, due to this diversity, the first meta-analysis in the field, including studies assessing NK cell biology prior IVF treatment, showed no significant difference in NK cell count and activity between fertile and infertile women (Seshadri and Sunkara, 2014). Emerging evidence now put in doubt the initial notion that NK cell density and activity may predict RIF (Donoghue et al., 2019; Zhang et al., 2020), while a recent meta-analysis highlights that studies reporting interventions based on NK status are heterogeneous and lack the quality to produce solid evidence (Woon et al., 2020).

The role of differential expression of several cytokines in implantation has been well described in the literature. A constant shift to a Th1 cytokine pro-inflammatory profile contributes to implantation failure or miscarriage (Liang et al., 2015). On the contrary, a shift toward a Th2 anti-inflammatory cytokine profile supports implantation and early fetal development. Interestingly, a successful implantation requires a T-regulatory (Treg) cell profile, while a shift toward a Th17 phenotype is associated with poor reproductive outcomes (Ali et al., 2018). It is reported that up to 80% of RIF cases may present with an abnormal cytokine profile (Lédée et al., 2016). It must be pointed out though, that, even well-studied, the above mentioned findings should be met with caution. The correlation of the immune profile with reproductive success has not been principally validated; it has been proven within special research settings.

Non-immunological Disorders

Several signaling pathways have been reported as impaired in case of repeated implantation failures. A recent transcriptome analysis has revealed that, in case of RIF, leukemia inhibitory factor (LIF) was reduced along with the expression of S100 calcium binding protein P (S100P), Chemokine (C-X-C motif) ligand 13 (CXCL13), SIX homeobox 1 (SIX1) and signal transducer, and activator of transcription 3 (STAT3) (Choi et al., 2016). Additionally, the endometrium of RIF patients has been characterized as of low MUC1 expression, this being an independent prognosticator of implantation failure (Wu et al., 2018). Furthermore, platelet and endothelial cell adhesion molecule 1 (PECAM1) and transforming growth factor β1 (TGF-β1) were also significantly reduced in RIF (Guo et al., 2018). Apart from altered implantation markers, it has been previously shown that prostaglandins’ synthesis is deranged in case of RIF; implying
a defective endometrial inflammation in favor of implantation failure (Achache et al., 2010; Demiral Keleş et al., 2020). Finally, studies evaluating metabolomics (RoyChoudhury et al., 2016) and microRNAs (Shi et al., 2017) have shown that RIF may be featured by a significant different profile that could be associated with poor reproductive outcome.

**Chronic Endometritis**

Chronic endometritis (CE) is an emerging entity considered to negative affect reproductive outcomes in case of IVF treatment. Especially in RIF, CE has been reported at an incidence of ranging from 14 to 30% with decreased pregnancy success rates (Quaas and Dokras, 2008; Bouet et al., 2016). EC diagnosis is rather complicated. Endometrial cavity assessment is initially performed via hysteroscopy, recognizing subtile endometrial lesions like micropolyps, stromal edema and profound vascularity attributed to inflammatory angiogenesis (Gkrozou et al., 2020). The gold standard in establishing CE diagnosis is the recognition of increased plasma cell density in the endometrial stroma, either by standard histology (Kasius et al., 2012), or even better, by immunohistochemically marking plasma cells with anti-CD163 (Fan et al., 2019; Li et al., 2020; Xu et al., 2020). A complementary approach is endometrial sampling for microbial culture. The most common pathogens identified are so far group B Streptococcus, Escherichia Coli, Streptococcus Faecalis, Mycoplasma (Cicinelli et al., 2015). Of note is the constant risk of sample contamination with vaginal or cervical pathogens. CE is considered to affect endometrial receptivity via establishing a dysbiotic endometrial environment featured by dense lymphocyte populations along with a shift toward inflammatory cytokine profiles (Th1/Th17) (Mor and Kwon, 2015; Al-Nasiry et al., 2020).

**Thin Endometrium**

Although there is no universal consensus about the threshold of thin endometrium, an endometrial thickness below 8 mm is generally accepted as characteristic for thin endometrium. Thin endometrium is a risk factor for implantation failure (Liu et al., 2018). Thin endometrium could be initially attributed to previous endometrial infection or intra-cavitary intervention. In the absence of an obvious cause, it is suggested that it could be the end result of defective angiogenesis, depriving the endometrium from the necessary nutrients and oxygen (Miwa et al., 2009). Several approaches have been proposed including adhesiolyis and estrogen treatment (Lebovitz and Orvieto, 2014). However, thin endometrium remains a challenge and as such novel approaches need to be devised and properly evaluated.

**Dysbiotic Microbiome: A Novel Pathophysiologic Approach in RIF**

During the last decade, with the technological evolution of new generation sequencing, it became feasible to evaluate the microbiome of the reproductive system. It is currently known that the vagina microbiome is considered of low diversity, having predominantly lactobacillus species (Ravel et al., 2011). Lactobacillus is the natural guardian of the vagina, since it metabolizes glycogen released by vaginal epithelium to lactic acid, securing a low pH which in turn inhibits the growth of local pathogens. Several controlled studies have been published, demonstrating that altered vaginal or even endometrial microbiome could be associated with poor reproductive outcomes. Of note is the study by Moreno et al. (2016) showing that pathological endometrial microbiota are associated with implantation failure. These studies have been recently systematically reviewed with the conclusion being supportive for the altered lactobacillus population to be a potential cause of impaired endometrial receptivity (Bracewell-Milnes et al., 2018). Normal endometrial microbiome is expected to be of low biomass, exerting a moderate local immune stimulation in favor of normal tissue remodeling (Einenkel et al., 2019). Furthermore, it is expected to support the endometrium via production of metabolites, while concurrently it blocks pathogen migration via spatial antagonization (Benner et al., 2018). On the contrary, dysbiotic microbiota are featured by abundance and a powerful immune stimulation with a local destructive result (Einenkel et al., 2019). It has been noted that normally, the endometrial microbiome contributes to a cytokine profile toward Th2/Treg immunity (Al-Nasiry et al., 2020). On the other hand, dysbiosis, induces a Th1/Th17 profile exerting a negative effect on tissue remodeling and trophoblast invasion (Al-Nasiry et al., 2020). Finally, the dysbiotic microbiome contributes to local oxidative stress with detrimental effect on endometrial cell homeostasis (Baker et al., 2018).

Despite the initiated enthusiasm, there are several issues regarding the procedure of sampling and evaluating endometrial microbiota. It has been reported that endometrial microbiome may fluctuate according to the circulating estrogen and progesterone (Molina et al., 2020). Additionally, it may be altered by infectious agents, increasing age, physical activity, pregnancy and childbirth (Molina et al., 2020). More importantly, endometrial microbiome evaluation is under the influence of technical details that need standardization. There is always the risk of contamination by the vaginal microbiome (Salter et al., 2014; Glassing et al., 2016). This demands a careful sampling along with setting proper negative controls (Kim et al., 2017). The platform used for sequencing may also affect the results (Clooney et al., 2016). Moreover, since endometrial microbiome is of low biomass, special DNA isolation kits are needed in order to minimize the risk of misinterpretation of the results along with the risk of inserting bias via the statistical method applied (Eisenhofer et al., 2019; Weyrich et al., 2019). Finally, the results need further critical analysis, since detecting 16s rRNA does not mean that the strains identified are necessary viable or abundant. To this direction, no “core endometrial microbiome” has been presented so far, nor has this been correlated with normal/fertile endometrium or any uterine pathology (like polyps or retarded decidualization).

**MATERIALS AND METHODS**

The aim of the current study was to highlight novel approaches in the field of unexplained RIF treatment. The Pubmed database was screened with the following searches: ("Endometrial injury"
OR "Endometrial scratching"), (["HCG" OR "human chorionic gonadotropin") AND ("intrauterine" OR "infusion" OR "injection" OR "administration")], (["PBMC" OR "peripheral blood mononuclear cells" OR "peripheral blood monocytes") AND ("intrauterine" OR "infusion" OR "injection" OR "administration")], (["G-CSF" OR "Granulocyte colony stimulating factor") AND ("IVF" OR "assisted reproduction")], (["atosiban" AND ("IVF" OR "assisted reproduction")], (["GH" OR "Growth hormone") AND ("IVF" OR "assisted reproduction")], (["PRP" OR "platelet-rich plasma") AND ("IVF" OR "assisted reproduction")], ("antibiotics" AND "chronic endometritis"), (["microbiome" AND ("IVF" OR "assisted reproduction")]. The publications were screened by title relevance and thereafter by abstract relevance. Only controlled trials and meta-analyses were included. Meta-analyses' references were also screened forPubmed publications.

**APPROACHES TO IMPROVE ENDOMETRIAL RECEPTIVITY IN UNEXPLAINED RIF PATIENTS**

**The Role of Endometrial Injury in Improving Reproductive Outcomes in Women With RIF**

The concept of performing an endometrial injury as a means of improving endometrial receptivity has been reported by Barash et al. (2003). The authors demonstrated, for the first time, a significant improvement both in implantation and clinical pregnancy rates. This report triggered a massive positive reaction by clinical research teams aiming to incorporate this simple and low cost approach in everyday clinical practice. As a result, both basic science teams and clinicians published a significant number of studies in the view both to delineate possible potential pathophysiological mechanisms, along with producing solid clinical evidence for endometrial injury to be accepted as a therapeutic procedure.

