Recurrent Implantation Failure
Recurrent Implantation Failure

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What is recurrent implantation failure?

BASIL C. TARLATZIS, JULIA K. BOSDOU, and EFSTRATIO M. KOLIBIANAKIS

INTRODUCTION

Successful implantation

Implantation is a complex phenomenon, still not thoroughly understood, involving the embryo and the endometrium. Implantation has been considered as a gradual process requiring embryo adhesion to the luminal surface of the endometrium, followed by invasion of the trophectoderm cells from the embryo, through the luminal epithelium, into the deeper layer of the endometrium. These complex molecular interactions between the uterus and the mature blastocyst are necessary to establish the uteroplacental circulation and finally lead to implantation, approximately six or seven days after fertilization. By the 10th day after fertilization, the blastocyst is completely embedded in the stromal tissue of the uterus, leading to cytotrophoblast invasion into the entire endometrium and the inner third of the myometrium, as well as the uterine vasculature.

In clinical practice, implantation can be confirmed by a rising human chorionic gonadotrophin (hCG) level, which occurs eight to ten days after ovulation or 14 days after oocyte retrieval in assisted reproductive technology (ART) cycles. It is considered to be successful when there is ultrasonographic evidence of an intrauterine gestational sac after five weeks of gestation, or usually three weeks after oocyte retrieval in ART cycles.

Unfortunately, pregnancy achievement in the human is a relatively inefficient process, with a probability of conception approximately 25%–30% per cycle. Thus, it is more likely for a human embryo not to implant than to lead to establishment of pregnancy.

Implantation failure

From a clinical point of view, implantation failure may occur very early during the adhesion stage, with no detectable hCG production and, thus, no objective evidence of pregnancy. It may also occur at a later stage, after initiation of hCG production from the implanting embryo, without, however, formation of an intrauterine gestational sac visible on ultrasonography, which is clinically recognized as biochemical pregnancy.

Implantation failure may be due to embryo and/or maternal factors. Problems due to the embryo can be associated either with poor oocyte quality or with paternal factors. On the other hand, implantation problems associated with the female may be due to uterine, tubal, or immunological factors; endometriosis; or thrombophilia. Surprisingly, the high implantation rate of donated oocytes in women of advanced reproductive age suggests that oocyte quality rather than endometrial receptivity largely determines the success of implantation.

In non-oocyte donation cycles and despite the technological advances in in vitro fertilization (IVF), only about one-quarter of IVF cycles will result in live births, leading many couples to experience multiple IVF failures. After each failed IVF attempt, pregnancy rates decrease significantly in subsequent cycles, with the most remarkable decrease after the third cycle. Failure to achieve pregnancy after multiple IVF attempts is recognized as recurrent implantation failure (RIF).
What is recurrent implantation failure?

FACTORS CONTRIBUTING TO RIF

The embryo

The embryo has been implicated in RIF in the presence of poor oocyte or spermatozoa quality. Poor oocyte quality has been associated with advanced maternal age, known to lead to a low number of oocytes that are usually aneuploid. On the other hand, poor spermatozoa quality has been associated with sperm DNA damage, also leading to the formation of poor-quality embryos.

Uterine pathologies and endometrial receptivity

A number of congenital uterine abnormalities, including Müllerian-duct malformations as well as submucous fibroids, endometrial polyps, intrauterine adhesions, or adenomyosis, may seriously affect endometrial receptivity and contribute to RIF. A functioning and receptive endometrium is of crucial importance for successful embryo implantation. Endometrial receptivity refers to the synchronized interaction among ovarian hormones, growth factors, adhesion molecules, cytokines, and lipids, allowing embryo adhesion, invasion, placentation, and therefore pregnancy initiation to occur. Impaired endometrial receptivity is responsible for approximately two-thirds of implantation failures.

Whether or not a thin endometrium is associated with impaired endometrial receptivity is still a matter of debate. The minimal adequate endometrial thickness for successful implantation varies between studies, ranging from 6–8 mm. Significantly higher embryo implantation and pregnancy rates have been demonstrated in patients with an endometrial thickness of >9 mm compared with those with a thickness <9 mm. On the other hand, two large prospective studies failed to confirm such an association.

Hydrosalpinges

Hydrosalpinges have also been recognized as a contributing factor to RIF due to their harmful impact on implantation. Hydrosalpinges may negatively influence implantation either by a direct toxic effect on the preimplantation embryo or mechanically by flushing the embryo out of the uterus. Moreover, it has been shown that endometrial receptivity is impaired in the presence of hydrosalpinges due to the abnormal expression of cytokines, which are necessary for successful implantation.

Immunological factors/thrombophilia

Endometrial decidualization is a process in which the differentiation of endometrial stromal cells occurs. It is of vital importance for pregnancy establishment and maintenance, enabling maternal immune tolerance, fetus survival, and placentation process regulation. In this respect, the impact of various immunological factors on implantation has attracted a lot of attention as a cause of RIF, although no consensus exists on whether or not immunological investigations and treatments may be beneficial.

The role of thrombophilia in implantation failure has been also the focus of research. The association of inherited thrombophilia, antiphospholipid or other autoantibodies, with RIF has been investigated with, however, controversial results. Hence, the value of thrombophilia screening in patients with RIF still remains a matter of debate.

VARIABLES USED IN THE DEFINITION OF RIF

Quality and number of embryos transferred

Generally, the probability of an embryo to implant is 30%, and thus, in order to increase the probability of pregnancy, it is imperative that the best-quality available embryo(s) are selected for
transfer. A good-quality embryo has been defined as an embryo with the number of cells corresponding to the day of its development, blastomeres of equal size with regular distribution, and less than 10% fragmentation. In addition, day-5 embryos (blastocysts) have been graded according to the expansion and quality of the inner cell mass and the trophectoderm.

Due to the higher implantation potential of a blastocyst compared with that of a cleavage-stage embryo, the developmental stage of the embryo transferred has also been taken into account in the definition of RIF. Single-blastocyst transfer has been suggested to result in a similar probability of live birth compared to the transfer of two cleavage-stage embryos.

Several definitions of RIF have been proposed including various numbers of embryos transferred per cycle or a cumulative number of embryos transferred overall. Controversy exists regarding the inclusion or not of frozen embryos in the number of total embryos used to define RIF. It has been suggested that frozen embryos should not be included in the definition of RIF due to their lower implantation rate compared with fresh embryos. However, the opposite has also been suggested since transfer of frozen embryos contributes to the cumulative pregnancy rate achieved.

**Number of unsuccessful IVF/ICSI cycles**

Many investigators have preferred to base the definition of RIF on the number of unsuccessful IVF/intracytoplasmic sperm injection (ICSI) cycles alone using different numbers, ranging from one to multiple cycles, usually in combination with other variables.

**Maternal age**

Maternal age has been incorporated into the definition of RIF, given the fact that maternal age is closely related to embryo quality. It has been demonstrated that implantation rates remain constant until the age of 35, followed by a linear decrease of 2.77% per year. Moreover, it has been shown that aneuploidy significantly increases with maternal age, and thus, implantation failure in women of advanced maternal age may be largely attributed to this problem. Although the age limit of 40 years has been proposed as an upper age limit for defining RIF in combination with other factors, it still has not been widely adopted.

**DEFINITION OF RIF**

As already discussed, many variables have been used for the definition of RIF, including number of embryos transferred, number of IVF attempts, embryo quality, or maternal age. Using these variables in different combinations, several definitions of RIF have been proposed without, unfortunately, a universally accepted definition being present. Consequently, the exact prevalence of patients with RIF is difficult to be determined.

According to an early definition, dated more than 20 years ago, RIF is defined as a cumulative transfer of eight cleavage-stage embryos or four blastocysts, not leading to a positive pregnancy test 14 days after oocyte pick-up (OPU). On the other hand, the ESHRE Preimplantation Genetic Diagnosis (PGD) Consortium has defined RIF as a failure to achieve clinical pregnancy after ≥3 unsuccessful transfers of high-quality embryos or after the cumulative transfer of ≥10 embryos in multiple transfers.

In a more recent approach, RIF has been defined as the failure of pregnancy achievement after the transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in women under the age of 40 years.

A systematic review performed in 2014 attempted to standardize the definition criteria for RIF by evaluating all published definitions, aiming to assist in the design and to enhance the quality of future studies focusing on RIF. A total of 119 studies, with a significant heterogeneity in the definition of RIF, were included in this systematic review. All definitions of RIF in these studies were arbitrary. The studies were categorized in multiple definition groups, based on the number
of unsuccessful cycles or the number of unsuccessful cycles in combination with the number of embryos transferred (Table 1.1).

The number of failed cycles, used as a single variable to define RIF, ranged from two to seven. The most common single-variable definition of RIF in the above systematic review was that using “three or more unsuccessful or failed cycles.”

Regarding the combination of the number of embryos transferred with the number of unsuccessful cycles, this ranged considerably from one to more than three embryos transferred or transfer of four to 10 or more cleavage-stage embryos, cumulatively. The most commonly

Table 1.1 Studies using a definition of RIF based on the number of unsuccessful cycles alone or on the combination of unsuccessful cycles with the number of embryos transferred/cumulative transfer of embryos.

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(Continued)
Conclusions

Successful implantation is considered to stem from an efficient combination of various factors. Repeated failure of any of these factors or of their combination might decrease the chance of implantation and eventually lead to recurrent implantation failure.

Unfortunately, an enormous variability in the definition of RIF exists, necessitating the adoption of a widely accepted definition. This would assist in enhancing our understanding of implantation and in developing effective treatments that would decrease significantly the psychological, emotional, and financial burden of couples with RIF experience. Until then, the evaluation of any proposed treatment for RIF should always take into account the definition used.

### Table 1.1 (Continued)

Studies using a definition of RIF based on the number of unsuccessful cycles alone or on the combination of unsuccessful cycles with the number of embryos transferred/cumulative transfer of embryos.

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<th>≥2 unsuccessful cycles &amp; transfer of ≥1–2 embryos</th>
<th>≥3–7 unsuccessful cycles &amp; transfer of ≥1–2 embryos</th>
<th>≥3–5 unsuccessful cycles &amp; transfer of ≥3 embryos</th>
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* Good/high embryo quality.
What is recurrent implantation failure?

REFERENCES


What is recurrent implantation failure?

INTRODUCTION

Although the goal of every initiated in vitro fertilization (IVF) cycle is a viable singleton pregnancy, in many cases that won’t be the outcome. With the exception of the most ideal circumstances, most patients who garner the physical, financial, and psychological resources to initiate treatment will meet with disappointment. For those who have the stamina to try again, some will have the same experience. And for the patients who undergo cycle after cycle, which happens often when insurance covers multiple attempts, there are still going to be individuals and couples who never achieve genetic parenthood. How does one cope when one has such an experience? What are the short- and long-term psychological consequences of multiple IVF failures?

A recent research study should serve as a wake-up call about the long-term psychological impact of IVF treatment, most especially in women who never achieved a pregnancy. In this study of 470 Swedish women who had undergone at least one IVF cycle 20–23 years before being assessed, all women, when compared to a reference group, were at increased risk for depression, obsessions-compulsions, and somatization. However, the women who had not conceived had significantly higher levels of depression and phobic anxiety. Given that their IVF failure(s) had happened more than two decades previously, it is alarming that they were still experiencing such profound psychological distress. In a similar study, but with follow-up four to five years after IVF treatment, individuals who had experienced IVF failure were compared to those who had succeeded, versus a control group that had not undergone IVF treatment. Women who had experienced IVF failure had higher levels of both depression and anxiety, as well as a lower sense of coherence than women in the successful group. They also reported more depression and less coherence than the control group.

We know that women who experience IVF failure report high levels of emotional distress. The purpose of this chapter is to describe what we know about the impact on women who experience recurrent implantation failure.

THE PSYCHOLOGICAL IMPACT OF RECURRENT FAILURE

Unfortunately, recurrent implantation failure (RIF) has not caught the attention of mental health researchers in the reproductive medicine field. A PubMed search including the words “recurrent implantation failure” with “depression,” “anxiety,” or “distress” turned up zero references. Another search on “post IVF syndrome” also did not turn up any studies on psychological distress as a factor. A recent study might explain the paucity in the field. In a recent prospective cohort study of 174 women undergoing IVF, participants were assessed for major depressive disorder (MDD) repeatedly over an 18-month study period, and 39% of women met the criteria for MDD on at least one time point. Although the mean number of failed treatment cycles was 2.5, there was no association between the number of failed cycles and the development of MDD. The only variable that predicted the development of depressive symptoms was a past history of MDD. Thus, it was hypothesized...
that it is the infertility experience itself, especially in women with a prior history of depression, that leads to depressive symptoms, not the experience of multiple failed cycles.

However, there was one study that did assess the psychological status of women who had experienced RIF with women who had experienced recurrent pregnancy loss (RPL), comparing both groups to fertile controls. As expected, both the RIF and RPL participants reported significantly higher levels of psychological distress than the control group. The only significant difference between the RPL and RIF patients was that the perceived stress score of the RPL group was higher than that of the controls, and that of the RIF patients was not.

In a study which examined the impact of IVF failure on emotional status, 64 couples who had experienced at least one failed cycle were compared to 56 couples who had not yet had an unsuccessful cycle. The two groups did not differ with respect to anxiety and depression scores.

**IVF FAILURE = EMOTIONAL DISTRESS?**

Although there have been millions of women and men who have undergone unsuccessful IVF treatment, and there are 1333 citations in PubMed on “IVF failure,” there are no citations on measures of depression or anxiety and only three on stress. However, the heightened levels of depression and anxiety of individuals undergoing IVF is well known. In one of the larger studies on the prevalence of distress in individuals undergoing infertility treatment, 56.5% of women and 32.1% of men reported clinical levels of depressive symptoms and 75.9% of women and 60.6% of men reported clinical anxiety symptoms. The majority of other recent research on the psychological profiles of infertility patients shows similar findings—infertility patients routinely experience symptoms of anxiety and depression.

It is understandable that IVF failure commonly leads to intensive feelings of sadness and loss of hope. In a survey of 66 women who had recently experienced at least one IVF failure, the most frequently reported grief responses were bargaining, acceptance, depression, anger, denial, and isolation. In a study on 372 Chinese women who had at least one unsuccessful IVF cycle, participants scored significantly higher on assessments of depression after the failed cycle compared to levels prior to treatment. What is alarming about this study is that 13% of the participants reported ideas about self-harm. In fact, self-harm and suicidal ideation are not uncommon in infertile women. In a recent study on the prevalence of suicidal risk, a total of 9.4% of women receiving infertility treatment reported suicidal ideation and/or attempts. In a small study of 21 couples who had experienced unsuccessful IVF treatment who were compared to 20 successful couples, the unsuccessful couples reported a lower quality of life, with the women reporting the lowest scores.

There have been a number of studies that assessed the emotional status of women who stopped treatment, although the reason for the failure was not cited. In one of the earliest studies on 40 couples with an average length of follow-up of 1.5 years, although 60% of the women reported symptoms of depression, the severity of the symptoms did decrease over time. However, 13% of the women had thought about suicide because of their inability to have children.

In another follow-up study of IVF treatment failure, 76 women were assessed four to nine years after treatment. In comparison to social norms, the 52 women who never conceived or adopted reported significantly higher levels of stress but also higher self-esteem. When the successful IVF patients were compared to the unsuccessful ones however, the unsuccessful ones reported themselves as significantly more stressed, more depressed, and with lower levels of life satisfaction and self-esteem.

There are a number of factors that can have an impact on emotional recovery after unsuccessful treatment. In a study of 187 women who were assessed prior to treatment, the women who reported the most negative psychological symptoms after failed treatment had the highest neuroticism scores pretreatment. Other factors associated with more distress were feelings of helplessness and marital dissatisfaction, while social support and acceptance of the situation were associated with less distress. In another study of 184 women who had failed IVF treatment, immediately after the last failed cycle, marital quality was positively related to depression while resilience was inversely
correlated. During the follow-up period, however, marital quality became negatively correlated with depressive symptoms. The authors concluded that marital quality may allow patients to experience the psychological consequences of failure while resilience can tamper distress. Longer term, however, marital quality can enhance the psychological recovery.

Making the decision to end treatment is a complicated process for many individuals and couples. For those without insurance, the main reason for treatment termination is financial, but for those with insurance or significant resources, it can be a challenging decision. In a study of 25 women who decided to end treatment, many reported that they had had unrealistic expectations of the possibilities of success. The main benefit of deciding to stop treatment was the end of the emotional pain of IVF, but many of the women found it challenging to confront issues that they had previously not been able to contemplate. Interestingly, the women who adopted perceived less pressure from others about their decision than those who remained childless. In another study of women who experienced treatment failure and were followed for three to five years, those who didn't conceive but did focus on new life goals had lower levels of depression and anxiety than those who continued to try to conceive.

A review, published in 2002, proposed a theory to explain why women may not express significantly more distress after multiple failed cycles when compared to one. It is possible that when an individual is exposed to multiple failures, it forces them to truly confront their infertility and move into acceptance.

**PSYCHOLOGICAL INTERVENTIONS TO REDUCE DISTRESS**

There are a number of meta-analyses that examined the impact of various psychological interventions on distress levels of infertile women, but none specifically for those who have experienced RIF. In addition, the results of the different analyses don't necessarily agree with each other, mostly because of the studies selected for inclusion.

In the first meta-analysis on this topic, 25 studies were included. The conclusions were that there were no differences in efficacy between men and women, psychological interventions were not associated with improvements in pregnancy rates but were for psychological symptoms, skills-based interventions were the most effective, and group interventions were better than individual.

Another review published six years later included only 21 studies and had different conclusions. These conclusions were that psychological interventions were not correlated to less distress and that non-ART (assisted reproductive technology) patients who received an intervention did experience higher pregnancy rates. In addition, the interventions of six or more sessions were more effective than shorter ones.

A 2016 review included 20 randomized trials and concluded that there were significant methodological issues with the studies that had significant findings for psychological improvement or increased pregnancy rates. The authors strongly encouraged more research on interventions aimed during the waiting period between transfer and pregnancy test. A Cochrane review, published in 2016, also reported on methodological limitations in the research in this area.

However, the largest review to date had more definitive conclusions. This review included 39 studies on 2746 men and women. Eligible studies assessed the impact of various psychological interventions on pregnancy rates and/or psychological symptoms including anxiety, depression, stress, and marital function. The conclusions were that there were significant impacts of psychological interventions on both psychological symptoms and pregnancy rates. The effect size for pregnancy was large ($p < 0.001$). The effect sizes were larger for women than men and the highest pregnancy rates were associated with the greatest decreases in anxiety. The authors reported that there were no obvious differences between cognitive behavioral therapy (CBT), mind/body interventions, and other intervention types but, in their conclusions, noted that the most effective intervention included instruction in CBT.

Finally, a recent small review had similar findings, including the association of psychological interventions with less distress and higher pregnancy rates.
Unfortunately, the research on psychological interventions with infertility patients has focused on the treatment and waiting phases, rather than on women who had stopped treatment due to lack of success. It is entirely plausible that any of these interventions, perhaps most likely CBT, could lessen the short- and long-term suffering of women who experienced treatment failure. Given that past research has indicated that women who move ahead after infertility by focusing on different life goals have more positive psychological outcomes, future research should focus on interventions that encourage such reframing.

**ALTERNATIVE FORMS OF INTERVENTIONS**

It can be challenging to recruit infertile women to attend on-site interventions, either for research purposes or in a clinical setting. Although insured infertile women who drop out of treatment report that they desire stress-reduction interventions, the vast majority of women, either during treatment or after treatment failure, do not participate in such interventions. Thus, the possibility of more advanced technological interventions has been proposed. In a pilot study on the efficacy of an online mind/body intervention, participants who were randomized to the intervention group reported significantly less psychological distress and higher pregnancy rates than women who were randomized to the control group. A new app, FertiCalm, which is designed to apply cognitive behavioral and relaxation techniques to women experiencing infertility, has one section devoted exclusively to coping after treatment failure. However, there are no data yet on the efficacy of any apps on improving the psychological status of women who use them.