As far as basic science is concerned, endometrial injury has initially been proposed to induce an aseptic inflammatory reaction possibly shifting the endometrial immune profile toward a Th2/M2 state (Granot et al., 2012). It was, thus, shown that endometrial injury may up-regulate the endometrial expression of several pro-decidualization molecules, including MUC-1, crystalline aB, APOD, and PLA2 (Kalma et al., 2009). MUC-1 is known to be up-regulated by prostegosterone affecting endometrial receptivity, acting at the same time as an independent receptivity marker in case of RIF (Wu et al., 2018). To the same direction, it has been shown that endometrial injury may induce uroplakin Iα expression, a molecule up-regulated mainly during the window of implantation (Kalma et al., 2009). Further studies have shown an induction of the endometrial repair mechanism involving the up-regulation of TNFα (Gnainsky et al., 2010). This in turn initiates the chemo-attraction of monocytes and dendritic cells, thus increasing the number of endometrial macrophages in favor of the implantation process, since they trigger the endometrial expression of osteopontin, a well-known receptivity marker (Gnainsky et al., 2015). More recent studies have also highlighted the activation of local angiogenesis as this is identified by elevated expression of VEGF, a phenomenon attributed to elevated HIF-1α expression as a result of inflammatory hypoxia (Yu et al., 2019). The complex network of aseptic inflammation and angiogenesis mediators has been considered as a positive contributor to receptivity (Yang et al., 2019).

In the field of clinical trials, many non-randomized and randomized controlled trials have been published (summarized in Table 1). All the trials have employed endometrial injury following various protocols in terms of: (a) number of procedures prior to embryo transfer, and (b) the timing of the procedure (follicular or luteal phase or both). Following the time line of the trials published since 2003 up to present, it can be recognized that initially the results were very supportive of the procedure and this was further presented in the first meta-analyses presented in 2012 (El-Toukhy et al., 2012; Nastri et al., 2012; Potdar et al., 2012). The initial enthusiasm was followed by comments regarding the quality of the included randomized trials, along with concerns upon a possible selection bias (Simón and Bellver, 2014). Since then, further studies of different sizes and methodology have been added, increasing the heterogeneity. Due to the lack of uniformity in performing the procedure, the most recent meta-analyses have noted the weaknesses of the randomized trials, pooling data that lead to rather discouragement (Gui et al., 2019; Sar-Shalom Nahshon et al., 2019; van Hoogenhuijze et al., 2019; Vitagliano et al., 2019). To this direction, a critical review of the randomized controlled trials published so far, revealed several issues in trials’ design, underlying that caution is needed especially when pooling low-quality evidence (Li et al., 2019). Very recently, a properly powered randomized trial has been published (Lensen et al., 2019a). Having recruited 1,364 patients randomized to receive or not an endometrial injury prior to embryo transfer, the authors state that performing an endometrial injury in everyday practice does not significantly alter the reproductive outcomes (Lensen et al., 2019a).

The evidence produced from this study (Lensen et al., 2019a), has initiated a long series of debates in terms of the endometrial injury application, along with the ethical dilemma of offering a procedure proven as useless or even possibly harmful (Yeung et al., 2014; Frantz et al., 2019; Lensen et al., 2019b; Mackens et al., 2020). This is especially important in case of selected groups receiving assisted reproduction treatments like women with RIF. Although Lensen et al. reported that endometrial injury was not efficient in women with RIF (Lensen et al., 2019a), this result was extracted by a sub-group analysis of the population. Despite the fact that the study was properly powered to identify a significant difference of 15% between the study and the control groups, there are always methodological issues in sub-group analyses, mainly due to lack of stratified randomization (VanderWeele and Knol, 2011; Lensen et al., 2019b). The clinical evidence to support endometrial injury in women with RIF is based on significantly fewer studies compared to the total number of studies published so far (see Table 1). The heterogeneity of these studies was addressed in a previous systematic review (Panagiotopoulou et al., 2015). Most of the studies have been summarized in a recent meta-analysis which clearly demonstrates that in case of
### TABLE 1 | Salient features of the included studies on endometrial injury as an intervention in improving endometrial receptivity.

| Year | PMID | Publication type | Participants | RIF | Outcome |
|------|------|------------------|--------------|-----|---------|
| 2020 | 32468267 | RCT | 352 | Not exclusively | Non-significant |
| 2020 | 32372078 | RCT | 200 | Not exclusively | Negative-premature end |
| 2020 | 32216503 | Non-randomized | 518 | Not exclusively | Significant |
| 2020 | 32003122 | RCT | 200 | YES | Significant |
| 2020 | 31897673 | Non-randomized | 300 | Not exclusively | Significant in RIF |
| 2019 | 31843072 | RCT | 304 | Not exclusively | Significant in RIF |
| 2019 | 31532321 | Non-randomized | 62 | YES | Significant in RIF |
| 2019 | 31450870 | Non-randomized | 137 | Not exclusively | Non-significant |
| 2019 | 31405721 | RCT | 239 | YES | Significant in RIF |
| 2019 | 30895265 | Meta-analysis | 2537 | Not exclusively | Non-significant |
| 2019 | 30683590 | Meta-analysis | 1354 | Not exclusively | Non-significant |
| 2019 | 30673547 | RCT | 1364 | Not exclusively | Non-significant |
| 2019 | 30661093 | RCT | 51 | Not exclusively | Non-significant–premature end |
| 2019 | 30515920 | Non-randomized | 266 | Not exclusively | Significant |
| 2019 | 30421580 | Meta-analysis | 4057 | Not exclusively | Non-Significant in RCTs Significant overall |
| 2019 | 30388238 | Meta-analysis | 1260 | Not exclusively | Non-significant |
| 2019 | 30496629 | RCT | 191 | Not exclusively | Non-significant–Premature end |
| 2018 | 29048754 | RCT | 300 | Not exclusively | Significant |
| 2018 | 30197017 | Meta-analysis | 1468 | YES | Significant in RIF |
| 2017 | 29259499 | RCT | 77 | YES | Significant in RIF |
| 2017 | 28964963 | RCT | 80 | Not exclusively | Non-significant |
| 2017 | 28551840 | RCT | 144 | Not exclusively | Significant |
| 2017 | 28511086 | RCT | 111 | Not exclusively | Non-significant |
| 2017 | 2847502 | Non-randomized | 576 | Not exclusively | Non-significant |
| 2017 | 28397081 | RCT | 106 | Not exclusively | Significant |
| 2017 | 2836815 | Non-randomized | 429 | YES | Significant in RIF |
| 2017 | 28612975 | RCT | 169 | Not exclusively | Non-significant |
| 2016 | 28101111 | RCT | 120 | YES | Non-Significant in RIF |
| 2016 | 27917011 | Non-randomized | 103 | YES | Significant in RIF |
| 2016 | 2736928 | RCT | 120 | Not exclusively | Non-significant |
| 2016 | 27738660 | RCT | 63 | Not exclusively | Negative |
| 2016 | 2755229 | RCT | 93 | Not exclusively | Non-significant |
| 2016 | 27296541 | Meta-analysis | 1512 | Not exclusively | Uncertainty due to low quality |
| 2016 | 27294218 | RCT | 400 | Not exclusively | Significant |
| 2015 | 27658405 | Non-randomized | 345 | YES | Significant in RIF |
| 2015 | 27146582 | RCT | 360 | Not exclusively | Significant |
| 2015 | 26542054 | RCT | 154 | Not exclusively | Significant |
| 2015 | 26725857 | RCT | 60 | YES | Significant implantation rate |
| 2015 | 26653889 | RCT | 251 | Not exclusively | Significant |
| 2015 | 25803542 | Meta-analysis | 2128 | Not exclusively | Significant |
| 2015 | 25561347 | RCT | 387 | Not exclusively | Significant only in RIF |
| 2015 | 2654351 | RCT | 332 | Not exclusively | Non-significant |
| 2014 | 25469138 | RCT | 144 | Not exclusively | Non-significant |
| 2014 | 25205759 | RCT | 300 | Not exclusively | Non-significant |
| 2014 | 25064410 | Non-randomized | 737 | Not exclusively | Non-significant in RIF |
| 2014 | 24791967 | Non-Randomized | 80 | Not exclusively | Non-significant |
| 2014 | 24289893 | Non-randomized | 118 | Not exclusively | Significant |
| 2013 | 24639710 | RCT | 217 | Not exclusively | Significant |
| 2013 | 23754314 | RCT | 158 | Not exclusively | Significant |
| 2013 | 23106834 | RCT | 101 | Not exclusively | Significant |
| 2013 | 23494199 | RCT | 150 | Not exclusively | Significant |
| 2013 | 24283157 | Non-randomized | 89 | YES | Significant in RIF |
| 2012 | 25246928 | Non-randomized | 83 | Not exclusively | Significant |

(Continued)
women with RIF, endometrial injury may significantly improve reproductive outcomes (Vitagliano et al., 2018a). Interestingly, the same research group has demonstrated in a separate meta-analysis that the positive effect of the procedure does not exist in case of women receiving their first IVF treatment (Vitagliano et al., 2019). This is in line with the report of Lensen et al. (2019a), strengthening the notion that endometrial injury should not be an everyday practice anymore. A properly designed randomized controlled trial is expected to delineate whether offering endometrial injury is beneficial to women with RIF. Until then, the patients should be properly informed about the potential benefits of the procedure and the lack of solid evidence.