**THE PSYCHOLOGICAL IMPLICATIONS OF RECURRENT IMPLANTATION FAILURE**

Although the vast majority of research studies on the psychological consequences of IVF failure do not differentiate between recurrent implantation failure versus other causes, it is most plausible that the psychological impact of unsuccessful treatment is fairly universal. Unsuccessful treatment is associated with significant distress, and in some women, this distress can be manifested with suicidal thoughts, with most women expressing symptoms of anxiety, low self-esteem, and depression. However, most women do adapt over time. Unfortunately, a subset continues to report symptoms for decades.

It is clear that research in this field is inadequate; although multiple studies confirm the long-lasting psychological consequences of treatment failure, there are no intervention studies that aim at decreasing this distress long after treatment is terminated. Since many women appear to be loath to physically attend any kind of intervention designed to decrease distress, more innovative forms of therapy must be assessed. Online interventions and apps need to be subjected to randomized controlled trials in an effort to determine the most effective way to improve the psychological status of women who fail high-tech treatment, both for short- and long-term efficacy. Women should not have to suffer for decades after failing to conceive.

**REFERENCES**

The role of lifestyle factors in recurrent implantation failure

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INTRODUCTION

Implantation is a remarkably complex, highly organized process that relies on precise coordination between the embryo and the host endometrium. Although over the last decade our understanding of the implantation process has improved dramatically, many unanswered questions remain, and we still have little knowledge about the molecular mechanisms involved in endometrial receptivity and in regulation of embryo-endometrium crosstalk.1

Recurrent implantation failure (RIF) is referred to a clinical condition when implantation fails repeatedly despite several transfer cycles of good-quality embryos.2 Multiple etiological factors of RIF have been described, of which suboptimal embryonic capacity and reduced endometrial receptivity are thought to be the key determinants. In a substantial proportion of couples, however, the exact cause of RIF is not identified, as we do not have universally accepted markers that accurately assess embryo competence and receptive status of the endometrium.3 Limited understanding of the cellular events underlying implantation failure results in lack of effective therapeutic and preventive strategies, which is frustrating for both patients and health-care providers.

There is an increasing concern that modern lifestyle and changes within the environment influence reproductive function of the couple and can have a detrimental effect on pregnancy outcomes.4–7 Although the couples undergoing assisted reproductive technology (ART) treatments are thought to be more conscious of their health and are less likely to engage in negative lifestyle behaviors, a survey of more than 12,000 ART patients in United States revealed that during fertility treatment, up to 23% of patients drank alcohol, up to 7% smoked, 68% consumed caffeine, and less than 1% used recreational drugs.8

Both oocytes and sperm are susceptible to the environmental influences, which can subsequently affect embryo development.9,10 Nutrition and other factors originating from the environment can adversely influence maternal health, the intrauterine milieu, and, possibly, maternal response to the implanting embryo.11 It has been recently proposed that women with RIF have a different metabolic status compared with women who have highly receptive endometria.12 RIF has been associated with higher levels of serum glucose and altered levels of the metabolites related to energy, amino acid, and lipid metabolism, suggesting a possibility of metabolic dysfunction.12 At the endometrial level, impaired glucose uptake has been implicated in abnormal decidualization, poor implantation, and pregnancy loss,13 whereas normal glucose metabolism was associated with successful decidualization.14 Certain lifestyle factors such as smoking, alcohol, and obesity are known to induce chronic oxidative stress, which has been extensively linked with reproductive dysfunction on oocyte, tubal, and endometrial levels.15 Elevated levels of adipic acid, a by-product metabolite of reactive oxygen species (ROS) has been detected in the circulation of women with RIF, indicating that women with RIF have an increased oxidative stress state, and the condition can be possibly linked with environmentally induced oxidative damage.12

Taken together, it is not unreasonable to assume that lifestyle, environmental, and occupational factors contribute to RIF. Currently, however, there is no clear evidence on association between lifestyle or environmental factors and RIF, with the ART outcomes and recurrent pregnancy loss.
Obesity being a primary focus in most studies. Although repeat in vitro fertilization (IVF) failure, recurrent miscarriage, and RIF represent distinct clinical entities, their underlying causes overlap, and supposedly these conditions have a similar link to the environmental exposures.

This chapter provides an up-to-date overview of the lifestyle, occupational, and environmental factors known to adversely affect reproductive function and discusses their possible contribution to RIF. The evaluated factors include obesity, caffeine intake, smoking, alcohol consumption, psychological stress, periodontal disease, and endocrine-disruptive chemicals as environmental pollutants.

**OBESITY**

Obesity is widely recognized as a global epidemic affecting around third of women and men of reproductive age.\(^{16}\) The clinical impact of obesity on reproduction, pregnancy outcomes and health of the mother and offspring have been well characterized.

Obesity increases the rate of anovulation, has a pronounced negative effect on endocrine and fertility parameters in women with polycystic ovarian syndrome (PCOS), and decreases the probability of spontaneous conception in the absence of ovulatory dysfunction.\(^{17,18}\) In women undergoing ART treatments, obesity has been associated with decreased ovarian response, higher rate of cycle cancellations, lower oocyte yield, larger number of immature oocytes, impaired fertilization rate, and poor embryo development.\(^{19}\) The analysis of 239,126 fresh IVF cycles revealed that increased body mass index (BMI) was associated with worsening of implantation, pregnancy, and live birth rates.\(^{20}\) Probability of live birth is inversely related to severity of obesity, with a 50% decrease in the odds for live birth in women with BMI \(>40\) kg/m\(^2\).\(^{21}\)

Compelling evidence links obesity with a risk of miscarriage.\(^{20,22-25}\) A recent umbrella review of systematic reviews and meta-analyses on the association between obesity and any type of obstetric or gynecological morbidity demonstrated highly suggestive evidence for a negative association between BMI \(>25\) kg/m\(^2\) and increased risk of miscarriage among IVF patients.\(^{22}\) A systematic review of 30 studies encompassing 115,158 participants concluded that paternal obesity negatively affects fertility and is associated with reduced live birth and increased pregnancy loss in ART cycles.\(^{26}\) Obese individuals don’t seem to have an increased rate of aneuploid embryos\(^{27}\) and have a high frequency of euploid miscarriage,\(^{23,25}\) implying that the negative effect of obesity on reproductive outcomes relies on factors other than embryo aneuploidy.

Obesity-related hyperinsulinemia stimulates production of ovarian androgens that aromatize to estrogen in adipose tissue and lead to negative feedback on the hypothalamic-pituitary-ovarian axis. Ovarian steroidogenesis is also affected by leptin, a cell-signaling protein produced in adipose tissue, acting on the level of the hypothalamus.\(^{19}\)

Raised inflammatory markers, lipids, and insulin have been observed in the follicular fluid of obese women.\(^{28}\) In animal models, obesity has been linked with oocyte mitochondrial abnormalities reflective of metabolic stress, derangements in the meiotic spindle, abnormal transcriptional activity in cumulus-oocyte complexes, and increased apoptosis of granulosa cells.\(^{19}\) In addition, the negative influence of obesity on oocyte competence and on the preimplantation embryo is mediated by leptin-induced apoptosis and lipotoxicity. Lipotoxicity is manifested by accumulation of excess fatty acids outside adipocytes with subsequent toxic damage to the surrounding cells through increase in oxidative stress, activation of the apoptosis cascade, and induction of inflammatory pathways.\(^{19}\)

In males, increased BMI has been associated with a decrease in androgens, an increase in estradiol due to aromatization of testosterone in adipose tissue, and an inverse correlation with semen parameters.\(^{26,29,30}\) Impaired steroidogenesis, oxidative stress, insulin resistance, apoptosis, and inflammation are thought to mediate the adverse effects on sperm parameters and DNA integrity.\(^{31}\)

The data from animal and human in vitro experiments demonstrate that obesity is implicated in endometrial decidualization defects and possibly affects endometrial receptivity.\(^{32}\) The exact mechanism is unclear, but it has been proposed that leptin may be involved in endometrial derangements by modulating cellular signaling involved in proliferation and apoptosis.\(^{33}\) Furthermore, obesity
induces low-grade systemic inflammation, which in turn can negatively influence implantation and embryo development. The studies on oocyte donation with normal-weight donors that isolate the effect of embryo on implantation and hence reflect the effect of obesity on endometrial milieu are equivocal. A systematic review of five studies encompassing 4758 women reported no difference in implantation rate between obese recipients and controls with normal BMI. In contrast, a more recent cohort analysis of 9587 oocyte donation cycles demonstrated significantly reduced implantation, clinical pregnancy, and live birth rate in obese recipients.

Despite obvious deleterious effects of obesity on a wide range of reproductive outcomes independent of methods of conception, there has been very little exploration of the role of obesity in RIF. Theorizing that gametes and embryos from obese individuals are subject to alterations, coupled with clear evidence on increased rate of euploid miscarriages, it can be supposed that obesity may contribute to RIF, although there are no data to support a direct association.

Weight-reduction strategies include lifestyle changes through diet and exercise as a first-line option and additional interventions such as medical therapies and bariatric surgery. To date, most studies that have evaluated weight-loss interventions in a context of fertility were significantly underpowered with high dropout rate and generally achieved modest weight loss. Weight reduction has been positively associated with improved endocrine parameters, increased rate of ovulation, and higher likelihood of spontaneous pregnancy in anovulatory women, although the results with regard to the effect of interventions on live birth or miscarriage rate are largely disappointing. It also remains unclear what degree of weight loss improves reproductive performance and what is the optimal timing and type of intervention. The interventional meta-analysis concluded that the existing studies on preconception weight-loss interventions failed to demonstrate improvement in the outcomes of fertility treatments. More work is clearly required for firm conclusions to be drawn as to whether fertility-related outcomes are amenable to risk-reduction interventions.

Guided by the evidence on multiple health benefits of normal weight, and considering reproductive challenges and obstetrics complications of obesity, multiple societies advocate that obese individuals should be engaged in weight-loss programs before attempting conception.

**CAFFEINE**

Caffeine is present in multiple food products and beverages and is naturally occurring in many plants. The main sources of caffeine include coffee, tea, chocolate, and soft or “energy” drinks. Caffeine is consumed by up to 90% of reproductive-aged women with 7%–18% consuming above 300 mg/day. Caffeine has been associated with a negative effect on fertility and pregnancy, although the data are inconsistent, and the findings of most studies are hampered by serious methodological flaws. A meta-analysis of 60 publications demonstrated a modest association between caffeine intake (within the range of intake currently recommended in most countries) and adverse reproductive outcomes. Likewise, there was no clear negative association between caffeine and sperm parameters or sperm DNA damage. The umbrella review of 201 meta-analyses of observational studies and 17 meta-analyses of interventions concluded that coffee intake within usual levels is generally safe, except for the relationship of high consumption during pregnancy with pregnancy loss and low birthweight. The negative effect of increased caffeine consumption on pregnancy loss was suggested in a more recent systematic review of 35 studies on dose-response association between caffeine and reproductive events, with significant risk observed for a caffeine dose of 300 mg/day and above.

The relationship between caffeine and ART outcomes is unconvincing. In high coffee and tea consumers, caffeine was detected in follicular fluid and was associated with reduced number of oocytes and good-quality embryos but did not affect live birth rate. One study in 221 IVF couples showed that caffeine consumption in women led to reduced live births and an increased chance of miscarriage in a dose-dependent manner, although a negative effect on IVF endpoints were not observed with male caffeine intake. These findings were not confirmed by others, and there are no publications on caffeine consumption in couples with RIF.
The proposed biological mechanisms by which caffeine could influence reproductive events involve alterations in secretion and metabolism of reproductive hormones and disruption of ovarian function. Increased levels of circulating catecholamines, homocysteine, cholesterol, and cellular c-AMP have also been reported, although the magnitude of these changes with the typically consumed caffeine doses and their direct effect on the embryo or endometrium are unclear.

The evidence linking preconception caffeine restriction with reproductive outcomes is inadequate, and there are no evidence-based recommendations for caffeine intake. Most guidelines recommend that women who are pregnant or trying to become pregnant limit caffeine consumption to less than 200–300 mg per day, and there is no evidence to suggest caffeine avoidance.

SMOKING

The epidemiological data indicate that about 2%–6% of women undergoing ART smoke, and a large proportion of the nonsmokers are exposed to second-hand smoke. Smoking has been repeatedly associated with decreased fertility and reduced success rate of ART cycles. Several meta-analyses reported that clinical pregnancy and live birth rate were reduced by approximately half in smokers compared with nonsmokers, with about a three-fold increase in miscarriage risk.

A growing body of literature suggests a detrimental effect of second-hand tobacco smoke exposure on fertility and IVF outcomes, which is similar to that observed in smokers. Cotinine, a metabolite of nicotine, was detected in follicular fluid of nonsmoking women who reported second-hand exposure to smoke and was associated with 52% higher risk of implantation failure and a 24% reduction in live births. Likewise, a reduction greater than 50% in live birth rate was demonstrated among IVF couples in which only male partners smoked, and this effect was only mildly improved with intracytoplasmic sperm injection (ICSI). The influence of smoking on uterine receptivity was demonstrated in oocyte donation cycles, where the recipients who smoked had a lower chance to conceive proportionally to the number of cigarettes smoked. Both active and passive smoking were reported in association with reduced implantation and increased risk of implantation failure. Despite compelling evidence that smoking has a negative effect on fertility and implantation, the extent to which smoking contributes to RIF is less clear.

Smoking cessation in both partners is considered an important strategy to improve fertility and is a universal recommendation for the couples presenting for preconception or fertility counseling. Current evidence indicates that reproductive risks associated with smoking are reversible within up to one year of smoking cessation, although the uptake of smoking reduction strategies is disappointingly low. The data on smoking reduction strategies in couples who attempt conception through ART are sparse, and the effect of these interventions on reproductive outcomes and broader lifestyle changes remains to be established.

ALCOHOL

Alcohol is one of the most frequently abused substances in the Western world. Epidemiological reports demonstrate that around 25% of couples planning a pregnancy consume alcohol at potentially unsafe levels and a large proportion of the population reports mild to moderate intake. Numerous studies have documented the negative effect of male and female alcohol consumption...
The role of lifestyle factors in recurrent implantation failure on reduced fertility, increased risk of spontaneous miscarriage, and adverse neonatal outcomes.\textsuperscript{5–7} The safe level of consumption associated with reproductive risk is unclear, and the most susceptible window of exposure has not been identified. Some investigators have reported that alcohol consumption levels as low as one drink per week reduced the chance for conception, but this has not been confirmed by others.\textsuperscript{5,59} A large, prospective, observational study in 27,580 pregnancies reported no association between low to moderate alcohol intake pre-pregnancy ($<12$ g/d) and early or late pregnancy loss, irrespective of maternal age, BMI, and smoking status.\textsuperscript{60} A systematic review of 15 observational studies in a total of 16,395 participants demonstrated that semen volume and normal morphology were affected by occasional alcohol consumption in fertile or infertile men, but daily drinking had a definite adverse effect versus both occasional and never alcohol consumers.\textsuperscript{61}

Among the couples participating in IVF programs, up to 45% of women and 67% of men reported alcohol consumption during their treatment, of which 4% women and 9% men drank daily.\textsuperscript{62} The direct effect of alcohol on IVF is less clear, and there is conflicting evidence on the impact of alcohol intake on treatment outcome.\textsuperscript{5,59} Alcohol drinkers had lower numbers of oocytes retrieved, lower fertilization rates, abnormal blastocyst development, and lower implantation rates.\textsuperscript{62} Consumption of four or more drinks per week was associated with 16% lower chance for live birth following IVF, with a more prominent effect (21% reduction in live birth) if both partners drank.\textsuperscript{62} Conversely, several studies failed to confirm a negative association between alcohol and live birth rate in IVF cohorts.\textsuperscript{59,63}

The exact mechanism is unclear, although chronic alcohol consumption is thought to affect feedback regulation of the hypothalamic-pituitary-gonadal axis by increasing circulating estrogen levels, with subsequent consequences for folliculogenesis and oocyte maturation. Alcohol has been shown to affect sperm parameters in some, but not other, studies\textsuperscript{59,61} and has been associated with increased sperm DNA damage and deranged male reproductive hormones.\textsuperscript{64}

In animal models, excess alcohol exposure resulted in amenorrhea, uterine atrophy, decreased ovarian weights, and suppression of luteinizing hormone (LH) levels.\textsuperscript{6} Ethanol-exposed male animals had reduced testicular volume, lower testosterone, gamete abnormalities, and decreased litter size.\textsuperscript{6,7} Oxidative damage has been observed in both the testes and epididymides of the exposed animals.\textsuperscript{65} The adverse effects on implantation were manifested by production of aneuploid embryos, delayed attachment of blastocysts, absence of the decidual reaction, and resynchronization of the implantation process.\textsuperscript{6,7}

Based on the evidence derived from animal studies, there is biological plausibility to support an adverse effect of alcohol on implantation. However, little is known on the effect of alcohol intake on implantation defects in humans, and the association of male or female alcohol consumption with RIF is unclear.

Excessive alcohol consumption is linked with higher rates of mental-health disorders and polysubstance abuse, while consumption during pregnancy may lead to serious health problems in the unborn child. Most guidelines recommend a cautious approach to alcohol consumption in couples planning pregnancy, which includes avoiding binge drinking, limiting number of weekly standard drinks, or complete abstinence for both partners.\textsuperscript{38–40} The therapeutic strategies to reduce alcohol intake include counseling, psychosocial intervention, and pharmacological therapy, although there are limited data on the effectiveness of preconception interventions and their impact on reproductive outcomes.\textsuperscript{66,67}

\textbf{PSYCHOLOGICAL STRESS}

Infertility is a stressful experience. Fertility treatments are commonly associated with significant stress for the couple, which can be further exacerbated by underlying anxiety, or a range of societal, energetic, and psychological stressors. Overall, one-fifth of the couples undergoing ART have clinically significant psychological distress or anxiety.\textsuperscript{68} Failed treatments result in higher psychological burden, which can lead to treatment discontinuation or higher levels of depression and anxiety in subsequent pregnancy. Women experiencing RIF had increased stress levels compared to fertile
controls, similarly to those in women with recurrent pregnancy loss, but the investigators were not able to demonstrate whether increased stress was a cause or consequence of reproductive failure.\(^6^9\)

Increased stress levels were associated with deranged levels of reproductive hormones and anovulation,\(^7^0\) while modified behavior led to restored ovulation.\(^7^1\) Long working hours and moving of heavy loads were also linked with increased time to conceive, with a more pronounced negative effect in overweight or obese women.\(^7^2\) The literature on the effect of stress on pregnancy loss is conflicting, and there is no conclusive evidence regarding the effect of stress on IVF outcomes.\(^6^9,^7^3\) One systematic review of 43 studies found a correlation between psychological stress and negative IVF outcomes, although the authors reported substantial inter-study heterogeneity with respect to the outcome measures and the tools of stress measurement.\(^7\) A more recent meta-analysis concluded that pretreatment emotional distress in women undergoing ART did not compromise treatment outcomes.\(^7^4\) Similarly, The Cochrane Library systematic review evaluated 39 studies comprising 4925 participants and reported uncertainty regarding the benefits of psychological or educational interventions on mental health or reproductive outcomes in subfebrile couples, mainly due to the overall low-quality evidence.\(^7^5\)

Stress may affect reproductive function through a corticotrophin releasing hormone (CRH)-induced effect on the hypothalamic-pituitary-gonadal axis, and this can be further modified by other stress-related mediators (namely, glucocorticoids, opioid peptides, and catecholamines).\(^4,^7^3\) It is thought that deleterious effect of stress is mediated by cortisol and prolactin, although the exact neuroendocrine mechanism is unclear.\(^7^6\) In women subjected to stress, urine cortisol levels negatively correlate with the levels of luteal progesterone, which could subsequently interfere with endometrial decidualization.\(^7^0\) Stress mediators may also alter cytokine expression in the endometrium and increase activity of uterine natural killer (NK) cells, which could contribute to impaired endometrial receptivity.\(^7^4\) Detection of adrenergic receptors in mice preimplantation embryos suggests that stress-related release of maternal catecholamine could affect early embryonic development and influence embryo implantation.\(^7^7\) Despite a plausible pathophysiological mechanism, little is known about the effect of stress and stress-relieving therapy on RIF. There are little epidemiological data, and no human studies have yet been performed to assess the effect of stress hormones on embryo developmental competence and implantation.