**Intrauterine Administration of Human Chorionic Gonadotropin (HCG)**

The concept of administering HCG in the uterine cavity before embryo transfer was based on evidence produced during the last two decades. It is well-established that HCG is the first molecule to participate in the cross-talk between the embryo and the maternal decidua. HCG is expressed even at the stage of the 8-cell embryos (Bonduelle et al., 1988; Lopata and Hay, 1989), following a specific pattern of augmentation during implantation and trophoblast invasion, inducing the differentiation of the cytotrophoblast to syncytiotrophoblast. At the same time a switch from protocol heterogeneity, patient characteristics differ as well, ranging from infertile women to patients experiencing repeated implantation failures. These differences can initially explain the contradicting results of the published trials along with the opposing conclusions drawn by the meta-analyses performed so far (summarized in Table 2), with the majority of them being randomized controlled trials (Mansour et al., 2011; Hong et al., 2014; Santibañez et al., 2014; Zarei et al., 2014; Aaleyasin et al., 2015; Wirleitner et al., 2015; Dehghani Firouzabadi et al., 2016; Navali et al., 2016; Huang et al., 2017a; Mostajeran et al., 2017; Boonsuk et al., 2018; Hafezi et al., 2018; Laokirkkiat and Thanaboonyawat, 2019). Despite the anticipation for solid evidence, an in-depth evaluation reveals high heterogeneity due to different methodologies applied. Indeed, treatment protocols differ in terms of HCG dosage, timing of intrauterine administration and the stage of the embryos transferred. Apart from protocol heterogeneity, patient characteristics differ as well, ranging from infertile women to patients experiencing repeated implantation failures. These differences can initially explain the contradicting results of the published trials along with the opposing conclusions drawn by the meta-analyses performed during the last 5 years. So far four meta-analyses (Ye et al., 2015; Osman et al., 2016; Hou et al., 2018; Gao et al., 2019) and one Cochrane review (Craciunas et al., 2018) have been performed with opposing results. Only 2 out of the 4 meta-analyses present a significant benefit (Ye et al., 2015; Gao et al., 2019). An in-depth analysis of the most recent meta-analyses has revealed differences in the included studies, mainly being reports published as

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### Table 1 | Continued

| Year | PMID | Publication type | Participants | RIF | Outcome |
|------|------|-----------------|--------------|-----|---------|
| 2003 | 12798877 | Non-randomized | 134 | Not exclusively | Significant |
| 2002 | 22985017 | Meta-analysis | 901 | Not exclusively | Significant |
| 2002 | 22943664 | RCT | 36 | Not exclusively | Negative in RIF |
| 2002 | 2285632 | RCT | 200 | Not exclusively | Significant in RIF |
| 2011 | 22014393 | Non-randomized | 30 | Not exclusively | Significant |
| 2011 | 26396577 | Non-Randomized | 74 | Not exclusively | Non-Significant |
| 2010 | 20407003 | RCT | 100 | Not exclusively | Significant |
| 2010 | 19568761 | RCT | 77 | Not exclusively | Negative |
| 2009 | 20070722 | RCT | 115 | Not exclusively | Significant in RIF |
| 2008 | 17681303 | RCT | 121 | Not exclusively | Significant |
| 2007 | 17197286 | Non-randomized | 117 | YES | Significant in RIF |
| 2003 | 12798877 | Non-randomized | 134 | Not exclusively | Significant |
in vitro available receptivity assays (Bielfeld et al., 2019). Interestingly to fertile controls even in molecules not included in commercially women with RIF showed a different proteomic profile compared RIF is even more perplexed. A recent proteomic analysis of embryo transfer (Volovsky et al., 2018).

HCG was administered in non-RIF patients followed by a fresh randomized trial reported a significantly negative outcome when in implantation (Evans and Salamonsen, 2013), a recent non-randomized urging for a potential negative effect of HCG after having primed the endometrial cavity with at least 500IU improve reproductive outcomes only in case of cleavage embryos and HCG ≥ 500IU

Meta-analysis, Craciunas et al. (2018) conclude that HCG may transfered (Craciunas et al., 2018). To the direction of increased heterogeneity, the source of HCG administered (recombinant vs. urinary) could also be pointed out. By performing sub-group meta-analysis, Craciunas et al. (2018) conclude that HCG may reproduce outcomes only in case of cleavage embryos after having primed the endometrial cavity with at least 500IU of HCG. Surprisingly, being in line with the findings of Evans and Salamonsen urging for a potential negative effect of HCG in implantation (Evans and Salamonsen, 2013), a recent non-randomized trial reported a significantly negative outcome when HCG was administered in non-RIF patients followed by a fresh embryo transfer (Volovsky et al., 2018).

The issue of HCG administration in case of women with RIF is even more perplexed. A recent proteomic analysis of women with RIF showed a different proteomic profile compared to fertile controls even in molecules not included in commercially available receptivity assays (Bielfeld et al., 2019). Interestingly it was shown in vitro that HCG could alter the proteomic profile in terms of endocytosis, HIF signaling and chemokine production (Bielfeld et al., 2019). This proves that RIF patients constitute a distinct population not to be treated simply as “infertile.” Such an approach dictates clinical trials to be designed exclusively for RIF patients. So far, only one full-paper RCT has been published addressing the issue of HCG efficacy in women with RIF, reporting HCG as significantly beneficial compared to controls; however benefit was shown even from placebo, implying an underlying endometrial injury effect (Huang et al., 2017a). The major core of evidence stems from non-randomized trials. A recent meta-analysis has summarized this evidence, supporting the use of HCG as an endometrial primer prior to embryo transfer in women with RIF (Xie et al., 2019). However, this meta-analysis has included both RCTs (including 2 RCTs published as abstracts) and non-randomized trials, a fact that poses a question upon the level of evidence produced. Further properly powered studies are needed to clarify the role of intrauterine HCG administration as a treatment option in women with RIF. Until such solid evidence so far, until properly designed randomized controlled trials verify such finding, HCG is not to be incorporated in clinical practice. It could be offered as a treatment option within the frame of a research protocol.

### Intra-Uterine Administration of Peripheral Blood Mononuclear Cells (PBMC)

Early reports based on in vitro and in vivo experiments have suggested that PBMC may modulate endometrial receptivity by (a) inducing a Th2 cytokine profile (Hashii et al., 1998), and (b) regulating trophoblast invasion (Nakayama

| Year | PMID | Publication type | Participants | RIF | Outcome |
|------|------|------------------|-------------|-----|---------|
| 2019 | 31704529 | Meta-analysis | 1,432 | YES | Significantly Favorable in RIF |
| 2019 | 31277770 | Meta-analysis | 2,763 | Not exclusively | Significantly Favorable |
| 2019 | 30659362 | Non-randomized | 305 | YES | Significantly favorable in RIF |
| 2019 | 30449012 | RCT | 200 | Not exclusively | Significantly favorable only for implantation rates |
| 2018 | 30291482 | Meta-analysis | 2,759 | Not exclusively | Non-significant |
| 2018 | 30341915 | Meta-analysis | 4,751 | Not exclusively | Significantly favorable only in case of cleavage embryos and HCG ≥ 500IU |
| 2018 | 29626233 | RCT | 180 | Not exclusively | Non-significant |
| 2018 | 29288552 | Non-randomized | 225 | YES | Significantly favorable for RIF |
| 2018 | 29148440 | Non-randomized | 1,207 | Not exclusively | Significantly unfavorable in FET and women without RIF |
| 2017 | 28400828 | RCT | 100 | Not exclusively | Non-significant |
| 2017 | 27921090 | RCT | 159 | Not exclusively | Non-significant |
| 2017 | 27680029 | RCT | 158 | Not exclusively | Significantly favorable |
| 2017 | 27449969 | RCT | 161 | YES | Significantly favorable compared to controls. Placebo also improved the outcomes |
| 2016 | 27317131 | Meta-analysis | 3,087 | Not exclusively | Non-significant |
| 2016 | 26359294 | Meta-analysis | 1,387 | Not exclusively | Significantly favorable |
| 2016 | 26141379 | RCT | 1,186 | Not exclusively | Non-significant |
| 2015 | 25531413 | RCT | 483 | Not exclusively | Significantly favorable |
| 2014 | 24799855 | RCT | 182 | Not exclusively | Non-significantly |
| 2014 | 25234040 | RCT | 300 | Not exclusively | Non-significant |
| 2014 | 24476536 | RCT | 210 | Not exclusively | Significantly favorable |
| 2011 | 22047664 | RCT | 260 | Not exclusively | Significantly favorable |

Salient features of the included studies on HCG as an intervention in improving endometrial receptivity.
Further postulations have been expressed: being a heterogeneous cell population (B- and T-lymphocytes, monocytes and macrophages) PBMC were considered ideal in mimicking the implantation process, namely an acute Th1 reaction to facilitate blastocyst adhesion followed by a Th2 modulation to achieve maternal-fetal immune tolerance and controlled blastocyst invasion (Mor et al., 2011). The first landmark study in the field was published by Yoshioka et al. (2006), showing that intrauterine administration of HCG-treated PBMCs could significantly improve reproductive outcomes in women with RIF. Since then a number of studies have been performed so far, with a moderate degree of heterogeneity. The published studies (summarized in Table 3), differ in terms of (a) population characteristics (infertile vs. exclusively RIF patients), (b) the transferred embryos (cleavage embryos vs. blastocysts, fresh vs. frozen embryos), c) the PBMC activation protocol (no-activation, activation by HCG, activation by corticotropin-releasing hormone-CRH). A critical approach in the published literature reveals the fact that although the randomized controlled trials (mainly focusing at RIF) performed so far, with a moderate degree of heterogeneity.