Considering broad social impact of emotional distress and its known association with discontinuation of fertility treatments, support and appropriate counseling should be available for every couple undergoing fertility treatments, irrespective of their treatment outcomes. Until new evidence emerges, there are no recommended psychological interventions specifically targeted to the couples with RIF.

**PERIODONTAL DISEASE**

The relationship between periodontal disease and its systemic sequelae has been an area of research interest over the past two decades.\(^7^8\) Periodontal disease affects around 10% of the population, with a higher prevalence in smokers and low-income groups. It has been suggested that periodontal disease may have a negative impact on fecundity and time to conception, and it is linked with increased risk of preterm birth, low birth weight, and miscarriage by most but not all of the studies.\(^7^9,^8^0\)

The hypothesized connection between periodontal disease and adverse reproductive outcomes posits that oral bacteria cause systemic bacteremia that initiates an inflammatory cascade with local and systemic production of cytokines, which can lead to intrauterine inflammation, reduced embryo implantation or placental infection.\(^7^9\) Local and systemic oxidative stress have been implicated in the pathogenesis of periodontitis and may compromise intrauterine milieu.\(^8^1\)

Interventional randomized controlled trials and systematic reviews of these studies have reached mixed conclusions with regard to effect of periodontal treatment on reproductive outcomes, mainly due to multiple confounders and the multifactorial nature of the condition.\(^8^2\) It remains unclear what subgroups of women are the most responsive to treatment, which treatment works the best, and when is the most optimal time for intervention to be effective. Even though periodontal disease
has not been specifically evaluated in couples with RIF, in view of long-term health benefits, opportunistic counseling regarding oral health should be offered to the patients in every care setting.

ENDOCRINE DISRUPTIVE CHEMICALS

There is growing awareness that multiple chemicals within the environment interfere with hormone action and may affect reproductive function and fertility. Environmental endocrine disrupting chemicals (EDCs) have been detected in hair or biological fluids of a large proportion of the population worldwide.83 EDCs may exert their effect on reproduction through binding to hormone receptors, by altering cytochrome P450 activity, by modulating estrogen signaling, or by interfering with thyroid function.83,84 EDCs have been also shown to affect mitochondrial function, promote oxidative stress, and induce apoptosis. It has been suggested that some of these compounds may have transgenerational influences through epigenetic modifications by inducing DNA methylation, histone modification and dysregulation of noncoding RNAs. More than 800 potentially hazardous chemicals have been identified, of which bisphenol A (BPA), the phthalates, certain pesticides, the polychlorinated biphenyls (PCBs), and heavy metals are the most extensively evaluated in the context of reproductive toxicity.4

BPA and phthalates

BPA and phthalates are abundant synthetic compounds widely present in various household products, cosmetics, plastics, packaging, and pharmaceuticals. Exposure occurs through foods from plastic containers or via transdermal contact. BPA exposure has been linked with a higher prevalence of PCOS, endometriosis, recurrent miscarriage, and infertility.85,86 Increased concentrations of BPA in blood or urine in women undergoing IVF were associated with a lower number of antral follicles and mature oocytes, decreased fertilization, and deranged implantation.83 Likewise, increased peripheral levels of phthalates have been associated with implantation failure and adverse IVF outcomes.87 Epidemiological data on the effect of BPA and phthalates on sperm parameters and DNA damage are equivocal, although most studies reported dose-dependent negative associations.83 In animal models, BPA and phthalates demonstrated adverse effects on steroidogenesis, oocyte, sperm, embryo, and endometrium, but mechanisms underlying their reproductive toxicity in humans are unclear. It has been proposed that BPA affects implantation via downregulation of endometrial HOXA10, abnormal expression of endometrial tight-junction proteins, and by increasing the rate of embryo aneuploidy.88,89

Pesticides

Atrazine is the most common pesticide suspected to have an EDC effect. It is widely used in commercial agriculture and remains in soil and water for a long time. The use of another once commonly used pesticide, dichloro-diphenyl-trichloroethane (DDT), has been banned for years in many countries, but it persists within the environment for decades due to resistance to degradation. Unfiltered drinking water is the main source of contact, and there is a possibility for occupational exposure in individuals who work with pesticides or in pesticide-treated areas.

Exposure to pesticides has been linked with pubertal abnormalities, menstrual irregularities, decreased fecundity, miscarriage, and risk of endometriosis in human and animal studies.4,83 Occupational exposure to certain pesticides, including DDT, is inversely associated with sperm parameters and male reproductive hormones.83 The authors of a systematic review of the available literature concluded that DDT did not seem to affect oocyte quality, fertilization, embryo development, or pregnancy rate in IVF patients.90 More recent data showed a significantly reduced implantation rate in women with high follicular fluid of DDT and several other pesticides (lindane, diazinon, and chlorpyrifos).91 The authors also found a significant inverse correlation between follicular fluid concentrations of the examined pesticides (DDT, lindane, diazinon, malathion, chlorpyrifos, bioallethrin, pretilachor, and b-cyfluthrin) on endometrial thickness. However, the effect of these chemicals on clinical pregnancy and live birth rate has not been evaluated.
PCBs

PCBs are organic compounds that have been widely used in electrical equipment and different industries, are a recognized carcinogen in humans, and are currently banned in most countries. PCBs, however, are continuously detected within the soil, water, buildings, and animal products because of the long half-life and the ability to accumulate in adipose tissue. Therefore, both non-occupational exposures via contaminated food and occupational exposure during the contact with old electric equipment can still occur.92

Human and animal studies demonstrated that PCBs reduce circulating levels of thyroid hormone and exhibit estrogenic and antiandrogenic properties, with a more prominent negative effect in obese individuals due to fat tissue affinity.4,83 PCBs are inversely associated with sperm parameters and sperm DNA damage at any level of exposure.92

Several recent studies reported an association between PCBs and decreased implantation after ART treatments. High concentrations of PCBs in follicular fluid from 94 women were related to thinner endometrium, fewer retrieved oocytes, lower fertilization rate with ICSI, and decreased implantation rate, but they did not significantly influence clinical pregnancy rate.91 The latter could be explained by the limited sample size, which could lead to a type II error. Additional prospective valuation measured 43 PCBs in follicular fluid from 32 IVF patients and identified significant inverse associations between levels of PCBs and all IVF endpoints, such as ovarian response, number of oocytes retrieved, endometrial thickness, oocyte fertilization, embryo quality, implantation, and live birth, after adjusting for BMI, smoking, race, and age.93 Dose-dependent decrease in implantation rate with increased serum levels of several PCBs was also observed in 827 ART cycles in 765 women.94

Heavy metals

Environmental heavy metals, including cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), and copper (Cu), are derived from multiple sources, of which industrial pollution and smoking are the most common. Heavy metals can accumulate in water or animal products and persist for years. Current evidence supports a negative association between cadmium, lead, arsenic, and copper with reduced fecundability, spontaneous miscarriage, and male infertility,95,96 but this adverse relation was not confirmed for exposure to reasonably accepted environmental levels of these metals.97 Exposure to mercury in population at risk (high fish consumers and dental practitioners) has been linked with reduced fecundability in a dose-dependent fashion, but typical environmental exposure did not seem to alter ovulation, and it did not affect ovarian response, fertilization rate, embryo development, or pregnancy rates in women undergoing ART.4,95 This is in agreement with a study in rats that showed an altered estrous cycle with short-term exposure to mercury, but no significant effects on implantation or pregnancy.98

The biological mechanism by which environmental heavy metals influence implantation and the exact dose-response association are unclear. Heavy metals within cigarette smoke have been found within the endometrium of women who smoke, and the findings are proportional to the magnitude of exposure.99 It is possible that heavy metals from the other environmental sources aggregate within the endometrium and induce immune response or oxidative stress in a dose-dependent manner, which is likely to disturb the endometrial environment and lead to impaired implantation or abnormal maintenance of pregnancy. Currently, however, there is a relative paucity of information concerning the effect of EDCs on implantation, and studies on exposure to EDCs in couples with RIF are lacking.

CONCLUSIONS

In summary, detrimental effects on fertility and IVF success rates have been demonstrated for obesity, smoking, and heavy alcohol consumption. It remains unclear whether and to what extent these lifestyle factors affect implantation in couples with RIF. It can be supposed that EDCs such as...
BPA, phthalates, PCB, and heavy metals adversely affect implantation and may contribute to RIF, although the dose-effect association and the critical timing of exposure are unknown. There is no convincing evidence to support negative effects of pesticides and heavy metals at low to moderate, accepted environmental levels on implantation, and a possible negative impact of heavy occupational or unique geographical exposures remains to be determined. The data derived from animal models and studies on pregnancy loss provide indirect evidence for the contribution of stress and periodontal disease to RIF. There seems to be little association, if any, between low to moderate caffeine and alcohol consumption and reproductive events, including implantation failure; however the effects of excessive caffeine intake are still unclear.

More experimental and epidemiological studies are required to estimate the magnitude of the effect of these factors in RIF that would help to refine the counseling of the affected couples. It would be also interesting to identify individual susceptibility factors that modify the dose-response relationship between different environmental factors and RIF. Furthermore, it needs to be determined whether or not there exists a direct link between environmental exposures, metabolic dysregulation, and genetic factors that affect implantation in RIF women.

Preconception interventions for the majority of adverse lifestyle behaviors have not been assessed in the couples seeking fertility treatments, and there are no reports on specific interventions in the context of RIF. There is a need to evaluate the efficacy, intensity, and timing of lifestyle interventions that could potentially improve a likelihood of reproductive success in different subpopulations of infertile couples and in those presenting with RIF.

REFERENCES


INTRODUCTION

Implantation is a critical step in the reproduction process and requires synchronized development of both the embryo and the endometrium. A series of well-coordinated events are taking place simultaneously, such that failure of one or more of these events or of their coordination results in no further development of the embryo taking place. A spectrum of causes can result in recurrent implantation failure (RIF), including anatomic, endometrial, endocrine, immunologic, and hematologic factors. Similarly, a number of hormones have been implicated in the pathogenesis of RIF, including human chorionic gonadotropin (hCG), progesterone (PR), kisspeptin, activin A, activin B, follistatin, and periostin.

Nuclear-located receptors for progesterone (PR-A and PR-B) and estrogen (ER-α and ER-β) as well as non-nuclear PRs, involved in rapid progesterone responses, are expressed in the human endometrium. Their expression varies throughout the menstrual cycle. Other hormonal receptors located in the human endometrium include thyroid stimulating hormone (TSH), thyroid hormone, and insulin receptors. Though their exact role in the human endometrium is not fully understood, they underline the complex interactions between reproductive and endocrine systems.

Certain endocrine disorders have been suggested to be involved in the pathogenesis of RIF, such as thyroid disease, vitamin D deficiency, diabetes mellitus (DM), polycystic ovary syndrome (PCOS), and obesity. The current chapter aims to review the evidence of the association of endocrine disorders with RIF.

THYROID DISEASE

Thyroid diseases are common among women of reproductive age. Hypothyroidism or the presence of thyroid autoimmunity (TAI) have been linked to adverse reproductive outcomes, either after natural conception or in vitro fertilization (IVF). The close link between thyroid and reproduction is underlined by the wide expression of thyroid hormone and TSH receptors (THRs and TSHRs, respectively) in reproductive tissues. THRs and TSHRs are distributed in human endometrium, granulosa, and ovarian stromal cells, and their distribution differs according to the different developmental stages of the follicle. It has even been suggested that the human ovarian follicle is an independent thyroid hormone producing unit and that thyroid hormones may influence implantation and early development of the blastocyst, through endometrial tissue proliferation and maturation. It has been shown that thyroid hormones increase bovine blastocyst formation and embryo quality, as well as having an impact on angiogenesis and immune function. Furthermore, TSH increases the expression of key factors for embryo implantation, namely, the leukemia inhibitory factor (LIF) and its receptor (LIFR) in endometrial stromal cells.

In vitro studies of blastocyst cultures in the presence of thyroid hormone have demonstrated that thyroid hormone influences both the blastocyst and the endometrium at the time of implantation. In vivo studies of methimazole-induced (MMI) hypothyroid, pregnant mice have demonstrated that hypothyroidism may influence implantation and later adversely affect placental activity. In the setting of IVF/intracytoplasmic sperm injection (ICSI), ovarian stimulation (OS) leads to increased estradiol (E2) concentrations. High E2 concentrations result to an increase in thyroid-binding
globulin (TBG) and this, in turn, leads to a decrease in free TH concentrations. As a result, TSH production is enhanced.\(^{10,11}\) A systematic review on thyroid function before and after OS has demonstrated that TSH concentrations may rise during or within one month after OS, above the threshold of 2.5 mU/L suggested as the norm for the first trimester of pregnancy.\(^{12}\) When women undergoing OS were studied closely, at five different time points during OS, TSH concentrations did not change throughout OS (\(p = 0.066\)). However, this lack of significance may be due to the small sample size (\(n = 42\)) of the aforementioned study.\(^{13}\) In the same study, however, TSH was increased in women who became pregnant in comparison to their baseline concentrations (3.41 ± 1.20 vs. 2.01 ± 0.95 mIU/mL, \(p = 0.001\)).\(^{13}\) This observation, if confirmed, is of major clinical significance as it would affect the management of women undergoing OS, which should include universal screening.

In women with subclinical hypothyroidism, levothyroxine (LT\(_4\)) supplementation resulted in a higher delivery rate (pooled relative risk [RR] 2.76, 95% confidence interval [CI] 1.20–6.44; \(p = 0.018\); \(I^2 = 70\%\)) and a lower miscarriage rate (pooled RR 0.45, 95% CI 0.24–0.82; \(p = 0.010\); \(I^2 = 26\%\)). Even in the case of euthyroidism, it has been suggested that thyroid hormone replacement for TSH concentrations above 2.5 \(\mu\)IU/mL (high normal concentration) is a predictive factor for pregnancy rate following intrauterine insemination (IUI).\(^{14}\) However, evidence of the association of clinical pregnancy or live birth rate and high normal baseline TSH concentrations is conflicting.\(^{14,15}\)

Women with TAI carry a higher risk regarding the reproductive outcome after IVF/ICSI, as demonstrated by a meta-analysis of nine studies involving 4396 women.\(^{16–19}\) According to this meta-analysis, the live birth rate (LBR) of women with TAI was lower in comparison to that of those without TAI (odds ratio [OR] 0.73, 95% CI 0.54–0.99; \(p = 0.04\); \(I^2 = 41\%\)). In another meta-analysis of four prospective studies involving 1098 women undergoing IVF, the presence of TAI was associated with an increased risk for spontaneous miscarriage.\(^{20}\) In a study of 72 women with RIF, it was shown that women with TAI had a decreased percentage of CD3\(^+\)CD8\(^+\) T cytotoxicity (T\(_c\)) cells and an increased T helper (T\(_h\))/T\(_c\) ratio compared with women without TAI.\(^{21}\) This is clinically important as a lower percentage of CD3\(^+\)CD8\(^+\) T\(_c\) and higher T\(_h\)/T\(_c\) ratio has been associated with adverse pregnancy outcome, including recurrent spontaneous abortion.\(^{22}\) Furthermore, decreased T\(_c\) cells in women with TAI may result to an increase in the T\(_h\)/T\(_c\) ratio, thus leading to implantation failure.\(^{21,23,24}\)

**VITAMIN D DEFICIENCY**

There is increasing evidence demonstrating the role of vitamin D in female fertility and pregnancy.\(^{25}\) Vitamin D may also play a role in implantation, as there is evidence on its role as an immune modulator,\(^{26}\) and vitamin D metabolizing enzymes are present in human endometrium.\(^{25}\) Vitamin D receptor (VDR) and 1\(\alpha\)-hydroxylase are also expressed in reproductive tissues such as the human placenta, endometrium, and ovaries.\(^{27}\) CYP2R1, the enzyme that converts inert vitamin D into 25(OH)D, is expressed in endometrial cells, with an increasing expression during the secretory phase over the proliferative phase.\(^{28}\) An animal study has shown the expression of VDR, CYP24A1—the enzyme that degrades 1,25(OH)\(_2\)D and 25(OH)D—CYP2R1, and CYP27B1—which also converts inert vitamin D into 25(OH)D—at the mRNA level in the endometrium of pigs. Also, interestingly, calcitriol is synthesized in human endometrial decidua.\(^{29}\) It has been suggested that calcitriol influences specific genes’ expression (i.e., HOXA10 and the calbindin and osteopontin genes) that are involved to implantation and vitamin D and calcium metabolism.\(^{29}\) In early pregnancy, there is an upregulation of the production of vitamin D in the endometrium by the blastocyst.\(^{30}\) It is also suggested that the immunomodulatory effects of vitamin D may be linked to its protective role on abortion.\(^{31}\)

Vitamin D insufficiency is associated with miscarriage, as shown by the Odense Child Cohort.\(^{32}\) In this prospective cohort study of 1683 pregnant women, those with higher 25(OH)D concentrations presented a lower adjusted hazard ratio (HR) of first-trimester miscarriage (HR 0.98, 95% CI 0.96–0.99). Another cross-sectional study of 252 women undergoing ICSI has revealed a significant positive association of 25(OH)D with clinical pregnancy and endometrium thickness.\(^{33}\) It has been suggested that there is an increased risk of early spontaneous pregnancy loss when 25(OH)D
concentrations are lower than 20 ng/mL (RR 2.24, 95% CI 1.15–4.37), though the data are conflicting. A meta-analysis by Chu et al. on the association of vitamin D and live birth in women undergoing ART, although showing an increased likelihood of clinical pregnancy in women replete in vitamin D in comparison with those with vitamin D insufficiency or deficiency, failed to reveal any association between miscarriage and vitamin D concentration. Another study has detected differences on the association of vitamin D concentration and reproductive outcomes in different ethnic groups. According to Rudick et al., vitamin D status is positively associated with favorable reproductive outcomes after IVF in non-Hispanic white women. After adjustment for age and number and quality of embryos transferred, the odds of pregnancy in this ethnic group were four times higher in vitamin D replete versus deficient patients. However, no similar observation was made in Hispanic whites or Asian women. It has also been suggested that an excess of serum and follicular fluid vitamin D concentrations, together with a decrease in follicular fluid glucose levels, may lead to adverse IVF outcome. However, this finding is not always confirmed by other studies and the predictive role of follicular fluid vitamin D concentrations regarding pregnancy rate after IVF is limited.