Recently, an array of meta-analyses has emerged, three referring to infertile populations in general (Maleki-Hajiagha et al., 2019; Yakin et al., 2019; Yang et al., 2020) and one referring to patients with RIF (Pourmoghadam et al., 2020a). Of the meta-analysis, evaluating the method as an intervention for infertility in general, the one that supports a significant benefit to the general infertile population (Maleki-Hajiagha et al., 2019) involves fewer participants than the two that draw a non-significant result (Yakin et al., 2019; Yang et al., 2020). Interestingly, subgroup analysis has revealed a significant improvement in reproductive outcomes in women with RIF (Yakin et al., 2019; Yang et al., 2020). The only meta-analysis focused on RIF, having included 1,215 participants, has concluded that intra-uterine administration of PBMC significantly improves the reproductive result in women with RIF (Pourmoghadam et al., 2020a). The results of the meta-analyses, combined, regarding the significantly positive results on RIF, imply a future role for this procedure in treating women with RIF. However, these findings should be treated with caution rather than enthusiasm. The level of evidence is rather weak and thus properly powered randomized trials are needed to enhance the evidence base to an acceptable level.

### Intrauterine Administration of Platelet-Rich Plasma

Platelet-rich-plasma (PRP) is a platelet-rich whole blood extract, having removed red and white blood cells. It is considered an inexpensive means of delivering high concentrations of growth factors since activated platelets release, via their α-granules, high concentrations of VEGF, TGFβ and PDGF (Lang et al., 2017; Baba et al., 2019). As a result PRP is considered effective as a regeneration and anti-inflammatory agent (Vitagliano et al., 2019; Arora and Arora, 2020). Local administration of PRP has been used in several medical fields like orthopedics, otolaryngology and ophthalmology. Five years ago, PRP was successfully applied for the first time as an intervention for improving refractory endometrium of women to receive IVF (Chang et al., 2015). Since then, several case series have been published with promising results. So far, three randomized controlled trials (Eftekhar et al., 2018; Nazari et al., 2020; Zamanian et al., 2020) along with two non-randomized controlled trials (Chang et al., 2019; Cokssuer et al., 2019) have been published showing significant improvement of the reproductive outcomes. The single meta-analysis in the literature has included seven studies (625 participants) of which 3 were randomized controlled trials and four were cohort studies (Maleki-Hajiagha et al., 2020) (Table 4). One of these four studies compared PRP administration with G-CSF administration while the rest used untreated controls (Maleki-Hajiagha et al., 2020). Of the three RCTs included, one was available as abstract. It was shown that all reproductive outcomes were significantly improved in PRP-treated cases (Maleki-Hajiagha et al., 2020).

### Table 3

| Year | PMID | Publication type | Participants | PBMC activation | RIF | Outcome |
|------|------|------------------|--------------|----------------|-----|---------|
| 2020 | 32781360 | RCT | 100 | HCG | Yes | Significantly favorable in RIF |
| 2020 | 31893538 | Meta-analysis | 1215 | HCG | YES | Significantly favorable in RIF |
| 2020 | 31791175 | Meta-analysis | 1173 | HCG or non-activated | Not exclusively | Significantly favorable in RIF (Sub group analysis) |
| 2019 | 31322946 | RCT | 250 | CRH | Not exclusively | Significantly favorable in RIF (Sub group analysis) |
| 2019 | 30739317 | Non-randomized | 26 | CRH | YES | Significantly favorable in RIF |
| 2019 | 30684765 | Meta-analysis | 886 | HCG or non-activated | Not exclusively | Significantly favorable |
| 2017 | 27915038 | Non-randomized | 633 | HCG | Not exclusively | Significantly favorable in RIF with cleavage embryos (Sub group analysis) |
| 2016 | 27521928 | RCT | 198 | HCG | YES | Significantly favorable in RIF |
| 2015 | 25852716 | Non-randomized | 45 | CRH | YES | Significantly favorable in RIF |
| 2011 | 22035703 | Non-randomized | 253 | Non-activated | Not exclusively | Significantly favorable in RIF (Sub group analysis) |
| 2006 | 17021188 | Non-randomized | 35 | HCG | YES | Significantly favorable in RIF |
The data upon women with RIF are also rather weak. Two randomized controlled trials and one non-randomized controlled trial address the PRP treatment in women with RIF reporting significant improvement in IVF efficacy (Coksu et al., 2019; Nazari et al., 2020; Zamanian et al., 2020), however the patients recruited in total do not allow extraction of definite conclusions. Thus, until evidence of properly designed randomized trials emerge, PRP should be offered within the frame of a trial.

Granulocyte Colony Stimulating Factor (G-CSF)

The role of G-CSF in reproductive physiology has been mainly studied the last two decades. G-CSF is produced by the granulosa cells during ovulation (Robert et al., 2019). It can be identified in the uterus mainly on the uterine NK cells, which play a major role during implantation both by enhancing receptivity and enabling endometrial synchronization (Sharma and Das, 2014; Robert et al., 2019). More importantly, G-CSF has been shown to regulate Th2 immunity, contributing to maternal-fetal immuno-tolerance (Moldenhauer et al., 2010).

The positive effects exerted by G-CSF on endometrial receptivity and implantation, supported the idea of using G-CSF as a local or systemic immune-modulator during IVF, and thus several observational studies and thereafter randomized and non-randomized clinical trials have emerged. Of note is the heterogeneity of the studies. Different populations selected (infertile, RIF, cases with thin endometrium), different ways of administration (intrauterine, subcutaneously), different concentrations offered and different study endpoints made it difficult to extract a solid conclusion. So far 10 randomized controlled trials have been published (Barad et al., 2014; Aleyasin et al., 2016; Davari-Tanha et al., 2016; Eftekhar et al., 2016a,b; Sarvi et al., 2017; Arefi et al., 2018; Bakirarar et al., 2018; Huang et al., 2020a; Kalem et al., 2020), with conflicting results, followed by meta-analyses in a timely manner (presented in Table 5). The six meta-analyses published present rather supportive results (Zhuo et al., 2016; Kamath et al., 2017; Li et al., 2017a; Hou et al., 2018; Jiang et al., 2020; Kamath et al., 2020). However, most of the meta-analyses have included both randomized controlled trials and non-randomized trials, weakening the level of evidence. The most powered meta-analysis at the moment is a Cochrane review including 15 randomized control trials with 1,253 participants (Kamath et al., 2020). The authors have shown a weak positive impact of G-CSF in reproductive outcomes, advising caution due to low quality data and increased uncertainty. As far as RIF cases are concerned, it was found that there could be a benefit by G-CSF administration (Kamath et al., 2020).

To the same direction most of the meta-analyses have showed a positive impact of G-CSF administration in case of RIF. Due, though, to the poor quality studies included, an increased level of uncertainty is generally noted. This uncertainty is in line with a recent randomized controlled trial showing that in case of RIF patients with normal endometrial thickness, G-CSF does not alter reproductive outcome (Kalem et al., 2020). It is thus evident that G-CSF is not to be applied as infertility intervention in the general population. Even in case of RIF the evidence does not allow G-CSF incorporation to the everyday practice. Properly powered studies are needed to clarify to which group of infertile patients would G-CSF offer some benefit.

Growth Hormone (GH)

The role of growth hormone in endometrial receptivity is still under investigation. It has been shown that GH receptors are expressed by endometrial epithelium, selectively during the mid-luteal phase (possibly during the window of implantation) and thereafter during decidualization (Sbracia et al., 2004). This expression pattern is similar to other molecules linked to endometrial receptivity. Additionally, it has been recently reported that GH may act, directly or indirectly, as an inducer for VEGF and integrin B3 expression, both involved in endometrial receptivity (Cui et al., 2019). As a result, GH has been demonstrated to be a mediator toward endometrial thickening, being rather appealing as a research intervention for women with thin endometrium (Lan et al., 2019). The evidence supporting GH for treating infertile women stems mainly from studies focusing on poor ovarian responders, mainly due to the parallel action that GH exerts on the ovary (Altmäe and Aghajanova, 2019; Huang et al., 2020b). So far, only one randomized trial has been published studying the GH-impact on women with RIF (Altmäe et al., 2018). The authors report that GH-treated RIF patients presented with significantly thicker endometrium and achieved significantly better reproductive outcomes compared to untreated RIF patients or women receiving their first IVF cycle (Altmäe et al., 2018). Although GH treatment seems promising, the lack of evidence does not allow its use as a standard of care.