A recent original study by Li et al. has demonstrated that 25(OH)D, TGF-β concentrations, and VDR expression are significantly decreased in women with recurrent spontaneous abortions when compared with women with normal pregnancies. In the same study, IL-17 and IL-23 concentrations were significantly increased. In donor-recipient cycles, clinical pregnancy and live birth rates were lower in vitamin D deficient recipients in comparison to those with vitamin D sufficiency, implying a favorable role of vitamin D at the human endometrium. There is no direct evidence however on the association of vitamin D status and RIF. Of note, obesity may influence vitamin D status in pregnant women as lower 25(OH)D concentrations are reported in pregnant women with higher body mass index (BMI), in comparison with those with normal BMI, and thus, vitamin D may be the key player in some of the obesity-related adverse pregnancy outcomes.

**POLYCYSTIC OVARY SYNDROME**

In women of reproductive age, polycystic ovary syndrome (PCOS) is a highly prevalent endocrinopathy, with an incidence that varies between 6% to 10%, based on the National Institutes of Health (NIH) criteria, and 20% to 22%, based on the American Society of Reproductive Medicine/European Society of Human Reproduction and Embryology (ASRM/ESHRE) criteria. Early pregnancy loss is increased in women with PCOS, up to five-fold, compared with women without PCOS. It seems that hyperinsulinemia, together with hyperandrogenism, may alter the endometrial environment and may have an impact in the increased percentage of early pregnancy loss and infertility in women with PCOS. Hyperinsulinemia and insulin resistance, common features of PCOS, may be risk factors for early pregnancy loss or implantation failure by themselves, as implied by the favorable action of metformin in the reduction of early pregnancy loss in women with PCOS. Indeed, metformin administration throughout pregnancy could lower the risk of early pregnancy loss in women with PCOS, as shown by a meta-analysis of six studies (OR 0.19 [0.12–0.28], I² = 0%). Even in lean women with PCOS, the risk of early miscarriage has been reported to be increased and the chance of live birth decreased, as compared with controls. Animal data do show a direct favorable action of metformin in implantation.

Animal and human data demonstrate that uterine receptivity in women with PCOS is impaired. Li et al. have demonstrated not only high circulating estrogen in DHEA-induced PCOS mice, but also compromise in the uterine decidualization and changes in the expression of implantation-related genes. Women with PCOS show increased endometrial androgen receptor (AR) expression, possibly inducing endometrial abnormalities in women with PCOS. Oocyte and embryo quality after IVF is also reported to be compromised in women with PCOS, probably due to increased oxidative stress in granulosa cells. The exact mechanism is unknown, but it seems that the NADPH oxidase (NOX) pathway may play a role in reactive oxygen species (ROS) production by granulosa cells in women with PCOS. Elevated aggrecan, disintegrin, and metalloproteinase with thrombospondin motif-1 (ADAMTS-1) concentrations were found in the follicular fluid of
women with PCOS, indicating a potential role of aggrecan in the pathogenesis of PCOS. A positive predictive effect of ADAMTS-1 on implantation was noted, indicating a potential role as a marker of high-quality embryos in women with PCOS.52

DIABETES MELLITUS

Women with DM type 1 and 2 have been reported to present pregnancy complications, including miscarriage. Insulin resistance and hyperinsulinemia have been related to adverse outcomes following IVF, though the exact mechanism is yet to be elucidated.

Diminished oocyte and embryo quality, as well as impaired implantation have been suggested to play a role.53

Local factors in the endometrium of women with DM may lead to impaired endometrial receptivity and RIF. Insulin receptors are present in the human endometrium and have varying expression during the different phases of the menstrual cycle, thus implying functionality of the receptor locally.5 Immune and vascular defects in the endometrium of women with DM during gestation have been suggested to lead to fetal loss.54 In a hyperinsulinemic mouse model, it was demonstrated that the endometrium receptivity is adversely affected by maternal hyperinsulinemia.53 The implantation rate is reduced in diabetic mice,55 with uterine environment alterations being the main reason. The upregulation of lactoferrin (Ltf) and complement 3 (C3) estrogen-responsive genes in diabetic mice implies higher estrogen concentrations. The increased estrogen concentrations, in turn, may impair embryo implantation in diabetic animals. In pregnant diabetic rats, when uterine tissue was examined, immune staining density of CD68, CD45, and CD56 was found to be decreased, and significant degeneration of uterine tissue was present.56

LEPTIN

Obesity includes a risk factor for recurrent spontaneous abortion, and obese women may experience various reproductive adverse events. On the other hand, a weight loss that exceeds 10% of current body weight has been reported to improve female fertility and reduce the spontaneous abortion rate by 50%.57 It is suggested that obesity has an impact on reproductive ability in various ways, including worsening of oocyte quality and inducing changes in human endometrium receptivity.57 In women with PCOS, success rates and adjusted odds ratios for all pregnancy outcomes progressively worsen as BMI increases.58

A recent theory underlines the role of leptin and leptin receptor (OB-R) gene polymorphisms on successful implantation and ongoing pregnancy. Leptin is a 16-kDa protein secreted mainly by adipocytes and expressed in human endometrium, suggesting a probable autocrine/paracrine role during implantation. It is suggested that in human endometrium, OB-R levels are higher during the implantation window. Women with PCOS or endometriosis have been reported to achieve lower concentrations of OB-R during implantation in comparison to controls,59 possibly due to dysregulation of ion channel expression. It is reported that there are lower levels of OB-R in patients with implantation failure in comparison with those with implantation success.60 According to Muller et al.,61 genetic variability in the leptin receptor gene is associated with recurrent spontaneous abortions. When women with unexplained RIF were compared with fertile women, lower endometrium expression of leptin and higher endometrium expression of leptin receptors was noted in women with RIF.4 The presence of OB-R mRNA at all developmental stages of the embryo underlines the role of leptin at the preimplantation stage.62 It has been suggested that leptin expression by the embryo influences its own development and its interaction with the endometrium, thus also leading to implantation and placentation.62,63 As a result, leptin and OB-R gene expression are being studied as probable new markers for the prediction of implantation failure59 and leptin is now assessed as a contributor to the preparation of the endometrium for blastocyst implantation.62 Similarly, adiponectin is also suggested to have an impact in human uterine receptivity and embryo implantation.59

Other than that, leptin can regulate the hypothalamic—pituitary—thyroid axis,64 thus also influencing implantation through thyroid hormone. Studies on pigs have shown that exogenous leptin can upregulate luteinizing hormone (LH) secretion in early pregnancy, when applied alone,
and downregulate it, in the presence of gonadotropin-releasing hormone (GnRH). It is also demonstrated that leptin knockout mice are not able to reproduce, but fertility is recovered when recombinant leptin is administered exogenously.

In the setting of IVF/ICSI, OS seems to be associated with elevated leptin concentrations, however evidence is limited. This increase in leptin serum concentrations is suggested to lead to negative reproductive outcomes. Of note, leptin concentrations in peripheral blood and follicular fluid have also been shown to be increased in obese women undergoing IVF in comparison to non-obese women.

CONCLUSIONS

Endocrine disorders have emerged as probable causes of RIF. The fact that different hormones have a catalytic role in the process of implantation explains the observation of endocrine disorders being involved in the pathogenesis of RIF. Various hormones interact with the human endometrium, whereas hormonal receptors are distributed in reproductive tissues, including the endometrium. However well-designed human studies in women with RIF are limited.

Subclinical hypothyroidism and TAI may compromise the reproductive outcome after IVF. Indeed, LT₄ supplementation in women with subclinical hypothyroidism or in women with TSH concentrations above 2.5 µIU/mL (high normal concentration) leads to higher delivery and lower miscarriage rates, though application of this threshold has not been tested in women with RIF. TAI is also associated with adverse pregnancy outcomes after IVF. The association of vitamin D deficiency and RIF is complex, but vitamin D is present in the human endometrium and it seems that it has a favorable role regarding endometrium receptivity. Hyperinsulinemia and insulin resistance by themselves, or as features of PCOS, also affect endometrium receptivity and thus IVF reproductive outcomes. Leptin and adiponectin are newly recognized key players in the implantation procedure. Leptin concentrations are generally increased in obese women and may further increase after IVF, but the endometrium expression of leptin is lower in women with RIF, with endometrium expression of leptin receptors being higher. In any case, evidence on leptin concentrations in women undergoing IVF is limited, not only due to the small number of studies, but also due to heterogeneity and methodological issues of the studies.

As new evidence on the association of endocrine disorders with RIF is gathered, new therapeutic opportunities will emerge. Assessment by an endocrinologist of women with RIF is necessary and may prove helpful in better management of women with RIF, not only for better reproductive outcomes, but also for the prevention of long-term complications of endocrine diseases.

REFERENCES


INTRODUCTION
The uterus, being one of the two partners (together with the developing embryo) in the achievement of pregnancy, plays a key role in implantation and subsequent pregnancy outcome. It seems reasonable that anything that alters normal anatomy of the myometrium and/or endometrial cavity might have an effect on implantation and the achievement of pregnancy. This seems to be extremely important in the subgroup of patients having a history of recurrent implantation failure (RIF), since the possibility of restoring the cause might reverse the negative effect of a potential uterine factor.

Congenital uterine anomalies (CUA) are common entities resulting from embryological defects in one or more stages of normal embryological development of the female genital tract. They are characterized by a distorted anatomy of the endometrial cavity. The American Society of Reproductive Medicine (ASRM), formerly the American Fertility Society (AFS), classified the anomalies into six classes: class I, including aplasia and agenesis (formation defect); class II, unicor- nuate uterus with its subclasses (formation defect); class III, didelphys uterus (fusion defect); class IV, bicornuate uterus (fusion defect); class V, septate uterus (absorption defect); and class VI, arcuate uterus (absorption defect), keeping also a place in the classification scheme for T-shaped uterus as a result of diethylstilbestrol (DES) administration during fetal life. The main restriction of this system, which received wide acceptance and was used until recently in almost all published studies, was the absence of definitions; it was only a descriptive presentation of the classes through only a classification scheme. Moreover, the inclusion of two separate categories for fusion defects, septate as the clinically important variant and arcuate as the variant with no clinical importance, without defining the anatomical borders for differential diagnosis between them, creates a huge confusion in the evaluation of their clinical consequences.

Recently, the European Society for Human Reproduction and Embryology (ESHRE) and the European Society for Gynaecological Endoscopy (ESGE) published a new classification of female genital anomalies, with clear anatomical definitions of the categories, giving the opportunity to evaluate their clinical impact; uterine, cervical, and vaginal anomalies were classified in independent categories. Furthermore, the two societies published a consensus for their diagnosis as an additional tool to clinicians and researchers to avoid subjectivity in their management. Uterine anomalies were categorized into five classes: class I, dysmorphic uterus (including T-shaped and infantilis uteri); class II, septate uterus (absorption defects/arcuate deleted); class III, bicornoreal uterus (fusion defects/including former didelphys and bicornuate uteri); class IV, hemi of unico- rneal uterus (formation defect/former unicorneal uterus); and class V, aplastic uterus (formation defect/including only cases of uterine aplasia).

The aim of the chapter is to critically review whether or not the anatomically distorted endometrial cavity in patients with CUA might have an adverse effect on implantation and, consequently, be a cause of RIF.
METHODOLOGICAL COMMENTS

Ideally, the study question could be answered by studies comparing infertile patients with and without CUA, treated with in vitro fertilization (IVF) and having RIF as the primary outcome; however, it is not clear yet what is defined as RIF. In the absence of such a definition and, also, of relevant studies, an alternative study outcome could be implantation rate per se; it is reasonable to assume that, if CUA impairs implantation, they might be a cause of RIF. In the same line, as the achievement of pregnancy is the result of a successful implantation, conception rates in patients with and without CUA could be another indirect study parameter. The prevalence of CUA in the general and infertile population could also provide indirect evidence for their potential impact on the achievement of pregnancy and, consequently, on implantation.

It is important to note that most of the available studies have used the ASRM classification system. However, the absence of clear definitions for the different CUA categories, as well as of criteria for the differential diagnosis between them, especially between bicornuate, septate, and arcuate uteri, represents an important limitation. Furthermore, the use of different diagnostic methods with variable diagnostic accuracy for categorization of patients in the available studies is an additional limitation. However, with the increasing availability of high-accuracy diagnostic methods and clinicians’ awareness, the objectivity of CUA diagnosis has improved in more recent studies, though still not overcoming the problem of differential diagnosis within the different categories and, potentially, that of overdiagnosing arcuate uterus.

Taking into account the previous methodological limitations, the impact of CUA on implantation will be assessed based on:

1. Prevalence of CUA in the general and infertile population; and
2. Conception and implantation rates in patients with CUA.

PREVALENCE IN THE GENERAL AND INFERTILE POPULATION

Historically, the first review examining that issue was published in 2001, concluding that there is no difference in the prevalence of CUA between the general and the infertile populations. However, the pooled prevalence of CUA in the studied populations was estimated by including all the available studies independent of the diagnostic method used; this represented an obvious major limitation since, for example, gynecological examination (used as a method in one study) has extremely low diagnostic accuracy compared to three-dimensional ultrasound (3D US).

Two subsequent reviews tried to address this limitation. Saravelos et al. reviewed the accuracy of the available methods in diagnosis and differential diagnosis of CUA; endoscopy, as the historical “gold standard,” hydrosonography (HSG), and 3D US, having an accuracy >90%, were characterized as high-accuracy methods. However, in studies performed with high-accuracy diagnostic methods, a similar pooled CUA prevalence of 6.7% (95% CI 6.0%–7.4%) and 7.3% (95% CI 6.7%–7.9%) in the general and infertile population, respectively, was observed.

Three years later, Chan et al., including additional high accuracy studies, found a CUA prevalence of 5.5% (95% CI 3.3%–8.5%) and 8.0% (95% CI 5.3%–12.0%) in the general and infertile populations, respectively. Moreover, they observed a statistically higher CUA prevalence of 13.3% (95% CI 8.9%–20.0%) in recurrent aborters and the impressive statistically higher CUA incidence of 24.5% (95% CI 18.3%–32.8%) in women with infertility and recurrent pregnancy losses. Although a statistically increased prevalence of CUA in the infertile population compared with the general one was not shown, there were two clinically interesting points that should not be ignored: (a) the prevalence of CUA was higher in the infertile population compared with the previous review and (b) the coexistence of infertility and recurrent pregnancy loss further increases the prevalence of CUA. Thus, the increasing clinical awareness and the use of more effective techniques in the diagnosis of CUA seems to gradually alter the available data in the field.
It should be noted, however, that the degree of anatomical distortion is not the same in all type of CUA, and this might exert a different effect on implantation. Thus, the distribution of the various categories of CUA in the different populations is another clinically interesting parameter.

Saravelos et al.,10 based on data from high-accuracy studies, found that the pooled prevalence of septate uterus was 2% and 3.5% in the general and infertile populations, respectively. Furthermore, the observed prevalence of CUA excluding arcuate uterus was 2.3% in the general and 5.6% (more than double) in the infertile population (Figure 5.1). Chan et al.,11 in a similar type of analysis, found that the pooled prevalence of septate uterus was 2.3% in the general and 3% in the infertile population. Moreover, the observed prevalence of CUA excluding arcuate uterus was 3.2% in the general and 5.8% in the infertile population (Figure 5.2). It seems, therefore that the diagnosis of the “gray zone” arcuate uterus might be responsible for the vast majority of CUA observed in the general population (Figures 5.1 and 5.2).

Thus, indirect evidence from prevalence studies supports the notion that CUA might have an adverse effect on woman’s fertility, potentially leading to infertility. Furthermore, it seems that the more severe the anatomical distortion of the cavity is, the higher the possibility of impaired fertility potential of a woman; septate uterus and more severe types of CUA appear to be the clinically important variants that adversely affect fertility.

CONCEPTION AND IMPLANTATION RATES IN WOMEN WITH CONGENITAL UTERINE ANOMALIES

Direct published evidence on the effect of uterine anomalies on implantation is not available in the relevant literature. Thus, as pointed out previously, indirect evidence coming from the study of the conception rates in patients with CUA might mirror a potential effect on implantation; for spontaneous pregnancies, this relationship is almost linear, whereas for pregnancies after IVF, embryo transfer policy is an important variable.

Chan et al.,12 in their systematic review of evidence, found that patients with septate uterus have ~15% (RR 0.86, 95% CI 0.77–0.96) significantly lower risk of conception compared to the anatomically normal ones. Conception rates were not different in patients with unicorneate uterus, but this observation should not be considered as conclusive due to the insufficient sample size. Three years

Figure 5.1 Prevalence of arcuate uterus and congenital uterine anomalies without arcuate uterus in the general and selected populations. (Data from Saravelos SH et al. Hum Reprod Update. 2008;14:415–9.)
later, Venetis et al. examined again pregnancy rates in both natural and assisted conception cycles in a larger cohort of meanwhile-published relevant studies. They did not find any statistically significant difference in natural (RR 0.96, 95% CI 0.89–1.04) and assisted cycles (RR 0.66, 95% CI 0.37–1.19) when reviewed separately; nevertheless, when natural and assisted cycles were analyzed together, patients with CUA experienced ∼15% (RR 0.86, 95% CI 0.74–1.00) lower risk of pregnancy compared with those without CUA, confirming the findings of the previous meta-analysis.

In a recent prospective observational study, Prior et al. examined the outcome of assisted reproduction in patients with CUA compared with normal controls. Patients with arcuate uterus compared with those having normal uterus, had similar clinical pregnancy (43.2% vs. 43.7%, p = 0.78) and live birth (36.7% vs. 37.2%, p = 0.91) rates; according to them, arcuate uterus was defined as the presence of a concave fundal indentation at an obtuse angle, thus including patients with any indentation even as small as 1 mm. On the other hand, patients with real CUA (septate, bicornuate, didelphys, and unicornuate), characterized by them as “major,” had significantly decreased clinical pregnancy (28.8% vs. 43.7%, p = 0.048) and live birth (22.2% vs. 37.2%, p = 0.042) rates. Embryo transfer policy, stage, and number of embryos transferred were similar in all studied groups, indicating that the presence of uterine anatomy distortion in patients with CUA has an adverse effect on implantation.

The results of hysteroscopic treatment could offer some additional evidence for the potential role of these anomalies on patients’ fertility. However, although an increase in conception rates after surgical correction of the anomaly might be an indirect evidence of an impairment of woman’s fertility by the anomaly, a neutral effect might not, since this could be due to the “trauma” and healing process following the procedure.

Looking to the effect of septum excision, Venetis et al., in their systematic review of comparative studies, failed to detect any statistically significant increase in the probability of conception (RR 1.14, 95% CI 0.79–1.65) after hysteroscopic treatment; surgical correction of uterine septa was followed by ∼60% decrease (RR 0.37, 95% CI 0.25–0.55) in the risk of abortion. However, the fact that the presence of septum impairs the probability of conception as well as the evolution of
pregnancy combined with the observed effect size in this meta-analysis suggests that further evidence is necessary to properly access the value of hysteroscopic treatment for infertility.

In another type of analysis, Mollo et al. compared prospectively two groups of patients with unexplained infertility: patients with septate and with normal uteri. Conception (38.6% vs. 20.4%, \( p = 0.016 \)) and live birth (34.1% vs. 18.9%, \( p < 0.05 \)) rates after septum excision in the septate group were significantly higher than those in the normal group. These findings support the notion that the presence of septum has an adverse effect on fecundity and that, potentially, hysteroscopic treatment has a beneficial effect.

Although some of the existing evidence is coming from prospective studies, it is important to note that most of them, especially the comparative ones, included in the prementioned systematic reviews are retrospective studies. Thus, solid conclusions cannot not be drawn yet, but according to the best available evidence (level C/meta-analysis of retrospective comparative studies), the presence of CUA might adversely affect implantation as indicated by the decrease in the relative risk of pregnancy by \(~ 15\%\). Furthermore, concerning the achievement of pregnancy, it seems that septum excision might have a beneficial effect (class IIb recommendation).