Atosiban

Atosiban is a receptor inhibitor of oxytocin and V1a vasopressin. Based on the observation that embryo-transfer may trigger uterine contractions, which could be detrimental in embryonic apposition, the first case report of a successful pregnancy after atosiban was published in 2007 (Pierzynski et al., 2007). Since then four randomized controlled trials (Table 6) have been published (Moraglo et al., 2010; Ng et al., 2014; He et al., 2016; Yuan et al., 2019), with the most powered reporting a non-significant effect on reproductive outcomes on the general population (Ng et al., 2014). A recent meta-analysis combining six studies (1,754 participants) shows a rather weak improvement in clinical pregnancy rates but no effect on live birth rates in the general population (Huang et al., 2017b). Of note is the fact that this meta-analysis included both randomized and non-randomized trials, thus

| Table 4 | Salient features of the included studies on PRP as an intervention in improving endometrial receptivity. |
| Year | PMID | Publication type | Participants | RIF | Outcome |
| 2020 | 32965968 | RCT | 98 | Yes | Significantly favorable |
| 2020 | 30714427 | RCT | 97 | Yes | Significantly favorable |
| 2020 | 32006776 | Meta-analysis | 625 | No | Significantly favorable |
| 2019 | 30966843 | Non-randomized | 34 | Yes | Significantly favorable |
| 2019 | 30653117 | Non-randomized | 64 | No | Significantly favorable |
| 2018 | 30546532 | RCT | 83 | No | Significantly favorable |
TABLE 5 | Salient features of the included studies on G-CSF as an intervention in improving endometrial receptivity.

| Year | PMID | Publication type | Participants | RIF | Outcome |
|------|------|------------------|--------------|-----|---------|
| 2020 | 32862740 | RCT | 163 | | Significantly favorable |
| 2020 | 32663652 | Meta-analysis | 1164 | Yes | Significantly favorable in RIF |
| 2020 | 32198409 | RCT | 157 | Yes | Non-significant |
| 2020 | 31978254 | Meta-analysis | 1253 | Not exclusively | Significantly favorable in RIF (subgroup analysis) |
| 2019 | 31091064 | Non-randomized | 66 | Yes | RIF patients did not differ from patients with their 1st IVF cycle |
| 2019 | 30568355 | RCT | 150 | Not exclusively | Non-significant |
| 2018 | 30220024 | Meta-analysis | 1016 | Not exclusively | Significantly favorable, Significantly favorable in RIF (subgroup analysis) |
| 2018 | 30027145 | RCT | 50 | Not exclusively | Non-significant |
| 2018 | 28632452 | Meta-analysis | 255 | Not exclusively | Significantly favorable, Significantly favorable in RIF (subgroup analysis) |
| 2017 | 28784292 | Non-randomized | 62 | Not exclusively | Non-significant |
| 2017 | 28791050 | RCT | 28 | Not exclusively | Significantly improved implantation rate |
| 2016 | 27659067 | Meta-analysis | 1101 | Not exclusively | Significantly favorable (sc) Non-significant (intrauterine) |
| 2016 | 28068833 | RCT | 100 | Yes | Non-significant pregnancy rate |
| 2016 | 27981253 | RCT | 90 | Yes | Significantly favorable in RIF |
| 2016 | 27326420 | RCT | 100 | Non-significant |
| 2016 | 26698009 | RCT | 112 | Yes | Significantly favorable in RIF |
| 2014 | 24242347 | RCT | 141 | Not exclusively | Non-significant |
| 2014 | 25489123 | Non-randomized | 68 | Not exclusively | Non-significant |
| 2014 | 23885097 | Non-randomized | 59 | Not exclusively | Non-significant |

weakening the level of evidence of the reported findings. Only two non-randomized studies on RIF patients report a significant benefit after atosiban treatment (Chou et al., 2011; Lan et al., 2012). Interestingly, both the meta-analysis (Huang et al., 2017b) and a recent prospective study on women with at least one IVF effort (Wu et al., 2020), after performing subgroup analysis, report significant improvement on the reproductive outcomes in case of RIF patients. Taking into consideration the fact that subgroup analysis may include a risk of statistical bias, this observation implies a potential role for atosiban when treating women with RIF. Properly powered randomized controlled trials are needed to delineate atosiban efficacy.

Antibiotics for Chronic Endometritis

The field of chronic endometritis (CE) is emerging, especially in unexplained RIF, since CE presents a subtle course that can be easily missed. Few reports have been published so far upon antibiotic treatment in case of CE diagnosed in women with unexplained RIF (Johnston-MacAnanny et al., 2010; Yang et al., 2014; Cicinelli et al., 2015; Tersoglio et al., 2015; Bouet et al., 2016; Kitaya et al., 2017; Zhang et al., 2019). Analyzing this therapeutic approach, it can be easily seen that there is substantial heterogeneity in terms of antibiotic treatment: antibiotics are offered according to microbial cultures or empirically, including penicillins, cephalosporins, kinolones, metronidazole, clindamycin, tetracyclines (mainly doxycycline), and aminoglycodides (gentamycine). The course of treatment also varies. Interestingly, apart from the standard per os treatment, the intrauterine approach has also been presented (Zhang et al., 2019). All the studies published so far are non-randomized thus producing low level of evidence. Two recent meta-analyses have summarized the existing results, supporting antibiotic treatment as an approach to improve reproductive outcomes in women with RIF (Vitagliano et al., 2018b; Huang et al., 2020b). Additionally, it is highlighted that a stringent approach in setting the diagnosis may reveal lower CE incidences and reproductive outcomes compared to approaches with broader diagnostic criteria (Huang et al., 2020b).
Long-term treatments seem more beneficial compared to short-term antibiotic courses (Huang et al., 2020b).

**Efforts to Intervene in Case of Endometrial Dysbiosis**

The concept of endometrial dysbiosis is emerging during the last 5 years. Several approaches have been proposed as potentially effective in restoring normal endometrial microbiome. However, it must be taken under consideration the fact that to date there is no core endometrial microbiome, therefore restoring to normal could be a matter of question (Molina et al., 2020). Microbiome restoration is initially approached by the administration of antibiotics, considering that most of the time dysbiotic microbiota may include pathogens well-controlled by standard anti-microbial agents (Kyono et al., 2019). Several routes of administration have been proposed so far including oral, vaginal and intrauterine (Molina et al., 2020). Additionally, the administration of pro- and pre-biotics has been tested as auxiliary means of maintaining or amplifying the eubiotic bacteria. Of note is the mode of action of pro-biotics—bacteria involved in normal microbiome, administered in the context to colonize in an antagonizing fashion the dysbiotic microbial counterparts (Chenoll et al., 2019). On the contrary, pre-biotics are molecules uptaken by normal microbial populations, facilitating their survival. Pre- and pro-biotics are usually co-administered with antibiotics. A recent study investigated different routes of antibiotic administration (metronidazole) combined with prebiotic (lactoferrin) and probiotic administration, concluded that the combined vaginal and oral metronidazole administration along with a vaginal probiotic treatment could restore normal endometrial microbiome in women with RIF (Kadogami et al., 2020). New approaches have been proposed as promising, lacking evidence at the moment. Vaginal microbiome transplants have been considered as a possibility. A recent study showed the restoration of the vaginal microbiome in case of refractory vaginosis (Lev-Sagie et al., 2019), a fact that could imply further colonization of the endometrial cavity by the ascending route. Taking all the above into consideration, it is clear that there is no evidence at the moment to support both endometrial microbiome assessment and thereafter interventions toward restoration to normal. Such efforts have to be strictly performed in the frame of a research protocol.

**CONCLUSION**

All the novel interventions, aiming to treat unexplained RIF, lack the evidence required in order to be incorporated to standard of care. Properly designed randomized trials are therefore needed to clarify which could be beneficial in RIF treatment. RIF patients should be properly informed regarding potential benefits and risks.