**POSSIBLE BIOLOGICAL EXPLANATIONS**

Two main theories have been proposed to explain the potential association between CUA, implantation, and subsequent pregnancy. According to the first one, impaired implantation is the result of alterations in vascularization. The hypothesis of an impaired blood supply of the septum and of an overlying functionally defected endometrium was supported as an explanation of a poorer implantation; thus, when embryos implant on the septum site, pregnancy is less likely to occur. However, the poorer pregnancy outcome in patients with vascularized septa compared with the avascularized ones, the increased density of vessels found in tissue samples from septa by some investigators, and the potential impairment of implantation in other types of anomalies are not explained with this hypothesis. Thus, it is supported that it is not the decreased vascularization but the alterations of the vascular “bed” in the different types of anomalies which decreases receptivity to the invading trophoblast by impairing later, vascular stages of implantation; once the implanting embryo overcomes the endometrial barrier, uterine vasculature and stroma could represent a subsequent important barrier.

Another pathophysiological explanation for the impaired implantation focuses on the differences in the structure of the uterine musculature in patients with CUA. Uterine contractility seems to be an important factor for successful implantation and evolution of pregnancy; altered uterine anatomy and the resulting alterations of normal uterine contractility could exert a detrimental role in implantation and early stages of pregnancy. Decreased uterine volume does not seem to play a role in implantation but only, potentially, in the evolution of pregnancy and its later stages for complications from the second trimester onward.

The more sophisticated hypothesis of alterations in the expression of HOX genes at different age stages was also included in the “armamentarium” of pathophysiological explanations: HOX genes are important both for normal female genital tract embryogenesis and for development of the endometrium. However, it seems that the accumulation of more epidemiological data on the exact role of each type of uterine deformity on the reproductive potential of the woman is necessary for clarifying the contributing factors and the underlying biological mechanisms.

**CONCLUSIONS AND RECOMMENDED CURRENT PRACTICE**

The original AFS classification, being only a descriptive presentation of CUA, provided no definition for the classes of the system; this gave place to the adoption of a wide range of arbitrary criteria for their differential diagnosis, especially, but not only, between the arcuate and septate category. This, as a result, led to a great confusion in the relevant research. Although, ASRM recently tried to treat this restriction with the adoption of anatomical criteria for the septate group, the insistence
in having arcuate uterus as a separate category from the normal one is expected only to continue the existing research uncertainties.

As the need for further research on the “hot” issue of the reproductive impact of CUA is obvious, clear categorization of them is crucial. The ESHRE/ESGE classification of female genital anomalies with the adoption of clear anatomical definitions offers a research platform for studying their clinical impact;\textsuperscript{5,6,31} the deletion of gray zone anomalies should be considered as crucial and opens a new era. It is important to note that other groups also accepted the idea to abandon the gray zone arcuate category, proposing the normal/arcuate category as one entity and the septate one as the other, despite the fact that their anatomical criteria differ from that of the ESHRE/ESGE classification.\textsuperscript{32}

However, from the existing evidence, it seems that the presence of an anatomically defected uterus is associated with a $\sim 15\%$ decrease in the chance for achieving a pregnancy, either spontaneously or with the use of assisted conception, indicating an impaired implantation in women with CUA. Although it is not yet clear which is the exact impact of each different type of CUA on implantation and pregnancy outcome, it seems that the more severe the anatomical defect of the uterus, the more the probability of an impaired implantation and evolution of pregnancy. Although the contribution of minor defects is questionable, it is accepted that the presence of a septate uterus and more severe anatomical defects are associated with an adverse effect on the probability of pregnancy. Thus, women with repeated implantation failures should have an assessment of the uterine anatomy, preferably with the use of 3D US, which seems to be the diagnostic method of choice.\textsuperscript{7,8}

In the presence of an anomaly and especially of a septum, hysteroscopic treatment might be considered as a way of restoring anatomy and the implantation potential of the woman; patients with RIF are symptomatic women and, based on the existing evidence, it is reasonable to consider hysteroscopic restoration of the cavity.\textsuperscript{18,19} Finally, it is important to note that well-designed studies on that issue remain a necessity.

REFERENCES

INTRODUCTION

Recurrent implantation failure (RIF), during in vitro fertilization (IVF) and embryo transfer of fresh or frozen embryos, refers to unsuccessful implantation after repeated transfers of good-quality embryos. Until now, there has been no international consensus for a definition of RIF regarding a number of failed trials or transferred embryos. However, it is often defined as failure of embryos to implant in at least three consecutive IVF cycles in which at least four high-grade embryos are transferred. Due to the complexity of the underlying pathophysiology of RIF, various etiologies have been reported. Embryonic etiologies include genetic abnormalities, inadequate culture condition, suboptimal embryo development, zona hardening, improper embryo transfer technique, impaired embryo development in utero, male factor, immunological factors, etc. Maternal etiologies include endometrial and systemic factors, such as immunological, thrombophilic, metabolic, anatomical, and infectious factors, and multifactorial effectors including endometriosis and hydrosalpinges. With the recent development and the better understanding of immunology, it is more evident that immune inflammation significantly contributes to the underlying pathology of RIF.

Much progress has been made in in vitro embryogenesis and gamete and embryo cryopreservation technologies. However, little attention has been paid to improve the implantation after embryo transfer. During the implantation, maternal immune recognition and tolerance to the invading embryo play a major role. Both local and systemic immune effectors, cytokines, chemokines, and various immune factors actively contribute to embryonic implantation and maintenance of pregnancy. Hence, the dysregulated maternal immune responses to invading embryos may result in RIF, recurrent pregnancy losses (RPL), and second- and third-trimester obstetrical complications. RIF is a physically, emotionally, and mentally distressing disorder, and patients who suffer from RIF warrant further investigation, although diagnosis and management of RIF is still an evolving field. Treatment for RIF should be stratified based on underlying etiologies and personalized by individual characteristics. Possible immune etiologies and immunological therapeutic modalities for RIF are summarized in Figure 6.1. In this review, various immune etiologies for RIF and therapeutic modalities for these conditions are addressed.
Immune etiologies

Antiphospholipid antibody and antinuclear antibody

The most reliable marker for acquired thrombophilia is antiphospholipid antibodies (aPLs), including criteria-defined aPLs (such as anticardiolipin antibody (aCL), lupus anticoagulant, and β2-glycoprotein I antibody) and noncriteria-defined aPLs (such as antiphosphatidylserine antibody [aPS], antiphosphatidylethanolamine antibody, and other phospholipid antibodies that bind to negatively charged phospholipids nonspecifically). It has been suggested that maternal thrombophilia, such as aPL, may disturb blood flow to the endometrium and placenta, which results in altered endometrial receptivity and implantation failure. Patients (n = 74) with RIF showed a significantly higher prevalence of IgG-aPS and IgG-aCL as compared to women with a history of early RPL or controls. In a long-range study, women with two and more IVF failures (48% and 50%, respectively, n = 853) are associated with significantly higher serum levels of aPLs against phosphatidylinositol and L-phosphatidylserine (p < 0.01). When IgG, IgA, and IgM isotypes of antibodies against seven phospholipids (i.e., cardiolipin, L-phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, as well as against beta2-glycoprotein I) were investigated, a quarter of women with RIF had three or more positive aPLs. Therefore, autoimmune activation with positive aPL, including not only criteria-defined but noncriteria-defined aPLs, is common in women with RIF. Whether the association of aPL with infertility and RIF is causative needs to be elucidated further.

**Figure 6.1** Therapeutic targets and immune modulation treatment for recurrent implantation failure (RIF). Cellular immune etiologies involving T_h1, T_h2, T_reg, and NK cells and autoimmune abnormalities such as ANA, autoantibodies to nuclear antigens, and antiphospholipid antibodies are the major causes of RIF. These biomarkers can be potential therapeutic targets for immune modulation treatment including immnosuppressive and immunotropic treatment. Dotted lines represent the possible treatment with limited clinical data.
The presence of antinuclear antibody (ANA) and anti-centromere antibody was reported to interfere with the oocyte and embryo development significantly, and reduce implantation and pregnancy rates in patients undergoing IVF treatment. The levels of ANA in serum and follicular fluid were positively correlated, and IVF outcomes were markedly poorer in ANA+ women as compared with those of ANA− women. In a prospective controlled study of women undergoing IVF or intracytoplasmic sperm injection (ICSI) and embryo transfer (ET), pregnancy rate, clinical pregnancy rate, and implantation rate were significantly lower in follicular fluid ANA+ women (n = 36, 27.8%, 25%, and 15.9%, respectively) as compared with those of follicular fluid ANA− women (n = 50, 57.8%, 48.4%, and 31.7%, respectively; p = 0.004, 0.022, and 0.009, respectively). This effect became worse with an increased level of serum ANA, although a low-titer ANA was reported not to affect IVF outcome adversely. In another prospective controlled study of infertile women undergoing IVF/ICSI (n = 517), 39.34% of infertile women had ANA+, while 16.13% of controls (n = 186) had ANA+ (p < 0.001). In ANA+ women, the implantation rate (16.09% vs. 27.03%) and clinical pregnancy rate (27.72% vs. 45.03%) were decreased significantly (p < 0.001), while the early miscarriage rate (21.43% vs. 3.39%) was increased (p < 0.001) as compared with those of ANA− women. In RIF women with a failure of implantation after eight or more good embryos transferred, ANA was positive in 33.6% (n = 122), which was significantly higher than that of fertile controls (10%, n = 20).

Natural killer cell pathology

During the implantation period, a complex micromilieu is necessary including various cytokines, chemokines, and factors produced by the blastocyst and endometrium, which have multidirectional pleiotropic effects for the embryonic development and implantation. Inadequate or overactive immune responses and an unbalanced cytokine network may lead to implantation failure, pregnancy loss, and obstetrical complications. T helper (T_h) 1, T_h2, T regulatory (T_reg), T_h17, and natural killer (NK) cells are the major immune effectors affecting human pregnancy. Women with unexplained infertility had higher proportions of activated NK cells, and the percentages of peripheral blood CD56+ and CD56+CD16+ cells on the day of ET were higher in women with IVF failure (19.1 ± 0.7% and 12.0 ± 0.6%, respectively) than those with successful implantation (15.2 ± 1.2% and 9.5 ± 0.9%, respectively) (p < 0.02 and p < 0.05, respectively). In women with RPL and RIF, the proportion (%) of CD56brght/IFN-γ+/TNF-α+ cells was significantly higher than that of healthy controls. The TNF-α+/GM-CSF+ expressing CD56brght cell ratio was significantly higher in women with RPL and RIF than in controls. This finding indicated that NK-1 shift in peripheral blood NK cells was present in nonpregnant women with RPL and RIF. In a prospective study, the percentage of peripheral blood NK cells out of the total lymphocyte population was higher in women with idiopathic RIF (13.4 ± 1.2%; range, 2.63–29.01) than in controls (8.4 ± 0.7%; range, 5.72–13.28; p = 0.026). In this study, idiopathic RIF was defined as no abnormalities in karyotyping of both the man and the woman, hysteroscopy, endometrial culture and microbiological analysis of endometrial biopsy, sperm fluorescent in situ hybridization (FISH) for chromosomes 21, 18, 13, X, and Y, and sperm DNA fragmentation. In addition, peripheral blood CD56+ NK cells were positively correlated with endometrial CD56+ cells (rho = 0.707; p < 0.001). NK cells have been reported to contribute to trophoblast implantation during early pregnancy via vascular remodeling, control the embryo implantation, and provide local maternal immunity. Hence, dysregulated uterine NK (uNK) cells may contribute to IVF failures or RPL. In women who aborted compared with those who delivered, there was an increase in the percentage of CD56dimCD16+ cells and a decrease in the percentage of CD56brghtCD16− cells in the endometrial tissue. Testing for NK cells might be useful for the diagnosis of immune etiology RIF among women with idiopathic RIF and selection of patients undergoing assisted reproductive technology (ART) cycles, who can be benefit from adjuvant therapy. Indeed, intravenous immunoglobulin G (IVIG) treatment has been reported to reduce NK cell percentage and cytotoxicity significantly and increase killer inhibitory receptors while decreasing killer activating receptors, while significantly improving pregnancy outcome in women with RPL and IVF failures.
T cell immunity

The proportion of TNF-α producing CD3+CD4+ cells (p < 0.05), and TNF-α/IL-4 (p < 0.05) and TNF-α/IL-10 (p < 0.005) producing CD3+CD4+ were significantly higher in women with multiple IVF failures without a history of RPL as compared with those of controls. Recently, peripheral blood IFN-γ/IL-4, IFN-γ/IL-10, IFN-γ/TGF-β1, IL-6/IL-10, IL-6/TGF-β1, IL-13/TGF-β1 and TNF-α/TGF-β1 cytokine ratios have been shown to be significantly higher in women with RIF when compared to controls. The prevalence of dominant Th1 immune responses in peripheral blood lymphocytes may reflect the systemic contribution of Th1 cytokines to RPL or RIF in IVF cycles, and the Th1/Th2 ratios may affect clinical outcomes in IVF. Other T cell subsets, such as Treg and Th17 cells and their cytokine products have not been studied well despite their importance in pregnancy. Th17 cells are proinflammatory cells secreting effector cytokines, such as IL-17A, IL-17F, IL-22, IL-26, TNF-α, and IFN-γ. Contrarily, Treg cells suppress Th1- and Th17-mediated immunity and regulate maternal immune tolerance to the fetus. Previously, we reported that increased Th17 cells and decreased Treg cells in women with RPL as compared with fertile controls, and the IL-7/IL-7R signaling pathway plays a role in upregulating Th17 immunity, meanwhile downregulating Treg immunity. Hence, Th1, Th2, Th17, and Treg cells and their cytokine products could be explored as biomarkers for early pregnancy prognosis and should be evaluated in the context of RIF as well.

Endometrial immune responses

Local immune effectors and factors in endometrium may play a role in RIF. Inhibitor of DNA-binding protein 3 (Id3) is required for angiogenesis and proliferation of Treg cells. T lymphocyte-associated molecule-4 (CTLA-4) is expressed on the surface of Treg and conventional T cells and acts as a brake to shut down the activation of effector T cells. Id3+ and CTLA-4+ cells, but not forehead box P3 (FOXP3)+ cells, were significantly increased in the endometrium of women with RIF and RPL as compared with those in controls. There was neither coordinated expression of Id3+ cells and CD34+ vessels nor colocalization of Id3+ and FOXP3+ cells in the endometrium. Aberrant expressions of Id3 and CTLA-4 in peri-implantation endometrium may suggest a population of women with RIF may have chronic inflammatory microenvironments in endometrium during the embryo implantation period. Several endometrial biomarkers for the endometrial immune inflammatory status have been reported. Ratios of the IL-15/fibroblast growth factor-inducible 14 (Fn-14) mRNA expression in endometrium, together with the uNK cell count, were reported to reflect uNK cell activation and maturation status. Ratios of the IL-18/TNF-like weak inducer of apoptosis (TWEAK) mRNA expression in endometrium were reported to be a biomarker of both angiogenesis and the Th1/Th2 balance. In 81.7% of the RIF patients, at least one of these parameters (CD56+ NK cell numbers in the endometrium, the ratios of IL-15/Fn-14 mRNA, and IL-18/TWEAK mRNA) was reported to be dysregulated. Based on these data, women with RIF may have dysregulated immunotropism and angiogenesis at the implantation site, and further studies are needed to advance current knowledge and understanding of immune responses during implantation and early pregnancy.

Genetic polymorphisms affecting immune responses

Genetic polymorphisms have been reported to affect the implantation process after IVF-ET. In the meta-analysis of five studies for the correlation between HLA-G polymorphism and RIF, a significant association was found in the population of Caucasian origin under allele contrast (OR = 1.73, 95% CI 1.20–2.50) and genetic models of +14bp/+14bp versus −14bp/−14bp (OR = 3.09, 95% CI 1.43–6.65). HLA-G 14-bp insertion allele may increase the risk of RIF in Caucasians. Additionally, allelic variations, particularly in exons 3 and 4, and intron 2 of the HLA-G gene have been reported to be associated with ART failures. Further studies with a large sample size of different ethnic backgrounds are needed to explore the association between HLA-G polymorphism and RIF.

During embryo attachment and implantation, loss of mucin 1 (MUC1), transmembrane mucin expressed at the apical surface of uterine epithelium, is required. The MUC1 expression is modulated
via dynamic interplay among cytokine-activated transcription factors, nuclear factor κB (NF-κB), progesterone receptor isoforms, and transcriptional coregulators. Therefore, polymorphisms in the NF-κB gene may have important consequences in implantation failure and RPL. In women with RIF (n = 209), the allelic and genotypic frequencies of the rs28362491 promoter in the NF-κB gene were statistically significantly different from those of healthy controls. The frequencies of the del/del genotype and the rs28362491 del allele were significantly higher in RIF patients than in healthy controls (p < 0.004). In the haplotype analysis, the A-C haplotype occurred significantly more frequently, and A-G haplotypes occurred less frequently in RIF subjects than healthy controls.

MicroRNAs regulate gene expression post-transcriptionally and contribute to folliculogenesis and oocyte maturation. miR-146a is enriched in the oocyte and has been reported to be upregulated in the plasma and ovarian granulosa cells of premature ovarian failure. miR-196a2 is expressed during oocyte maturation and early bovine embryonic development and regulates the homeobox gene expression of the newborn bovine ovary during early embryogenesis. A case-control study of 354 Korean women investigating the association of microRNA polymorphisms (miR-146aC>G, miR-149T>C, miR-196a2T>C, and miR-499A>G) with RIF, demonstrated that the polymorphisms in miR-146a and miR-196a2 were related to RIF. Collectively, these polymorphisms may serve as biomarkers for detecting oocyte pathology of RIF via immune mechanisms.

**IMMUNOLOGICAL TREATMENT**

**Glucocorticoid**

Prednisolone, which belongs to glucocorticoids, is the most widely prescribed treatment for immunomodulation in patients with infertility and RIF due to its easy application, low cost, and relative safety in short-term treatment regimes. Prednisolone was reported to be effective in RIF women with high NK cell levels and/or activity. In a randomized controlled, prospective study, prednisolone 20 mg was given to women undergoing ICSI treatment. Patients with elevated NK cell activation marker (CD69+ >1% of total lymphocytes) by flow cytometric analysis were included. Women with an immunological disease, thrombophilia, or uterine or endometrial abnormalities were excluded. Clinical pregnancy rate of women with prednisolone (n = 58) was 48.3% as compared with 29.6% in controls (n = 54, p < 0.05, RR 1.63, CI 1.00–2.66). Prednisolone induces maternal immune tolerance by decreasing peripheral blood NK cell levels and activities. It has been hypothesized that elevated peripheral blood CD56<sup>bright</sup> NK cells in the failed IVF cycle may account for reduced decidual recruitment and predict implantation failure. One week after ET, women with failed IVF showed elevated peripheral blood NK (both CD56<sup>bright</sup> and CD56<sup>dim</sup>) and NKT-like cell proportions, increased perforin-containing CD56<sup>bright</sup> cells, more activated and degranulated CD56<sup>dim</sup> NK cells, and enhanced NK cell-activating receptor expression on both cell types and both NK cell subsets (CD160, NKG2D). All these findings reflect unfavorable type 1 changes of NK and NKT-like cells during this period.

It is suggested that uteroplacental thrombosis and vasoconstriction, resulting from binding of antibodies to platelet and endothelial membrane phospholipids, may contribute to adverse reproductive outcomes in women with autoantibodies. Corticosteroids are considered suppressing autoantibodies and thereby may contribute to successful implantation and placentation in those women. In a retrospective study that evaluated the effects of prednisolone in women with ANA+ who underwent IVF (n = 120), implantation and clinical pregnancy rates in the prednisolone-treated ANA+ women were significantly higher than those of untreated ANA+ women. The higher pregnancy rate in women with autoantibodies treated with prednisolone was noted in spite of no significant decrease in autoantibody titers. In a prospective controlled study of ANA+ women (n = 133), 60 women were treated with prednisone 10 mg/day and aspirin 100 mg/day, starting three months before ovulation induction, and 73 women served as an untreated control group. Fertilization, implantation, and clinical pregnancy rates of ANA+ women (71%, 27.4%, and 53.3%) with prednisone and aspirin treatment were significantly higher when compared with those of controls (58.0%, 13.6%, and 30.1%) (p < 0.01, p = 0.002, p = 0.007, respectively). Miscarriage rate
was significantly lower in the prednisone and aspirin treatment group (9.4%) as compared with that of controls (36.4%, p = 0.36, implies that the difference is NOT significant). In a prospective controlled trial, 66 women with ANA+ who had poor IVF/ICSI outcomes received a daily oral dose of prednisone 10 mg and aspirin 100 mg up to three months before the second IVF/ICSI cycle. Women who were treated with prednisone and aspirin had a significantly higher numbers of embryos (6.90 ± 3.24 vs. 4.81 ± 2.93, p = 0.006) and transferred embryos/cycle (2.76 ± 0.58 vs. 2.06 ± 0.44, p = 0.008) than women without any treatment. Pregnancy rate (57.1% vs. 12.5%, p = 0.006) and implantation rate (27.9% vs. 6.06%, p = 0.013) were significantly higher in women with prednisone plus low-dose aspirin treatment as compared with those of controls. In the meta-analysis of randomized controlled trials (RCTs), the efficacy of administering glucocorticoid in the peri-implantation period in subfertile women undergoing IVF or ICSI, but not women with RIF, failed to show a significant difference in pregnancy rates between the intervention and control groups. However, subgroup analysis revealed that couples undergoing IVF rather than ICSI showed a significantly higher pregnancy rate in women who received glucocorticoids (OR 1.50, 95% CI 1.05–2.13, p = 0.02, 6 RCT, 650 women, I² = 0%). In this analysis, patients with autoantibodies were excluded from the meta-analysis. Hence, the clinical efficacy of glucocorticoid in subfertile women with autoimmune etiology could not be assessed.

**Intravenous immunoglobulin G (IVIG) infusion**

IVIG, which is isolated from human plasma and contains more than 95% unmodified immunoglobulin G, has been established as an effective treatment for autoimmune and inflammatory diseases. The suggested mechanism of IVIG in reproduction is a reduction of peripheral NK cell cytotoxicities, enhancement of regulatory T cells, downregulation of antibody production, and favoring Th2-mediated immune response by decreasing Th1 immunity. For several decades, IVIG infusion has been utilized for the prevention of RPL potentially caused by immunological etiologies. However, the efficacy of IVIG treatment for RIF patients has not been well elucidated.

In a prospective controlled study, 8 of 30 infertile women with a history of three or more RIF or three or more RPL presented with high numbers of CD3+CD56+ NKT cells (>93.6 cells/µL), which declined after treatment with IVIG. Women with NK/NKT cell expansion with and without autoimmunity achieved a significantly higher pregnancy rate with IVIG treatment compared with women with average numbers of NKT cells and no evidence of autoimmunity (p = 0.018). In this trial, heparin and aspirin were also given if patients were positive for aCL or aPL. Elevated NKT cell levels alone were the independent predictor of success on IVIG treatment (p = 0.003). IVIG increased pregnancy and live birth rates in selected women with RIF who had NK/NKT cell expansion. Hence, the selection of the proper population of IVIG treatment may enhance the treatment outcome. In a prospective controlled study (n = 188), IVIG therapy in a cohort of women with RIF and elevated circulating NK (CD3−CD56−CD16+, >12% of lymphocytes) and lack of aPLs significantly improved the pregnancy rate to 50.5% and implantation rate to 21% compared with 15.0% and 9.3%, respectively, in women not receiving IVIG. In another prospective controlled study of women with a history of RPL or RIF (n = 157) undergoing IVF cycle, 64 (40.8%) women had elevated NK/NKT-like cells. Expansion of blood NK cells (CD3−CD56−CD16+, CD3−CD56+CD16+, or CD3+CD56+CD16+ lymphocytes) was defined as proportions above 12% and for NKT-like cells (CD3−CD56−CD16+), as proportions above 10% of total lymphocytes. Among the women with elevated NK/NKT-like cells and three or more RIF (n = 40), a total of 20 women received IVIG treatment, and 20 did not receive IVIG treatment (controls). The first IVIG infusion (400 mg/Kg of body weight) was given within 24 hr before the embryo transfer, then 15 days after with the confirmed biochemical pregnancy, and every three weeks during the first trimester of gestation. After gestational week 13, patients were given IVIG with a monthly dose of 200 mg/Kg of body weight until 35 weeks of gestation. The clinical pregnancy rate of RIF patients with IVIG treatment was 85% versus 10% in controls (p ≤ 0.0001, OR 34), and the live birth rate was 75% with IVIG versus 5% in controls (p ≤ 0.0001, OR 34). Meta-analysis of three published RCTs of IVIG in IVF failure patients shows a significant increase in the live birth rate per woman (p = 0.012, number needed to treat for one additional live birth, NNT = 6.0...
women). Using the live birth rate per embryo transferred, and adding data from two unpublished cohort-controlled trials to the meta-analysis, further supports this conclusion (overall $p = 0.000015$, NNT = 3.7 women). This study also revealed that properties and scheduling of the IVIG and selection of patients with abnormal immune test results were relevant variables for these trials.48 However, one of the RCT studies included in the meta-analysis compared the outcome of women with IVIG, heparin, and aspirin treatment with the controls who were treated with heparin and aspirin only.54 If this study is excluded due to the study design of using co-intervention, the difference reported is not significant. Hence, the data should be carefully interpreted. Recently, another systemic analysis was reported including two non-RCTs ($n = 129$). In this analysis of women with elevated NK cell numbers or activity, outcomes of IVIG treatment were sought. Individual reported outcomes of identified observational studies using IVIG and the results of pooled data had shown benefit of the respective intervention with a relative risk (RR) of 3.41 (95% CI 1.90–6.11) for clinical pregnancy rates (65.3% for IVIG vs. 19.1% for controls), and an RR of 3.94 (95% CI 2.01–7.69) for live birth rates (58.7% for IVIG vs. 14.9% for controls).50 This demonstrated that adjuvant therapies, such as IVIG, in women with elevated NK cell numbers or cytotoxicities seem to confer some benefit on ART outcome. However, well-designed RCTs with an appropriate population selection by the standardized NK testing are needed to verify these findings.

**Tacrolimus**

Tacrolimus inhibits T cell activation by binding to the intracytoplasmic FK-binding protein. Once the complex is formed, it inactivates calcineurin, which is calcium and calmodulin-dependent phosphatase and consequently inhibits transcription factors in activated T cells. Tacrolimus has been used for the treatment of T cell-associated diseases, such as ulcerative colitis, eczema, atopic dermatitis and vitiligo, and immunosuppression after transplantation.55 In a prospective cohort study of 42 patients with RIF and elevated peripheral blood $T_h^{1}/T_h^{2}$ cell ratios (CD4+$^+$IFN-$^+$+$^+$/CD4+$^+$IL-$^+$+$^+$+$^+$), 25 patients were treated with tacrolimus (treatment group) and 17 received no treatment (control group). In the treatment group, tacrolimus was initiated two days before embryo transfer and continued until the day of the pregnancy test for 16 days. The clinical pregnancy rate of the treatment group was 64.0%, which was significantly higher than that of the control group (0%) ($p < 0.0001$). In the treatment group, the miscarriage rate was 6.3%, and the live birth rate was 60.0% ($p < 0.0001$). There were no serious side effects from tacrolimus in the treatment group, and no women developed obstetrical complications during pregnancy.56 In a recent study from the same investigators, patients were divided into three groups: the low (CD4+$^+$IFN-$^+$+$^+$/CD4+$^+$IL-$^+$+$^+$ less than 22.8), the middle (22.8 to less than 28.8), and the high (28.8 or greater) $T_h^{1}/T_h^{2}$ groups, and the tacrolimus dose was adjusted (1–3 mg/day) based on peripheral blood $T_h^{1}/T_h^{2}$ ratios (CD4+$^+$IFN-$^+$+$^+$/CD4+$^+$IL-$^+$+$^+$). The clinical pregnancy rate of the tacrolimus treatment was 48.8% in the low, 43.9% in the middle, and 33.3% the high groups, with similar results among these groups. However, the ongoing pregnancy/delivery rate of the low $T_h^{1}/T_h^{2}$ group (46.3%) was significantly higher than that of the high $T_h^{1}/T_h^{2}$ group (21.4%).57 In these trials, no significant side effects were reported. Adverse effects of tacrolimus were demonstrated in a case study of 100 pregnancies of women with a history of organ transplantation, in which the incidence of malformations was similar to that reported with other immune suppressants in transplant recipients.58 Further clinical trials are required to verify these results.

**Hydroxychloroquine**

Hydroxychloroquine, an antimalarial drug, is often used for various autoimmune diseases, particularly systemic lupus erythematosus. Its use in pregnant patients with antiphospholipid antibody syndrome (APS) was reported to improve pregnancy outcomes.59 It has anti-inflammatory, antithrombotic, and immunoregulatory properties and impairs complement-dependent antigen-antibody reactions.60 Besides, hydroxychloroquine was reported to restore trophoblast fusion and differentiation caused by aPL and diminished toll-like receptor 4 (TLR-4) expression.61 Those immunomodulatory effects led to its use in patients with RIF or RPL; however, there is a lack of
robust clinical evidence to improve clinical outcome. One retrospective study has shown a 78% live birth rate in a few refractory APS patients after add-on treatment with hydroxychloroquine. Hydroxychloroquine reversed the aPL-inhibition of trophoblast IL-6 secretion and partially limited aPL inhibition of cell migration. Therefore, hydroxychloroquine may be beneficial to RIF patients, particularly with APS. Currently, the clinical data on the use of hydroxychloroquine in RIF are lacking, and prospective studies are necessary.

**TNF-α inhibitors**

TNF-α inhibitors (adalimumab, infliximab, golimumab, etc.) are TNF-α blocking biologics to treat diseases such as rheumatoid arthritis and inflammatory bowel disease. They interact and neutralize TNF-α, which is produced by T_h1 and NK cells and induces a cell-mediated immune response. TNF-α inhibitors were reported to promote trophoblast invasion by reducing proinflammatory immune responses at the maternal-fetal junction and have been used for RIF and RPL. There are no randomized trials for the efficacy of adalimumab on pregnancy outcomes in patients with RIF, but a few observational studies in IVF patients have been reported. In a study evaluating women with a high TNF-α/IL-10 producing T_h1 cell ratio undergoing IVF or ICSI, the implantation rates were 59% (50/85), 47% (21/45), 31% (4/13), and 0% (0/9) for the treatment groups using IVIG + anti-TNF-α, IVIG, anti-TNF-α, and no treatment, respectively. There was a significant improvement in implantation, clinical pregnancy, and live birth rates for the treatment group with IVIG + anti-TNF-α when compared with the no treatment group (p = 0.0007, 0.0009, and 0.003, respectively). TNF-α blockers can be safely used during the implantation period and pregnancy. The US Food and Drug Administration (FDA) has categorized the medication as pregnancy category B after evaluating the congenital anomaly rate in pregnant women exposed to the drug for the treatment of rheumatoid arthritis and inflammatory bowel disease. Since data on the actual mechanism of JAK-STAT pathways in inflammatory obstetric disorders, including embryo implantation, are scarce, for the time being, therapeutic interventions in this setting should be carefully determined.

**Intralipid solution**

The infusion of intralipid, which is a sterile, nonpyrogenic fat emulsion, has been reported to improve pregnancy outcomes in patients with RIF. A number of studies suggest that intralipid has immune modulatory functions including suppression of NK cell cytotoxicities and proinflammatory cytokine production. These studies indicated that intralipid could be a therapeutic option to modulate abnormal NK cytotoxicity in women with reproductive failure. In a retrospective review of pregnancy outcomes of 200 women including 162 women with RIF and 38 with RPL, who had elevated NK cell cytotoxicity, intralipid infusion treatment was as effective as IVIG treatment in the overall live birth/ongoing pregnancy rate per cycle between women treated with IVIG (56%) and intralipid (61%, p = NS). While there is potential for intralipid therapy as an economically affordable treatment strategy to improve pregnancy outcome, substantial datasets are hard to come by, rendering it difficult to make a firm recommendation. Although generally considered as a safe and easily accessible treatment by clinicians, in 2015, Public Health England became aware of three women who developed severe sepsis following administration of intravenous intralipid (20%), believed to be as a result of poor practice in the administration leading to contamination of the product.

**Granulocyte colony-stimulating factor**

Granulocyte colony-stimulating factor (G-CSF) is well known to play a significant role in a wide variety of granulocytic and myelocytic proliferation. G-CSF has been reported to proliferate chorionic villi, increase the T_reg cell density, activate dendritic cells, and reduce cytotoxicity of NK cells. With those perspectives, several studies using G-CSF treatment have been conducted. The results were contradictory, and furthermore, the potential malignoma, congenital fetal malformation, and fetal hematologic abnormalities could not be excluded. In one study, acromegaly, omphalocele, Down syndrome, Edward a syndrome, and Patau syndrome were observed in RIF
women treated with G-CSF. In a recent multicenter randomized controlled trial, 112 infertile women with repeated IVF failure were evaluated. The efficacy of systemic single-dose subcutaneous G-CSF administration on IVF success in implantation rate (18% vs. 7.2%, \( p = 0.007 \)), chemical pregnancy (44.6% vs. 19.6%, \( p = 0.005 \)), and clinical pregnancy (37.5% vs. 14.3%, \( p = 0.005 \)) rates was evaluated and shown to be significantly higher in the intervention group compared with the controls. However, in another randomized placebo-controlled clinical trial, the success in the G-CSF group was not statistically significantly different from the controls. Statistical models looking at treatment effects on clinical pregnancy and implantation rates demonstrated no effect of G-CSF treatment. In a randomized controlled study of normal IVF patients with normal endometrial thickness (\( n = 100 \)), the intrauterine infusion of G-CSF did not improve pregnancy outcome.

**Endometrial scratching**

Injuring the endometrium may enhance endometrial receptivity by modulating various local factors, including proinflammatory cytokines, in the endometrium. It has been proposed to increase vascularity and lead to increased sustained implantation as well. Based on these findings, endometrial scratching was recommended in women with low endometrial CD56+ NK cells (<10), decreased IL-15/Fn14 (<0.3) and IL-18/TWEAK (<0.02) gene expression ratios during a window of implantation. In a study of 173 women with RIF undergoing FET cycle, the clinical pregnancy rate per transfer was significantly higher in women with soft curettage to the endometrium twice (\( n = 38 \)) (42.1%, 16/38) compared with women without a curettage (22.2%, 20/90). The crude and adjusted ORs were 2.55 and 2.49, respectively (95% CI 1.13–5.78, \( p = 0.03 \) and 1.01–6.17, \( p = 0.048 \), respectively).

On the other hand, contradictory results were reported in a retrospective study with RIF patients (\( n = 208 \)). The clinical pregnancy rate in the subsequent ET cycle following the local endometrial injury (\( n = 94 \)) was not different from that of women without the local injury (\( n = 114 \)). In women with polycystic ovarian syndrome, the local endometrial injury was the most effective treatment to improve the pregnancy outcome in women suffering RIF with uncompromised ovarian reserve. In a recent meta-analysis, the endometrial injury done between day seven of the previous cycle and day seven of the ET cycle was reported to be associated with an improvement in the live birth and clinical pregnancy rates in women with more than two failed previous embryo transfers (RR = 1.42, \( p = 0.01 \)). Inconsistencies in the literature remain, and there is a lack of robust evidence. In a recent scientific impact paper, the Royal College of Obstetricians and Gynecologists suggests that available evidence points toward a potential benefit of endometrial scratch in women with RIF when done in the cycle preceding the IVF treatment cycle. However, further prospective randomized studies of sufficient power are required to rule out the clinical value of local endometrial trauma.

**CONCLUSIONS**

RIF remains one of the most enigmatic areas of ART, although many efforts have been made to explore underlying etiologies. In most cases, routine infertility evaluations fail to identify the underlying etiology of RIF. Immunological, thrombophilic, anatomical and genetic evaluations can be considered for women with RIF. Treatment should be established based on its potential etiologies and focused on improving the maternal environment to enhance embryonic implantation while optimizing embryogenesis. Since a variety of underlying etiologies are present, personalized therapeutic approaches should be considered. Maternal interventions targeting dysregulated humoral and cellular immunity may improve pregnancy outcomes in women with RIF. Immunological treatments, including corticosteroid, IVIG, tacrolimus, hydroxychloroquine, TNF-\( \alpha \) inhibitors, endometrial scratch, have been investigated and reported to demonstrate some beneficial effect. Laboratory studies also have demonstrated the pharmacological and immunological effects of these treatments in vitro. However, it has been a challenge to translate the laboratory findings to clinical medicine. Additionally, clinical data for the efficacy of these treatments are limited, partly due to the difficulties in RCT for ART cycles and pregnant women. Further studies with larger sample sizes and randomized approaches are needed in the future. Until that time, patients should be thoroughly informed and counseled about the limitation of clinical data prior to the treatment.
REFERENCES


Acquired uterine conditions, reproductive surgery, and recurrent implantation failure

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INTRODUCTION
The uterus plays a key role in the achievement and evolution of pregnancy. Successful implantation of an invading embryo relies on a normal functioning endometrium supported by a healthy myometrial bed. Factors that might affect normal response to ovarian steroids, anatomy, vascularization, and/or motility, altering uterine integrity and function, could act as “barriers” to the early and late stages of implantation. This is even more significant nowadays, as a result of the trend to delay parenthood in modern countries: a number of acquired conditions, not present when a woman is young, are expected to be more frequent with increasing age.

In their presence, surgical treatment aiming to restore the reproductive potential of the patient represents an important option; however, its application is dependent on the need for histological documentation of the lesion’s benignity and on the efficacy of the treatment to restore normality.

The uterus consists of two major anatomical entities: myometrium and endometrium. Acquired conditions of the myometrial layer are fibroids and adenomyosis. Furthermore, endometrial polyps (including the adenomyotic ones), adhesions, and chronic endometritis represent acquired conditions affecting the endometrium and endometrial cavity. All these uterine conditions might impair the implantation process, altering the chain of events necessary for the embryo to be accepted and invade the uterine partner.

The aim of this chapter is to critically evaluate the potential impact of each acquired condition on implantation and the efficiency of surgical, preferably endoscopic, treatment to restore the reproductive potential of the patient.

METHODOLOGICAL COMMENTS
The initial question for each acquired condition is if it has any association with recurrent implantation failure (RIF). Apart from the fact that the definition of RIF is still unclear, the existing evidence on the association between uterine conditions and RIF seems to be either poor or absent. Thus, another way of examining the potential effect is to review the existing evidence on implantation per se, as it is reasonable to conclude that if a condition impairs implantation, it might be a cause of RIF. Furthermore, the achievement of pregnancy might be used as an indirect parameter to assess the effect on implantation, as pregnancy is the end result of a successful implantation. If any effect is present, the existing proposals on the potential biological explanation will be reviewed.