**AUTHOR CONTRIBUTIONS**

AM had the idea for this review. AM, FM, and TV participated in literature search, drafted, and critically revised the manuscript, approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

**REFERENCES**

Aaleyasin, A., Aghahosseini, M., Rashidi, M., Safdarian, L., Sarvi, F., Najmi, Z., et al. (2015). In vitro fertilization outcome following embryo transfer with or without preinstillation of human chorionic gonadotropin into the uterine cavity: a randomized controlled trial. Gynecol. Obstet. Invest. 79, 201–205. doi: 10.1159/000363235

Achache, H., Tsafrir, A., Prus, D., Reich, R., and Revel, A. (2010). Defective endometrial prostaglandin identified in patients with repeated implantation failure occurring in vitro fertilization. Fertil. Steril. 94, 1271–1278. doi: 10.1016/j.fertnstert.2009.07.1668

Al-Nasiry, S., Ambrosino, E., Schlaepfer, M., Morré, S. A., Wieten, L., Voncken, J. W., et al. (2020). The Interplay Between Reproductive Tract Microbiota and Immunological System in Human Reproduction. *Front. Immunol.* 11:378. doi: 10.3389/fimmu.2020.00378

Aleyasin, A., AbediAsl, Z., Nazari, A., and Sheik, M. (2016). Granulocyte colony-stimulating factor in repeated IVF failure, a randomized trial. *Reproduction* 151, 637–642. doi: 10.1530/rep-16-0046

Ali, S. B., Jeelall, Y., Pennell, C. E., Hart, R., McLean-Tooke, A., and Lucas, M. (2018). The role of immunological testing and intervention in reproductive medicine: A fertile collaboration? *Am. J. Reprod. Immunol.* 79:12784. doi: 10.1111/aji.12784

Altmäe, S., and Aghajanova, L. (2019). Growth Hormone and Endometrial Receptivity. *Front. Endocrinol.* 10:653. doi: 10.3389/fendo.2019.00653

Altmäe, S., Mendoza-Tesarik, R., Mendoza, C., Mendoza, N., Cucinelli, F., and Tesarik, J. (2018). Effect of Growth Hormone on Uterine Receptivity in Women With Repeated Implantation Failure in an Oocyte Donation Program: A Randomized Controlled Trial. *J. Endocr. Soc.* 2, 96–105. doi: 10.1210/js.2017-00359

Arefi, S., Fazeli, E., Esfahani, M., Borhani, N., Yamini, N., Hosseini, A., et al. (2018). Granulocyte-colony stimulating factor may improve pregnancy outcome in patients with history of unexplained recurrent implantation failure: An RCT. *Int. J. Reprod. Biomed.* 16, 299–304.

Arora, G., and Arora, S. (2020). Platelet Rich Plasma - where do we stand today? A critical narrative review and analysis. *Dermatol. Ther.* 2020.e14343. doi: 10.1111/dth.14343

Baba, K., Yamazaki, Y., Sone, Y., Sugimoto, Y., Moriyama, K., Sugimoto, T., et al. (2019). In vitro long-term study of cryopreserved umbilical cord blood-derived platelet-rich plasma containing growth factors-PDGF-BB, TGF-β, and VEGF. *J. Cranio-maxillofac. Surg.* 47, 668–675. doi: 10.1016/j.jcms.2019.01.020

Baker, J. M., Chase, D. M., and Herbst-Kralovetz, M. M. (2018). Uterine Microbiota: Residents, Tourists, or Invaders? *Front. Immunol.* 9:208. doi: 10.3389/fimmu.2018.00208

Bakirarat, B., Kent, E., Makrigiannakis, A., Gurgan, T., Jain, S., Mahey, R., et al. (2018). Effect of Intrauterine Perfusion of Granulocyte Colony-stimulating Factor on Endometrial parameters and In Vitro Fertilization Outcome in Women Undergoing In Vitro Fertilization/Intracytoplasmic Sperm Injection Cycles: A Randomized Controlled Trial. *Sci. Rep.* 11, 254–260.

Barad, D. H., Yu, Y., Kushnir, V. A., Shohat-Tal, A., Lazzaroni, E., Lee, H. J., et al. (2014). A randomized clinical trial of endometrial perfusion with granulocyte colony-stimulating factor in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates. *Fertil. Steril.* 101, 710–715. doi: 10.1016/j.fertnstert.2013.12.016

Barash, A., Dekel, N., Fieldust, S., Segal, I., Schechtman, E., and Granot, I. (2003). Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. *Fertil. Steril.* 79, 1317–1322. doi: 10.1016/S0015-0282(03)00345-5
experienced previous embryo transfer failure. Fertil. Steril. 112, 1103–1111. doi: 10.1016/j.fertnstert.2019.08.006
Fazleabas, A. T., Donnelly, K. M., Srinivasan, S., Fortman, J. D., and Miller, J. B. (1999). Modulation of the baboon (Papio anubis) uterine endometrium by chorionic gonadotropin during the period of uterine receptivity. Proc. Natl. Acad. Sci. U.S.A. 96, 2543–2548.
Fluhr, H., Bischof-Isalmi, D., Kremer, S., Licht, P., Bischof, P., and Zygumnunt, M. (2008a). Human chorionic gonadotropin stimulates matrix metalloproteinases-2 and -9 in cytotrophoblastic cells and decreases tissue inhibitor of metalloproteinases-1, -2, and -3 in decidualized endometrial stromal cells. Fertil. Steril. 90(4 Suppl.), 1390–1395. doi: 10.1097/01.fert.0000368109.20947.6d
Fluhr, H., Carl, S., Deperschmidt, M., Wallwiener, D., Zygumnunt, M., and Licht, P. (2008b). Differential effects of human chorionic gonadotropin and decidualization on insulin-like growth factors-I and -II in human endometrial stromal cells. Fertil. Steril. 90(4 Suppl.), 1384–1389. doi: 10.1097/01.fert.2007.07.1357
Fogle, R. H., Li, A., and Paulson, R. J. (2010). Modulation of HOXA10 and other markers of endometrial receptivity by age and human chorionic gonadotropin in an endometrial explant model. Fertil. Steril. 93, 1255–1259. doi: 10.1016/j.fertnstert.2008.11.002
Frantz, S., Parinaud, J., Kret, M., Rocher-Escriva, G., Papaxanthos-Roche, A., Creux, H., et al. (2019). Decrease in pregnancy rate after endometrial scratch in women undergoing a first or second in vitro fertilization. A multicenter randomized controlled trial. Hum. Reprod. 34, 92–99. doi: 10.1093/humrep/dey334
Gao, M., Jiang, X., Li, B., Li, L., Duan, M., Zhang, X., et al. (2019). Intratubine injection of human chorionic gonadotropin before embryo transfer can improve in vitro fertilization-embryo transfer outcomes: a meta-analysis of randomized controlled trials. Fertil. Steril. 112, 89e–97e. doi: 10.1016/j.fertnstert.2019.02.027
Gkrozou, F., Tisonis, O., Dimitriou, E., and Paschopoulos, M. (2020). In women with chronic or subclinical endometritis is hysteroscopy suitable for setting the diagnosis? A systematic review. J. Obstet. Gynaecol. Res. 46, 1639–1650. doi: 10.1111/j.1447-0756.2019.10442.x
Glassing, A., Dowd, S. E., Galandiuk, S., Davis, B., and Chiodini, R. J. (2016). Glassing, A., Dowd, S. E., Galandiuk, S., Davis, B., and Chiodini, R. J. (2016). Peripheral blood mononuclear cells stimulate progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and interleukin-10 in corpus luteum function and differentiation. Hum. Reprod. 13, 2738–2744. doi: 10.1093/humrep/der240
He, Y., Wu, H., He, X., Xing, Q., Zhou, P., Cao, Y., et al. (2016). Administration of atosiban in patients with endometriosis undergoing frozen-thawed embryo transfer: a prospective, randomized study. Fertil. Steril. 106, 416–422. doi: 10.1016/j.fertnstert.2016.04.019
Hong, K. H., Forman, E. J., Werner, M. D., Upham, K. M., Gumeny, C. L., Winslow, A. D., et al. (2014). Endometrial infusion of human chorionic gonadotropin at the time of blastocyst embryo transfer does not impact clinical outcomes: a randomized, double-blind, placebo-controlled trial. Fertil. Steril. 102, 1591.e–1595.e. doi: 10.1016/j.fertnstert.2014.08.006
Hou, W., Shi, G., Cai, B., Ding, C., Song, J., Zhang, X., et al. (2018). Effect of intratubine injection of human chorionic gonadotropin before fresh embryo transfer on IVF and ICSI outcomes: a meta-analysis. Arch. Gynecol. Obstet. 298, 1061–1069. doi: 10.1007/s00404-018-4923-3
Huang, P., Wei, L., and Li, X. (2017a). A study of intratubine infusion of human chorionic gonadotropin (hCG) before frozen-thawed embryo transfer after two or more implantation failures. Fertil. Steril. 107, 67–69. doi: 10.1016/j.fertnstert.2016.12.07164
Huang, P., Yao, C., Wei, L., and Lin, Z. (2020a). The intratubine perfusion of granulocyte colony-stimulating factor (G-CSF) before frozen-thawed embryo transfer in patients with two or more implantation failures. Hum. Fertil. 2020, 1–5. doi: 10.1080/14472737.2020.1811904
Huang, Q. Y., Rong, M. H., Lan, A. H., Lin, X. M., Lin, X. G., He, R. Q., et al. (2017b). The impact of atosiban on pregnancy outcomes in women undergoing in vitro fertilization-embryo transfer: A meta-analysis. PLoS One 12:e0175501. doi: 10.1371/journal.pone.0175501
Huang, W., Liu, B., He, Y., Xie, Y., Liang, T., Bi, Y., et al. (2020b). Variation of diagnostic criteria in women with chronic endometritis and its effect on reproductive outcomes: A systematic review and meta-analysis. J. Reprod. Immunol. 140:103146. doi: 10.1016/j.jri.2020.103146
Jiang, Y., Zhao, Q., Zhang, Y., Zhou, L., Lin, J., Chen, Y., et al. (2020). Treatment of G-CSF in unexplained, repeated implantation failure: A systematic review and meta-analysis. J. Gynecol. Obstet. Hum. Reprod. 2020:101866. doi: 10.1016/j.jogoh.2020.101866
Johnston-MacAnany, E. B., Hartnett, J., Engmann, L. L., Nelsen, J. C., Sanders, M. M., and Benadiva, C. A. (2010). Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. Fertil. Steril. 93, 437–441. doi: 10.1016/j.fertnstert.2008.12.131
Kadogami, D., Nakaoka, Y., and Morimoto, Y. (2020). Use of a vaginal probiotic suppository and antibiotics to influence the composition of the endometrial microbiota. Reprod. Biol. 20, 307–314. doi: 10.1016/j.ripbj.2020.07.001
Kalem, Z., Namli Kalem, M., Bakirarar, B., Keni, E., Makriannakis, A., and Gurgan, T. (2020). Intrauterine G-CSF Administration in Recurrent Implantation Failure (RIF): An Ret. Sci. Rep. 10, 5319. doi: 10.1038/s41598-020-61955-7
Kalma, Y., Granot, I., Gnainsky, N., Or, Y., Czernobilsky, B., Dekel, N., et al. (2009). Endometrial biopsy-induced gene modulation: first evidence for the expression of bladder-transmembrane uroplakin Ib in human endometrium. Fertil. Steril. 91, 1042–1049. doi: 10.1016/j.fertnstert.2008.01.043
Kamath, M. S., Chittawar, P. B., Kirubakaran, R., and Mascarenhas, M. (2017). Use of granulocyte-colony stimulating factor in assisted reproductive technology: A systematic review and meta-analysis. Eur. J. Obstet. Gynecol. Reprod. Biol. 214, 16–24. doi: 10.1016/j.ejogrb.2017.04.022
Kamath, M. S., Kirubakaran, R., and Sunkara, S. K. (2020). Granulocyte-colony stimulating factor administration for subfertile women undergoing assisted reproduction. Cochrane Database Syst. Rev. 1:Cd0131226. doi: 10.1002/14651858.CD0131226.pub2
Kara, M., Sabah Ozcan, S., Aran, T., Kara, O., and Yilmaz, N. (2019). Decreased expression of LIF mRNA in patients with myoma uteri. J. Cell Biochem. 120, 3423–3427. doi: 10.1002/jcb.27613
Kasius, J. C., Broekmans, F. J., Sie-Go, D. M., Bourgain, C., Eijkemans, M. J., Fauser, B. C., et al. (2012). The reliability of the histological diagnosis of endometriosis in asymptomatic IVF cases: a multicenter observer study. Hum. Reprod. 27, 153–158. doi: 10.1093/humrep/ddr341
Kayisli, U. A., Selam, B., Guzeloglu-Kayisli, O., Demir, R., and Arici, A. (2003). Human chorionic gonadotropin contributes to maternal immunotolerance and
endometrial apoptosis by regulating Fas-Fas ligand system. J. Immunol. 171, 2305–2313.