Another important clinical dilemma, if impairment is present, is whether treatment could restore fertility; a review of the existing evidence on that issue will also be provided. Finally, based on the currently best available evidence, recommendations for the management of uterine acquired conditions in patients with RIF will be provided.
FIBROIDS
Uterine fibroids are benign monoclonal tumors arising from smooth muscle myometrial cells. Even though the prevalence of uterine fibroids among infertile women is high (26%), studies have reported the presence of uterine fibroids in 12%–25% of women of reproductive age. However, their true incidence and prevalence remain uncertain, due to the high rate of undiagnosed cases.1–3

Although not necessarily symptomatic, the clinical manifestations of fibroids as well as their impact on reproductive outcome can vary widely. Different parameters, such as fibroid size and number, can play a role in their clinical presentation, but it is generally accepted that the most important parameter is their location in the myometrium and their anatomical relation to the endometrium and endometrial cavity.

Thus, classical classification of fibroids includes grouping them into submucosal, intramural, and subserosal fibroids. However, for submucosal fibroids, a further subclassification for the needs of surgical treatment has been introduced: those being totally intracavitary (type 0), those being >50% intracavitary (type 1), and those being <50% intracavitary (type 2). Similarly, a more detailed subclassification of the other types could be also clinically useful. Hence, the International Federation of Gynaecology and Obstetrics (FIGO), in order to create a uniform classification system, focused on the topography, suggested the classification of fibroids into seven types: types 0–2 include the existing submucosal categories based on the percentage of the lesion protruding into the endometrial cavity; types 3 and 4 include fibroids with primarily intramural location, type 3 being those in contact with the endometrium but not distorting the cavity (involving the inner myometrium) and type 4 being those lying in the outer myometrium; types 5–7 include subserosal fibroids, which are further classified according to the proportion of their intramural component, with type 7 being the pedunculated ones.4 It seems that this classification could be a useful tool in evaluating clinical, especially reproductive, consequences.5,6

Evidence of association
Although there are not sufficient data in the literature about the correlation of fibroids with RIF, there is indirect evidence, through their impact on in vitro fertilization (IVF) outcome, that they are associated with an adverse effect on implantation and hence with RIF. However, as fibroids constitute a heterogeneous entity, their impact on fertility varies according to the fibroid type and size.

Somigliana et al.7 meta-analyzed the available data from 13 studies on the effect of fibroids on the probability of conception: submucosal types were found to be associated with a ~70% decrease in the odds of pregnancy (odds ratio [OR] 0.3, 95% CI 0.1–0.7) and delivery (OR 0.3, 95% CI 0.1–0.8) rates, intramural ones with a ~20% decrease in the odds of pregnancy (OR 0.8, 95% CI 0.6–0.9) and ~30% decrease in delivery (OR 0.7, 95% CI 0.5–0.8) rates. On the other hand, subserosal types had no effect on the achievement of pregnancy. In a subsequent systematic review and meta-analysis of 18 case-control studies, Pritts et al.6 further confirmed these findings. Submucosal fibroids reduce the relative probability of pregnancy up to ~60% (relative risk [RR] 0.36, 95% CI 0.18–0.74) and those not distorting the intrauterine cavity up to ~20% (RR 0.78, 95% CI 0.69–0.88). Focusing on intramural fibroids, they found a significant decrease in clinical pregnancy (RR 0.81, 95% CI 0.70–0.94) and live birth (RR 0.70, 95% CI 0.58–0.85) rates; interestingly, data from prospective only studies showed impaired implantation (RR 0.55, 95% CI 0.39–0.78) and live birth (RR 0.46, 95% CI 0.29–0.74) rates. Subserosal fibroids had no impact on the achievement and evolution of pregnancy.

As the detrimental effect of submucosal fibroids and the absence of any effect of the subserosal ones were considered obvious, Sunkara et al.9 conducted another meta-analysis focusing on the “gray” zone of intramural fibroids. They included 14 retrospective and five prospective case-control studies having IVF patients with intramural fibroids ranging between 0.7 and 0.5 cm. They concluded that even though intramural fibroids do not protrude in the intrauterine cavity, they have a detrimental effect on IVF outcomes, as they decrease the relative probability of pregnancy by 15% (RR 0.85, 95% CI 0.77–0.94) and live birth rate up to 20% (RR 0.79, 95% CI 0.70–0.88); the detrimental effect on the achievement of pregnancy was more pronounced when only the five
Prospective studies were analyzed, with a reduction of the relative probability of live birth rates up to 40% (RR 0.60, 95% CI 0.41–0.87). However, this study did not evaluate whether the different subgroups of intramural myomas have a different effect on implantation.9

It should be noted that other potentially important parameters of intramural fibroids, such as their size, number, and their location in relation to the endometrium, were not taken into account in all those meta-analyses. Rarely the mean or median fibroid diameter is above 3 cm in studies on IVF outcome and fibroids.7,9 Actually, the general empirical policy of IVF specialists is to recommend surgical excision of lesions exceeding 5 cm in diameter; thus, the detrimental effect of intramural fibroids that emerges from studies investigating IVF outcomes might be an underestimation of their true impact.

On the other hand, Oliveira et al., examining the impact of intramural fibroid size on IVF pregnancy rates, found that intramural fibroids >4 cm had significantly lower pregnancy rates than those <4 cm, supporting the notion that size is a potential critical parameter. Furthermore, Yan et al.11 found significantly lower live birth rates (adjusted OR 0.86, 95% CI 0.74–0.99) in patients with intramural fibroids with >2.85 cm in their largest diameter when compared with matched controls without fibroids. Christopoulos et al.,12 in an observational study, found that IVF patients with myomas not distorting the endometrial cavity had significantly lower live birth rates only if two or more fibroids were present (OR 0.47, 95% CI 0.26–0.83) and if the lesion was ≥3 cm in diameter in size (OR 0.41, 95% CI 0.19–0.89); no difference was observed in patients with single fibroids of <30 mm in diameter.

It seems, therefore, that submucosal fibroids of any type negatively affect IVF outcome and implantation. Intramural fibroids also have a negative impact, especially if there are more than one and larger than 3–4 cm in diameter; their effect in relation to their proximity to the endometrial cavity (FIGO type 3 and 4) has still not been investigated. Subserosal fibroids of reasonable size have no impact on implantation and IVF outcome.

**Potential mechanisms/biological explanation of the effect**

Several mechanisms have been proposed to explain the negative effect of fibroids on implantation and fertility. The distortion of normal architecture of the intrauterine cavity has been implicated in poor implantation outcomes. Additionally, functional changes such as impaired vascularization and increased contractility result in a hostile environment for blastocysts and impair their invasion into the endometrium. Thus, histological examination of endometrium reveals gland atrophy and chronic inflammation, possibly reflecting an endocrine dysregulation and alterations of the local secretion of inflammatory and vasoactive substances.

Moreover, even though gene expression profiling of leiomyomas revealed upregulation and downregulation of genes affecting cellular differentiation and proliferation, such as TGF-β, only a few alterations were found in genes related to the window of implantation.13–15

**Treatment results**

Taking into consideration that myomectomy is associated with myometrial (intramural) or endometrial trauma (submucosal), followed by a healing process, whether myomectomy is able to restore fertility is another important clinical question. The reported pooled spontaneous pregnancy rates post laparoscopic/abdominal myomectomy in published comprehensive reviews ranged from 49% (95% CI 46–52)16 to 57% (95% CI 48–65), whereas in women with otherwise unexplained infertility, the pregnancy rate was 61% (95% CI 51–70); furthermore, the pooled pregnancy rates post hysteroscopic myomectomy were 45% (95% CI 40–50).16

However, these were data from observational noncomparative studies; comparative studies are rare and randomized controlled trials (RCTs) are still an exception. Bulettii et al. compared patients with fibroids who underwent laparoscopic excision (group 1) with patients who were not treated (group 2) and patients with unexplained infertility without fibroids (group 3). The group of laparoscopically treated patients had significantly higher delivery rates (42%) than the untreated
group (11%, p < 0.001) and the group without fibroids (25%, p < 0.001). In a subsequent study, the same investigators allocated IVF patients with at least one intramural-subserosal fibroid >5 cm to myomectomy or expectant management and found that women who underwent surgery had higher pregnancy rates than those who did not (25% vs. 12%, respectively; p < 0.01). Despite the absence of randomization, the beneficial effect of fibroid excision appears to be obvious.

In their prospective study, Casini et al. examined the pregnancy rates in women with fibroids who underwent laparoscopic and/or hysteroscopic myomectomy, compared with those who did not. Women with hysteroscopically treated submucosal fibroids had significantly higher pregnancy rates than those who did not undergo treatment (43.3% vs. 27.2%, respectively; p < 0.05); also, patients with laparoscopically treated submucosal/intramural myomas had significantly higher pregnancy rates than those who did not undergo treatment (36.4% vs. 15%, respectively; p < 0.05). This study, obviously, provides good quality evidence on the beneficial effect of surgical treatment of fibroids. Furthermore, three different groups of researchers reported similar delivery rates in IVF patients with submucosal fibroids after hysteroscopic myomectomy compared with controls without fibroids; this indicates that the negative effect of fibroids on IVF is alleviated by surgery, or at least that myomectomy does not seem to negatively affect the chances of conception in IVF cycles.

Pritts et al., in their systematic review, found significantly higher pregnancy rates after surgical excision of submucosal fibroids, although they failed to demonstrate a beneficial effect after excision of intramural ones due, apparently, to scarcity of data. In a Cochrane systematic review, Metwally et al. reported that the existing RCTs assessing the effect of myomectomy on fertility did not provide sufficient evidence due to the very low number of studies. On the other hand, laparoscopic and abdominal myomectomy have the same results on fertility restoration but the laparoscopic approach has faster postoperative recovery and lower morbidity.

ADENOMYOSIS

Adenomyosis is an acquired, benign gynecological condition of the myometrium. It is characterized by the presence of endometrial glands and stroma within the myometrium. The neighboring muscle cells respond to this intrusion with hypertrophy and hyperplasia. It can be either diffuse, where endometrial foci are scattered throughout the myometrium or focal, taking the form of adenomyoma (defined as a circumscribed nodule within the myometrium) or adenomyotic cyst, covering different zones of the myometrium (inner or outer myometrium). Histologically, it could range from mostly solid to mostly cystic.

Although histology is the gold standard of diagnosis, as this is mainly based on hysterectomy specimens, it could not be used for the diagnosis of the disease in infertile patients. Currently, two noninvasive imaging techniques have proved to be highly accurate for the diagnosis of adenomyosis: ultrasound and magnetic resonance imaging (MRI). Campaneria et al., in their systematic review, found that ultrasound has a pooled sensitivity of 0.72 (95% CI 0.65–0.79), specificity of 0.81 (95% CI 0.77–0.85), and an area under the curve (AUC) of 0.85. In a more recent review, Andres et al. found that two-dimensional ultrasound (2D US) had a pooled sensitivity of 0.84 (95% CI 0.79–0.86) and a specificity of 0.64 (95% CI 0.64–0.69), whereas 3D US had a pooled sensitivity of 0.85 (95% CI 0.76–0.91) and a specificity of 0.81 (95% CI 0.73–0.88). Considering MRI, Campaneria et al. found a pooled sensitivity of 0.77 (95% CI 0.67–0.85), specificity of 0.89 (95% CI 0.84–0.92), and an AUC of 0.93. An additional advantage of MRI is its high positive predictive value and its excellent correlation with histological findings. Thus, diagnosis of adenomyosis could be based on the noninvasive ultrasound and MRI evaluation of the uterus.

Evidence of association

The recognized clinical manifestations of adenomyosis include abnormal uterine bleeding and pelvic pain (dysmenorrhea and/or chronic pelvic pain). The presence of adenomyosis is found to be associated with infertility and impaired implantation. By considering the pregnancy rate as a means
to evaluate implantation, Vercellini et al.,\textsuperscript{26} in a systematic review, examined the impact of adenomyosis on IVF results. Either ultrasound or MRI was used to diagnose adenomyosis. They found that patients with adenomyosis had significantly lower clinical pregnancy rates (RR 0.72, 95% CI 0.55–0.95), although the study was unable to detect a difference in implantation rates. Furthermore, a limitation of the results reported was the significant heterogeneity among the studies.

Younes and Tulandi,\textsuperscript{27} in a recent meta-analysis, also examined the impact of adenomyosis on IVF outcome including, additionally, the effect on implantation; two more studies were included, one of them being prospective and well designed. They found that patients with adenomyosis have significantly lower pregnancy (OR 0.73, 95% CI 0.60–0.90) and implantation (OR 0.66, 95% CI 0.49–0.88) rates compared with those without. They also observed that patients with diffuse adenomyosis have a tendency for lower pregnancy rates than those with focal disease (OR 1.36, 95% CI 0.67–2.75).

In another study, Mavrelos et al.\textsuperscript{28} found that IVF patients with ultrasound findings of adenomyosis had significantly decreased clinical pregnancy rates (29.2% vs. 42.6%; \( p = 0.044 \); OR 0.68, 95% CI 0.47–1.00) and that the presence of \( \geq 4 \) ultrasound features was an independent predictor of clinical pregnancy (OR 0.35, 95% CI 0.15–0.82) as compared with those with no adenomyosis features.

It seems, therefore, that the presence of adenomyosis is a significant factor affecting implantation and the more severe the disease, the higher the possibility of decreased pregnancy rates.

**Potential mechanisms/biological explanation of the effect**

The biological explanation of the potentially impaired implantation linking adenomyosis with infertility and decreased pregnancy rate after IVF is not very clear. An abnormal inflammatory reaction, as indicated by the observed increased density of stromal macrophages, being the result of myometrial infiltration by endometrial glands as well as the elevated levels of nitrogen and oxygen-free radicals accumulating in the endometrial cavity of women with adenomyosis has been proposed as potential pathophysiological explanations.\textsuperscript{29–31} Other biological interpretations for this effect include local hyperestrogenism changing the estrogen/progesterone balance in the secretory phase and the density of endometrial capillaries; defective expression of implantation markers and defective decidualization through overexpression of P450 aromatase; hypervascularization;\textsuperscript{32} impaired utero-tubal transport; and altered uterine contractility due to the destruction of the normal architecture of the inner and/or outer myometrium.\textsuperscript{33}

**Treatment results**

The gold standard treatment for symptomatic adenomyosis is hysterectomy, but in cases of women desiring to preserve their fertility, this is obviously not an option. Uterine-sparing alternatives in the management of patients with adenomyosis include medical maneuvers aiming to control or temporarily regress the disease process and surgical solutions aiming to excise the diseased myometrium.

Obviously, from the different medical therapies proposed up until now (oral contraceptive pills, selective progesterone receptor modulators, progestins, aromatase inhibitors, levonorgestrel-releasing intrauterine device, danazol, etc.), gonadotropin-releasing hormone agonists (GnRHa) are the most appropriate for the management of infertile patients with adenomyosis. Vercellini et al.,\textsuperscript{26} in their systematic review, reported that patients with adenomyosis who stimulated for IVF with the long GnRHa protocol had no difference in pregnancy rates compared with those without the disease (RR 1.05, 95% CI 0.75–1.48) and those stimulated with the short GnRHa protocol had significantly lower pregnancy rates (RR 0.58, 95% CI 0.38–0.88). In a more recent systematic review, which included all the meanwhile published studies, Rocha et al.\textsuperscript{34} supported those findings: IVF/intracytoplasmic sperm injection (ICSI) patients with adenomyosis, stimulated with the long GnRHa protocol, when compared with those stimulated with the short GnRHa protocol, had higher pooled clinical pregnancy (43.3% vs. 31.8%, respectively; \( p < 0.001 \)) and live birth/ongoing pregnancy (43% vs. 23.1%; \( p < 0.05 \)) rates. Based on that, an extra-long protocol has been proposed for patients with adenomyosis;\textsuperscript{35} freezing all embryos from the stimulated cycle and frozen embryo transfer after downregulation with GnRHa up to three months.
Adenomyomectomy represents the conservative surgical therapeutic option for the treatment of infertility in patients with adenomyosis. Due to disease characteristics (infiltration of the myometrium by adenomyotic tissue), any surgical procedure always excises myometrial tissue as well; thus, adenomyosis surgery is limited by the need to preserve as much as feasible of the myometrial layer and to avoid defective myometrial reconstruction. It is noteworthy to mention that surgical treatment is quite demanding and the risk of uterine rupture in a subsequent pregnancy should always be taken into account. At first, Rocha et al., in their systematic review, reported an overall pooled clinical pregnancy rate after adenomyomectomy of 38.8% and a pooled live birth rate of 30.4%, both spontaneous and after assisted reproductive technology (ART). However, the pooled spontaneous clinical pregnancy rate was low (18.2%), explained potentially by mechanical reasons such as tubal patency. A high proportion of patients needed ART after surgery to achieve pregnancy. The use of GnRHa after surgery resulted in a significantly higher spontaneous conception rate and seems, also, to have a beneficial effect on the results of surgery and ART. It has also been supported that in infertile patients, the combination of surgery and GnRHa gives better results than GnRHa administration alone (OR 6.22, 95% CI 2.34–16.54).

It seems, therefore, that GnRHa might offer a window of improved implantation by temporary regression of adenomyosis, thus, exerting a beneficial effect on IVF outcome; this might be the result of the decrease in the size of adenomyotic lesions and the positive effect on endometrial implantation markers. Obviously, any beneficial effect of surgical treatment is due to the reduction of adenomyotic foci and their impact on implantation. 

ENDOMETRIAL POLYPS

Endometrial polyps (EPs) are focal, benign overgrowths of endometrium consisting of endometrial glands, stroma, and blood vessels. Their prevalence in the general female population is estimated near 10%. However, it is reported to be significantly higher in subfertile women undergoing IVF, approximately 30%, although there is a wide discrepancy in the reported prevalence among different studies, which can be explained by the diversity of diagnostic means used. Similarly, EP prevalence appears to be higher in women with recurrent pregnancy loss (RPL), indicating a possible negative effect of EP on reproductive outcome. Furthermore, as there is a causative relationship between estrogen effect and EP growth, the incidence of EPs is higher in obese women and, as recently shown, in those suffering from endometriosis.

Their most common clinical manifestation is abnormal uterine bleeding, although EPs are often diagnosed in asymptomatic women during a routine ultrasound examination. Their surface, projecting from functional endometrium, is often asynchronous to the normal and provokes abnormal uterine bleeding, possibly as a result of disproportion in the expression of estrogen/progesterone receptors. Additionally, atypical necrosis of a polyp may be another cause for intermenstrual bleeding.

Evidence of association

Due to the lack of high-quality prospective studies in the field of IVF in women with endometrial polyps, the hypothesis of their unfavorable effect on implantation is currently based on studies comparing IVF outcome in patients with and without polyps. Tiras et al. studied IVF patients with untreated endometrial polyps <14 mm diagnosed during ovarian stimulation and a control group of IVF patients with normal cavity; they found similar pregnancy rates in both groups, suggesting that the presence of polyps does not affect implantation. On the other hand, Yang et al. compared IVF patients with endometrial polyps diagnosed incidentally during ovarian stimulation with age-matched IVF patients having normal cavity. They observed significantly higher clinical pregnancy rates after hysteroscopic polypectomy followed by frozen embryo transfer than in normal controls followed by fresh embryo transfer (63% vs. 41%, p = 0.009), indicating that the presence of polyps interferes with the achievement of pregnancy and potentially implantation despite the obvious bias
in the embryo transfer policy. Furthermore, the achievement of pregnancy was similar 1–3 months postpolypectomy. Elias et al.,54 although they failed to find any difference between IVF patients having polyps compared to those with normal cavity, observed significantly higher biochemical pregnancy rates indicating that polyps could interfere with the evolution of pregnancy.

Nevertheless, the role of EP in RIF is indirectly shown by the fact that uterine abnormalities in women submitted to their first ART attempt range between 11% and 22%,43 but it is significantly higher in RIF patients reaching 45%, with the most frequently detected defect being EP,55 suggesting a potential correlation between EP and RIF.