Kim, D., Hofstaedter, C. E., Zhao, C., Mattei, L., Tanes, C., Clarke, E., et al. (2017). Optimizing methods and dodging pitfalls in microbe research. Microbiome 5:5. doi: 10.1186/s40168-017-0267-5

Kitaya, K., Matsuabashiyi, H., Takaya, Y., Nishiyama, R., Yamaguchi, K., Takeuchi, T., et al. (2017). Live birth rate following oral antibiotic treatment for chronic endometritis in infertile women with repeated implantation failure. Am. J. Reprod. Immunol. 78:12719. doi: 10.1111/aji.12719

Koot, Y. E., van Hooff, S. R., Boomsma, C. M., van Leenen, D., Groot Koerkamp, M. J., Godijn, M., et al. (2016). An endometrial gene expression signature accurately predicts recurrent implantation failure after IVF. Sci. Rep. 6:19411. doi: 10.1038/srep19411

Kyono, K., Hashimoto, T., Kikuchi, S., Nagai, Y., and Sakuraba, Y. (2019). A pilot study and case reports on endometrial microbiota and pregnancy outcome: An analysis using 16S rRNA gene sequencing among IVF patients, and trial therapeutic intervention for dysbiotic endometrium. Reprod. Med. Biol. 18, 72–82. doi: 10.1002/rmb2.12250

Lan, K. C., Lin, P. Y., Chang, Y. C., Chen, Y. J., Tsai, Y. R., Ismael Mohamed, I. S., et al. (2019). Growth hormone supplementation may improve the pregnancy rate and endometrial receptivity among women aged more than 40 years undergoing in vitro fertilization. Biomed. J. 42, 411–416. doi: 10.1016/j.bj.2019.05.003

Lan, V. T., Khang, V. N., Nhu, G. H., and Tuong, H. M. (2012). Atosiban improves embryo transfer cycles of patients with repeated implantation failure. J. Reprod. Immunol. 90, 106–111. doi: 10.1016/j.jreim.2012.05.014

Lang, S., Herrmann, M., Pfeifer, C., Brockhoff, G., Zellner, J., Nerlich, M., et al. (2017). Leukocyte-reduced platelet-rich plasma stimulates the in vitro proliferation of adipose-tissue derived mesenchymal stem cells depending on PDGF signaling. Clin. Hemorheol. Microcirc. 67, 183–196. doi: 10.3233/ch-170246

Läkkirikiat, P., and Thanabooynawat, I. (2019). Increased implantation rate after intrauterine infusion of a small volume of human chorionic gonadotropin at the time of embryo transfer: a randomized, double-blind controlled study. Arch. Gynecol. Obstet. 299, 267–275. doi: 10.1007/s00404-018-4962-7

Lebovitz, O., and Orvieto, R. (2014). Treating patients with “thin” endometrium - an ongoing challenge. Gynecol. Endocrinol. 30, 409–414. doi: 10.3109/09513590.2014.906571

Lédée, N., Petitbarat, M., Chevrier, L., Vitoux, D., Kiehne, K., Rahmati, M., et al. (2017). The effect of G-CSF on infertile women undergoing IVF treatment: A meta-analysis. Syst. Biol. Reprod. Med. 63, 239–247. doi: 10.1080/19337191.2017.1287225

Li, J., Mo, S., and Chen, Y. (2017a). The Uterine Immune Profile May Help Women With Repeated Unexplained Embryo Implantation Failure After In Vitro Fertilization. Am. J. Reprod. Immunol. 75, 388–401. doi: 10.1111/aji.12483

Liang, P. Y., Yin, B., Cai, J., Hu, X. D., Song, C., Wu, T. H., et al. (2015). Increased circulating Th1/Th2 ratios but not other lymphocyte subsets during controlled ovarian stimulation are linked to subsequent implantation failure after transfer of in vitro fertilized embryos. Am. J. Reprod. Immunol. 73, 12–21. doi: 10.1111/aji.12320

Liu, J., Losch, A., Dittrich, R., Neuwinger, I., Siebenthulb, E., and Wildt, L. (1998). Novel insights into human endometrial paracarcinoid and embryo-maternal communication by intrauterine microdialysis. Hum. Reprod. Update 4, 532–538.

Liu, K. E., Hartman, M., Hartman, A., Luo, Z. C., and Mahutte, N. (2018). The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: an analysis of over 40 000 embryo transfers. Hum. Reprod. 33, 1883–1888. doi: 10.1093/humrep/dey281

Lopata, A., and Hay, D. L. (1989). The potential of early human embryos to form blastocysts, hatch from their zona and secrete hCG in culture. Hum. Reprod. 4(8 Suppl.), 87–94.

MacKens, S., Racca, A., Van de Velde, H., Drakopoulos, P., Tournaye, H., Stoop, D., et al. (2020). Follicular-phase endometrial scratching: a truncated randomized controlled trial. Hum. Reprod. 35, 1090–1098. doi: 10.1093/humrep/deaa018

Madkour, A., Bouamoud, N., Louanjli, N., Kaarouch, I., Copin, H., Benkhalfa, M., et al. (2016). Intrauterine insemination of cultured peripheral blood mononuclear cells prior to embryo transfer improves clinical outcome for patients with repeated implantation failures. Zygote 24, 58–69. doi: 10.1017/ s096719441600719

Makrigiannakis, A., BenKhalifa, M., Vrekoussis, T., Mahjub, S., Kalantaridou, S. N., and Gurgan, T. (2015). Repeated implantation failure: a new potential treatment option. Eur. J. Clin. Invest. 45, 380–384. doi: 10.1111/eci.12417

Maleki-Hajiagha, A., Vrekoussis, T., Mrekousis, T., Mahjub, S., Kalantaridou, S. N., and Gurgan, T. (2019). Intrauterine CRH-treated PBMC in repeated implantation failure. Eur. J. Clin. Invest. 49:e13084. doi: 10.1111/eci.13084

Maleki-Hajiagha, A., Razavi, M., Rezaeinjedad, M., Rouholamin, S., Almassi-Hashiani, A., Pirjani, R., et al. (2019). Intrauterine administration of autologous peripheral blood mononuclear cells in patients with recurrent implantation failure: A systematic review and meta-analysis. J. Reprod. Immunol. 131, 50–56. doi: 10.1016/j.jri.2019.01.001

Maleki-Hajiagha, A., Razavi, M., Rouholamin, S., Rezaeinjedad, M., Maroufizadeh, S., and Sepidarkish, M. (2020). Intrauterine infusion of autologous platelet-rich plasma in women undergoing assisted reproduction: A systematic review and meta-analysis. J. Reprod. Immunol. 137:103078. doi: 10.1016/j.jri.2019.103078

Mansour, R., Tawab, N., Kamal, O., El-Faissal, Y., Serour, A., Aboughar, M., et al. (2011). Intrauterine injection of human chorionic gonadotropin before embryo transfer significantly improves the implantation and pregnancy rates in in vitro fertilization/intracytoplasmic sperm injection: a prospective randomized study. Fertil. Steril. 96, 1370.e–1374.e. doi: 10.1016/j.fertnstert.2011.09.044

Mao, X., Wu, L., Chen, Q., Kuang, Y., and Zhang, S. (2019). Effect of hysterectomy before starting in-vitro fertilization for women with recurrent implantation failure: A meta-analysis and systematic review. Medicine 98:e14075. doi: 10.1097/md.0000000000001475

Miwa, I., Tamura, H., Takasaki, A., Yamagata, Y., Shimamura, K., and Sugino, N. (2009). Pathophysiological features of “thin” endometrium. Fertil. Steril. 91, 998–1004. doi: 10.1016/j.fertnstert.2008.01.029

Moldenhauer, L. M., Keenihan, S. N., Hayball, J. D., and Robertson, S. A. (2010). GM-CSF is an essential regulator of T cell activation competence in uterine dendritic cells during early pregnancy in mice. J. Immunol. 185, 7085–7096. doi: 10.4049/jimmunol.1001374

Molina, N. M., Sola-Leyva, A., Saez-Lara, M. J., Plaza-Diaz, J., Tubic-Pavlovic, A., Romero, B., et al. (2020). New Opportunities for Endometrial Health by Modifying Uterine Microbial Composition: Present or Future? Biomolecules 10:1004593. doi: 10.3390/biom10040593

Mor, G., Cardenas, I., Abrahams, V., and Guller, S. (2011). Inflammation and pregnancy: the role of the immune system at the implantation site. Ann. N Y. Acad. Sci. 1221, 80–87. doi: 10.1111/j.1749-6632.2010.05938.x
Moraloglu, O., Tonguc, E., Var, T., Zeyrek, T., and Batioglu, S. (2010). Treatment
Mor, G., and Kwon, J. Y. (2015). Trophoblast-microbiome interaction: a new
Makrigiannakis et al. Approaches to Improve Endometrial Receptivity
Nakayama, T., Fujiwara, H., Maeda, M., Inoue, T., Yoshioka, S., Mori, T., et al.
Osman, A., Pundir, J., Elsherbini, M., Dave, S., El-Toukhy, T., and Khalaf, Y. (2016).
Ng, E. H., Li, R. H., Chen, L., Lan, V. T., Tuong, H. M., and Quan, S. (2014). A
Navali, N., Gassemzadeh, A., Farzadi, L., Abdollahi, S., Nouri, M., Hamdi, K., et al.
Pier, B., Crellin, C., Katre, A., Conner, M. G., Novak, L., Young, S. L., et al. (2020).
Pierzynski, P., Reinheimer, T. M., and Kuczynski, W. (2007). Oxytocin antagonists
Potdar, N., Gelbaya, T., and Nardo, L. G. (2012). Endometrial injury to overcome

Wirleitner, B., Schuff, M., Vanderzwalmen, P., Stecher, A., Okhowat, J., Hradecký, Volodarsky-Perel, A., Buckett, W., and Tulandi, T. (2019). Treatment of Weyrich, L. S., Farrer, A. G., Eisenhofer, R., Arriola, L. A., Young, J., Selway, C. A., Vitagliano, A., Saccardi, C., Noventa, M., Di Spiezio, Sardo, A., Saccone, G., Valenti, G., Sapia, F., et al. (2018a).

VanderWeele, T. J., and Knol, M. J. (2011). Interpretation of subgroup analyses in van Hoogenhuijze, N. E., Kasius, J. C., Broekmans, F. J. M., Bosteels, J., and Toth, B., Roth, K., Kunert-Keil, C., Scholz, C., Schulze, S., Mylonas, I., et al. (2008).

Tersoglio, A. E., Salatino, D. R., Reinchisi, G., Gonzalez, A., Tersoglio, S., and Tapia-Pizarro, A., Archilles, S., Argandona, F., Valencia, C., Zavaleta, K., Cecilia transcervical resection of adhesions: a retrospective cohort study.

Frontiers in Cell and Developmental Biology | www.frontiersin.org 17 16 March 2021 | Volume 9 | Article 613277

Yu, N., Zhang, B., Xu, M., Wang, S., Liu, R., Wu, J., et al. (2016). Intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) for infertile women before embryo transfer: meta-analysis. J. Obstet. Gynaecol. 40, 961–968. doi: 10.1016/j.jogre.2019.1673711

Yang, H., Lei, C. X., and Zhang, W. (2013). Human chorionic gonadotropin (hCG) regulation of galectin-3 expression in endometrial epithelial cells and endometrial stromal cells. Acta Histochem. 115, 3–7. doi: 10.1016/j.acthis.2011.05.002

Yang, J. H., Chen, C. D., Chou, C. H., Wen, W. F., Tsao, P. N., Lee, H., et al. (2019). Intentional endometrial injury increases embryo implantation potentials through enhanced endometrial angiogenesis. Biol. Reprod. 100, 381–389. doi: 10.1093/biolre/iroy205

Yang, R., Du, X., Wang, Y., Song, X., Yang, Y., and Qiao, J. (2014). The hysteroscopy and histological diagnosis and treatment value of chronic endometritis in recurrent implantation failure patients. Arch. Gynecol. Obstet. 289, 1363–1369. doi: 10.1007/s00404-013-1311-2

Ye, H., Hu, J., He, W., Zhang, Y., and Li, C. (2015). The efficacy of intrauterine injection of human chorionic gonadotropin before embryo transfer in assisted reproductive cycles: Meta-analysis. J. Int. Med. Res. 43, 738–746. doi: 10.1177/0300060515592903

Yeung, T. W., Chai, J., Li, R. H., Lee, V. C., Ho, P. C., and Ng, E. H. (2014). The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in vitro fertilization: a randomized controlled trial. Hum. Reprod. 29, 2474–2481. doi: 10.1093/humrep/deu213

Yoshisaka, S., Fujiyama, H., Nakayama, T., Kosaka, K., Mori, T., and Fujii, S. (2006). Intrauterine administration of autologous peripheral blood mononuclear cells promotes implantation rates in patients with repeated failure of IVF-embryo transfer. Hum. Reprod. 21, 3290–3294. doi: 10.1093/humrep/del312

Yu, X., Gao, C., Dai, C., Yang, F., and Deng, X. (2019). Endometrial injury increases expression of hypoxia-inducible factor and angiogenesis in the endometrium of women with recurrent implantation failure. Reprod. Biomed. Online 38, 761–767. doi: 10.1038/s41148-018-0071-9

Yuan, C., Song, H., Fan, L., Su, S., and Dong, B. (2019). The Effect of Atosiban on Patients With Difficult Embryo Transfers Undergoing In Vitro Fertilization-Embryo Transfer. Reprod. Sci. 26, 1613–1617. doi: 10.1177/193719119831791

Zamaniyan, M., Peyvandi, S., Heidaryan Gorji, H., Moradi, S., Jamal, J., Yahya Poor, et al. (2020). Effect of platelet-rich plasma on pregnancy outcomes in
infertile women with recurrent implantation failure: a randomized controlled trial. Gynecol. Endocrinol. 2020, 1–5. doi: 10.1080/09513590.2020.1756247

Zarei, A., Parsanezhad, M. E., Younesi, M., Alborzi, S., Zolghadri, J., Samsami, A., et al. (2014). Intraperitoneal administration of recombinant human chorionic gonadotropin before embryo transfer on outcome of in vitro fertilization/ intracytoplasmic sperm injection: A randomized clinical trial. Iran J. Reprod. Med. 12, 1–6.

Zhang, H., Huang, C., Chen, X., Li, L., Liu, S., Li, Y., et al. (2020). The number and cytotoxicity and the expression of cytotoxicity-related molecules in peripheral natural killer (NK) cells do not predict the repeated implantation failure (RIF) for the in vitro fertilization patients. Genes Dis. 7, 283–289. doi: 10.1016/j.gendis.2019.03.005

Zhang, Y., Xu, H., Liu, Y., Zheng, S., Zhao, W., Wu, D., et al. (2019). Confirmation of chronic endometritis in repeated implantation failure and success outcome in IVF-ET after intrauterine delivery of the combined administration of antibiotic and dexamethasone. Am. J. Reprod. Immunol. 82:e13177. doi: 10.1111/aji.13177

Zhao, J., Xu, B., Xie, S., Zhang, Q., and Li, Y. P. (2016). Whether G-CSF administration has beneficial effect on the outcome after assisted reproductive technology? A systematic review and meta-analysis. Reprod. Biol. Endocrinol. 14:62. doi: 10.1186/s12958-016-0197-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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