**Potential mechanisms/biological explanation of the effect**

The biological explanation of the potential unfavorable effect of EP on reproductive outcome is not completely understood; hence there are different possible mechanisms that have been proposed.

Mechanical interference affecting sperm transportation, as well as embryo implantation, is in line with the hypothesis that the location of EP may influence pregnancy rate; Yanaihara et al. showed that removal of EP located near the utero-tubal junction provides the best results on fertility compared with other locations.52

Another alteration that may explain the effect of EP on implantation is the elevated levels of glycodeolin, a glycoprotein that inhibits natural killer (NK) cell activity and sperm-oocyte binding. In normal ovulatory endometrium, low levels of glycodeolin, six days before until five days after ovulation, permit fertilization followed by a rise in order to suppress NK cells and facilitate implantation. Elevated levels of glycodeolin may modify endometrial receptivity when EP are present.53

In addition to glycodeolin, alteration in expression of *HOXA10* and *HOXA11* genes that has been described in the presence of endometrial polyps may play a vital role in endometrial receptivity.54 Local inflammatory mediators, as well as other factors such as IGFBP-1 have also been proposed to impair implantation in EP.55,56

**Treatment results**

Hysteroscopy remains the gold standard for the diagnosis as well as the treatment of endometrial polyps. Given that the risk of complications after hysteroscopic polyp removal (adhesion formation, polyp recurrence, and decrease on endometrial thickness due to electrosurgery) is negligible, a detrimental effect on endometrial receptivity is not expected by hysteroscopy per se.57–59

Looking to the effect of treatment on natural conception, Varasteh et al. found that patients submitted to polypectomy have significantly higher spontaneous pregnancy rates than those who did not (78.3% vs. 42.1%, p < 0.05).45 Furthermore, other groups examined the effect of hysteroscopic polypectomy on clinical pregnancy rates after four cycles of intrauterine insemination (IUI) as compared to expectant management. Perez-Medina et al., in a well-designed RCT, found 63.4% cumulative pregnancy rates in the treated group and 28.2% in the expectant management group (p < 0.05).60 Similarly, Shohayeb and Shaltout observed 41.7% cumulative pregnancy rates in the treated group and 20% in the expectant management group (p < 0.05), confirming the beneficial effect of hysteroscopic polypectomy in the achievement of pregnancy.61 In a subsequent study, Kalampokas et al. also found statistically significant differences in cumulative pregnancy rates after IUI, favoring hysteroscopic polypectomy (40.7% vs. 22.3%, p < 0.05).62 The change of the endometrial environment required for embryo implantation seems to be the primary mechanism of this effect. The positive effect of hysteroscopic polypectomy on IUI results was confirmed in a Cochrane Review63 based on the results of the Perez-Medina et al. study.60

On the other hand, the results of hysteroscopic removal of endometrial polyps in IVF patients remain controversial. Lass et al. found 28.6% ongoing pregnancy rates after hysteroscopic removal of polyps >2 cm compared with 16.3% after expectant management (p = NS).64 Furthermore, Isicoglu et al.65 and Tiras et al.66 failed to find any difference in the clinical pregnancy rates whether or not endometrial polyps <1 and 1.5 cm, respectively, were removed. Furthermore, Ghaffari et al., also, found similar implantation, clinical pregnancy, and live birth rates in IVF patients who
underwent hysteroscopic polypectomy during ovarian stimulation compared with those who did not. But, the absence of any difference should be considered only as the result of treatment during the same cycle; recovery of endometrium from hysteroscopic trauma needs at least a period of five days, since in the same publication, no pregnancy was achieved if the interval between treatment and embryo transfer was shorter. The positive effect could be obtained from the next cycle on; the achievement of pregnancy is similar 1–3 months postpolypectomy.

It seems therefore that, although the role of polypectomy in women undergoing IVF remains unclear, its beneficial result in natural conception and in IUI is undisputable. Furthermore, Mouhayar et al. found that hysteroscopic removal of polyps when performed in IUI and IVF patients is cost effective, justifying its application also in IVF patients even in the absence of hard evidence.

**INTRAUTERINE ADHESIONS**

Intrauterine adhesions (IUA) are defined as the partial replacement of endometrium with fibrotic tissue together with adherence of the opposite sites. Although the term Asherman syndrome (AS) is often used as a synonym of IUA, AS includes those patients with IUA clinically manifested with amenorrhea, hypomenorrhea, dysmenorrhea, and recently proposed to include infertility, RPL, and abnormal placentation.

Adhesion formation might be the result of fibrosis following endometrial injury; infection at the time of injury is an augmenting factor as well, shifting the process from re-coverage of the trauma site with endometrium to a healing process with fibrotic tissue. The causes of IUAs and AS may be classified in two groups. The first one includes those following a gravid condition such as postabortion or postpartum curettage, miscarriage per se without any invasive procedure, postpartum and postabortion endometritis, and endometrial ischemia, as a consequence of postpartum hemorrhage or uterine artery embolization. The second one involves those caused by a nongravid condition such as surgical hysteroscopic procedures and infections, mainly tuberculosis of the female reproductive tract.

Their reported prevalence varies widely depending on the population studied, the diagnostic procedure and the classification method used. Although various classification systems have been suggested, further validation is needed in order to define their clinical utility.

**Evidence of association**

Although the effect of IUAs and AS on spontaneous pregnancy is very well documented, data regarding ART results in this group of patients are insufficient; thus, there is mainly indirect evidence that they have a detrimental effect on the achievement of pregnancy in patients undergoing ART.

Demirol and Gurgan found that IUAs occurred in 8.5% of women with RIF, indicating that their presence is related to impaired implantation. Schenker and Margalioth found that almost two-thirds of women with AS are infertile, whereas only 45% of patients with IUAs achieved a pregnancy, but 40% of them have ended in abortion. Moreover, 23% of these pregnancies have been complicated by preterm deliveries and 13% with placenta accreta. Acunzo et al. reported as low as 18.3% spontaneous live birth rates in patients with IUAs who did not have adhesiolysis, and Roy et al. suggested that there is an association between conception rates and the severity of adhesions.

The incidence of IUAs among ART patients reaches 16%, suggesting a link between adhesions and subfertility, although others support that it is more possible that the underlying cause of adhesions also result in subfertility, rather than IUAs as an independent factor.

**Potential mechanisms/biological explanation of the effect**

Apart from the mechanical obliteration of cervical canal and tubal ostia, the unfavorable effect of IUAs on fertility may be explained by the replacement of normal endometrium by fibrotic
tissue and, hence, although fertilization occurs, endometrium appears to be hostile for implantation. Additionally, impaired vascularization of the residual endometrial tissue and reduction of progesterone receptors decrease the chance of pregnancy and increase the risk of miscarriage.\textsuperscript{79,84} It is, also, suggested that IUAs interfere with the early stages of implantation, not allowing embryos to attach to the luminal surface of endometrium, and consequently, if present, may play a key role in RIF.

**Treatment results**

Although the endpoint of surgical treatment is the restoration of an anatomically normal cavity, from a functional perspective, it is the reversal of the presenting symptoms—in cases of abnormal menstruation, to achieve normal menses and in cases of infertility, to achieve a normal functioning endometrium with a normal implantation potential. It is obvious that, even in cases with a successful anatomically restored cavity, the extent of endometrial fibrosis and the level of endometrial regeneration play key roles in the functional result. Another factor determining the success is also adhesion recurrence.

Successful anatomical restoration ranges from 44% to 95%.\textsuperscript{57,71,85–88} However, the success rate is highly dependent on the severity of adhesions: the more severe the adhesions the lower the chances of achieving an anatomically normal cavity. Also, adhesion reformation varied widely from 3.5% to 20% for mild ones to almost 60% for severe cases, indicating a significant dependence on the adhesion’s severity and the extent of fibrosis.\textsuperscript{57,71,73,88} In this respect, referring severe cases for treatment to centers with expertise might improve the results.\textsuperscript{71}

Concerning reproductive outcome, Acunzo et al. reported that hysteroscopic adhesiolysis could significantly increase spontaneous live birth rates from 18.3% to 68.6%.\textsuperscript{80} Similarly, Pace et al., in women with AS, observed an increase in spontaneous pregnancy rates from 28.7% before to 53.6% after hysteroscopic surgery.\textsuperscript{87} Yu et al.,\textsuperscript{69} in their review, reported a pooled pregnancy rate, both spontaneous and after ART, of 74% after hysteroscopic treatment of IUA in women wishing pregnancy, much higher than the 46% observed in the nontreated cases; however, the pooled conception rates in infertile treated women was found to be $\sim$46% and for severe treated cases, the pregnancy rate was consistently lower, about 33%. Chen et al.,\textsuperscript{85} in infertile patients with IUA, found an overall spontaneous pregnancy rate after treatment of 48.2% with a mean time from treatment to conception of 9.7 ± 3.7 months; pregnancy rates decreased with IUA severity: 60.7% in mild, 53.4% in moderate, and 25% in severe cases of IUA. Although the existing evidence is not strong, it seems that hysteroscopic treatment offers better chances of conception in infertile patients, and this is inversely related to the degree of the existing endometrial damage and fibrosis.

Regarding prevention of IUA reformation, various methods have been suggested and appear to improve reproductive outcome. Recently, the American Association of Gynecologic Laparoscopists (AAGL) in collaboration with the European Society for Gynaecological Endoscopy (ESGE) published guidelines for prevention of IUA formation.\textsuperscript{89} It seems that posthysteroscopically, the use of mechanical (stent or heart-shaped balloons) and semisolid barriers (e.g., hyaluronic acid) as well as hormonal treatment (estrogens with or without progestin) might reduce recurrence of IUAs.\textsuperscript{89} Intrauterine devices (IUD) that contain progestin or copper should not be used; if any is used, it should be inert with a large surface area.\textsuperscript{89} Stem cell treatment at the moment should not be offered outside of rigorous research protocols.

**CHRONIC ENDOMETRITIS**

Chronic endometritis (CE), the chronic inflammation of the endometrial lining, reflects a loss of balance between the colonization of endometrium by microorganisms and the reaction of the local immune system, which leads to anatomic and functional alterations and, eventually, to an unfavorable effect upon fertility.\textsuperscript{90} The clinical manifestation of CE is usually subtle; the majority of patients are asymptomatic, or they may present with abnormal uterine bleeding, pelvic pain, and/or dyspareunia and leukorrhea.\textsuperscript{91}
Hysteroscopic findings suggestive of CE are micropolyps, stromal edema, hyperemia, and strawberry pattern of endometrium. Although the overall hysteroscopic accuracy seems to be satisfactory with a high negative predictive value, the positive hysteroscopic findings should always be complemented by endometrial biopsy, which confirms the diagnosis in 45%–90% of cases.\textsuperscript{92,93} Infiltration of endometrial stroma by plasma cells in endometrial biopsy specimens is considered fundamental for the establishment of diagnosis;\textsuperscript{94} immunohistochemical detection of CD38 and CD138 plasma cells seems to provide higher sensitivity than the conventional hematoxylin-eosin staining.\textsuperscript{95}

**Evidence of association**

The first suggestion of an association between CE and infertility was reported in 1978 by Czernobilsky.\textsuperscript{96} The reported prevalence of histologically confirmed CE in asymptomatic infertile patients before their first IVF attempt ranged between 3%\textsuperscript{97} and 15%,\textsuperscript{90} whereas a higher prevalence was found in patients with RIF, ranging between 15% and 60%,\textsuperscript{90,93,98,99} The prevalence of CE reported by Romero et al. was as high as 42% in RIF patients undergoing IVF,\textsuperscript{90} only 15% in infertile women, and even lower in the general population.\textsuperscript{94,100} Moreover, Conway et al. found this incidence even greater, above 60%.\textsuperscript{99}

The observed differences between the prevalence of CE in the general infertile population and in RIF patients indicates a potential unfavorable effect of this condition on implantation. However, more data are needed to elucidate the exact role of CE on implantation and the work-up for its diagnosis.

**Potential mechanisms/biological explanation of the effect**

In the majority of cases, common bacteria were found in endometrial cultures of patients with CE: gram-negative microorganisms in \(~60\)% and *Ureaplasma urealyticum* in \(\sim10\)% Chlamydia was detected in only 2.7% of cases, while genital tuberculosis could be responsible for CE in developing countries and in special groups of a population (e.g., immune-suppressed women). Those microorganisms, ascending from the lower genital tract, colonize the endometrial cavity and stimulate the immune system's reaction in order to restrict bacterial proliferation, such as formation of mucus plug and activation of epithelial cells, as well as neutrophils, macrophages, and NK cells. Bacteria form biofilms to resist the immune mechanisms causing chronic inflammation, secretion of cytokines, and further attraction of NK cells.\textsuperscript{92,101–105}

The disturbance of the normal balance of the paracrine milieu in case of CE could create a hostile environment for blastocyst invasion and successful implantation.\textsuperscript{106}

In addition to infection, gene reprofiling with upregulation of *IGFBP1* and downregulation of *IGF1*, as well as alteration of the expression of genes mediating the inflammatory process, could have a detrimental effect on successful implantation.\textsuperscript{107}

**Treatment results**

Since microorganism infection is the basis of CE, antibiotic treatment is considered fundamental. Targeted therapy based on endometrial cultures and antibiogram should be given; in the absence of cultures or negative cultures, blind therapy to cover potential chlamydia and mycoplasma infections, which are difficult to be isolated and cultured, should be given. Doxycycline as monotherapy, as well as ofloxacin in combination with metronidazole, has been found to attenuate the effect of CE on infertility. Doxycycline at a dose of 100 mg twice daily for 14 days had a beneficial result in 70% of patients with CE and a reported clearance of CD138 plasma cells in 96% of RIF patients.\textsuperscript{98,108} Combined therapy with ofloxacin 400 mg and metronidazole 500 mg twice daily for 14 days resulted in improvement of histopathological findings in 73% of patients.\textsuperscript{109} Alternatively, a five-day course of azithromycin at a dose of 500 mg orally on the first day and 250 mg from the second to the fifth days, is found to be effective for patients allergic to doxycycline.\textsuperscript{98,110}

Nevertheless, although histopathological and hysteroscopic findings could be cured with antibiotic therapy, successful pregnancy as a requested benefit remains the most vital concern of clinicians. In the category of patients with RPL having CE, Cicinelli et al. reported spontaneous clinical
pregnancy rates as high as 74.8% one year after antibiotic therapy as compared with only 24.4% in
the control group of untreated women,92 and McQueen et al., in their prospective study, found an
increase in spontaneous live birth rate per pregnancy from 7% before to 56% after antibiotic treat-
ment.95 However, despite the impressive results in RPL patients, clinical benefit from antibiotic
treatment in women with CE and RIF remains unclear. Thus, in a retrospective study, although
implantation rates increase after therapy, this increase was not statistically significant,100 while
Yang et al. found a significantly higher implantation rate after antibiotic treatment in patients with
RIF and CE diagnosed by hysteroscopy.68 It is also proposed that in this group of patients, hys-
teroscopic removal of bacterial biofilms could improve reproductive outcome, but there is not yet
enough evidence to support this hypothesis.35

CONCLUSIONS AND CURRENT PRACTICE RECOMMENDATIONS

From the reviewed evidence it seems that the uterus plays a significant role in implantation and that
acquired conditions of the myometrium and endometrial cavity could impair implantation poten-
tial of the invading embryo. Although the existing evidence is not always of high quality and there
is still place for further research in this field, it seems that surgery has a place in restoring normality
and improving the chances for increasing implantation rates and the achievement of pregnancy in
ART patients with RIF.

Based on the currently existing evidence and despite the lack of absolute agreement, the follow-
ing best clinical practice points could be recommended:

Myomas

- Submucosal fibroids negatively affect implantation and the achievement of pregnancy; their
  hysteroscopic excision improves the probability of successful pregnancy and clinicians should
  offer this option.
- Intramural fibroids not distorting endometrial cavity could negatively impair IVF outcome,
  especially if they are more than one and/or larger than 3–4 cm in diameter; clinicians should
  consider myomectomy, especially for patients with implantation failures; laparoscopic exci-
  sion, if feasible, appears to be the preferred surgical approach.
- Subserosal fibroids do not affect fertility; thus, myomectomy does not offer any beneficial effect
  and should be performed only as a treatment for other clinical manifestations arising from
  fibroid size and location, or in the case of simultaneous surgical excision of another fibroid.

Adenomyosis

- There is still no general consensus on how significant the impact is of adenomyosis on a wom-
  an’s implantation potential, if there is any difference between its different varieties and forms,
  which is the best treatment approach, and especially which patient is the best candidate for
  which treatment and what are the consequences to a future pregnancy.
- Infertile patients with adenomyosis could be stimulated for IVF with the long or extra-long
  GnRHa protocol by having a frozen embryo transfer after downregulation for three months
  before hormonal preparation of the endometrium.
- Adenomyomectomy could be offered as an alternative after implantation failures, mainly for
  focal disease, after careful estimation of the risks and benefits in experienced centers. GnRHa
  treatment after surgery seems to have an added value both for spontaneous conception and
  IVF outcome.

Polyps

- Hysteroscopic polypectomy could be offered as an option prior to ART based on the clinical
  benefits on fertility, the insignificant risk of complications, and the cost-effectiveness of this
  option.
In women diagnosed with endometrial polyps incidentally during ovarian stimulation, embryo cryopreservation and frozen embryo transfer after polypectomy could be recommended.

**Intrauterine adhesions**

- Hysteroscopy and hysteroscopic adhesiolysis, current “gold standards” for diagnosis and treatment of IUA, should be offered to women undergoing IVF presenting with the clinical features and, even more significantly, to patients with RIF.
- After surgery, intrauterine placement of mechanical and semiliquid barriers might prevent reformation of adhesions while estrogen therapy might enhance the regeneration of endometrium.

**Chronic endometritis**

- In patients with histologically confirmed CE after hysteroscopic-guided biopsy, targeted therapy, based on antibiogram when the etiology is known, should be given.
- In patients with hysteroscopic findings of CE with negative or no culture, a blind therapy with doxycycline or ofloxacin and metronidazole should be administered.
- Treatment should be completed by reevaluation of uterine cavity using hysteroscopy and endometrial sampling to confirm recovery; antibiotic therapy should be repeated in the case of persisting endometritis.

Nevertheless, patient counseling on RIF should be personalized, taking also into consideration other parameters, such as age, coexisting pathology, and, more importantly, the couple’s wishes.

**REFERENCES**


WHAT IS THROMBOSIS AND THROMBOPHILIA?
Thrombosis describes the clotting of blood inside the vessels. Thrombophilia is a condition where the blood has an elevated tendency to form clots (hypercoagulability). The origin of thrombosis was described in 1856 by the Virchow triad, which includes endothelial injury, hypercoagulability, and stasis. Clots usually appear in the venous vessels (e.g., deep vein thrombosis), while their circulation in the blood system can lead to pulmonary embolism. Arterial thrombosis can cause myocardial infarction, apoplectic stroke or in the case of the antiphospholipid syndrome, habitual abortion. Thrombophilia can either be acquired or inherited (Table 8.1). The cause of thrombophilia determines the risk of thrombosis.

During a women’s reproductive years, lifetime venous thromboembolism (VTE) is a major cause for morbidity and mortality. Its mean incidence ranges from 2/10,000 during the mid-teens to 1/1000 at 50 years of age. Estrogens enhance the risk for VTE, which explains the four-fold increase in relative risk with the use of oral contraceptives (7/10,000) and the increased incidence of VTEs in pregnancy (20/10,000). About 30% of patients with an event of VTE will develop recurrence in the next 10 years.

WHAT IS RECURRENT IMPLANTATION FAILURE?
Recurrent implantation failure (RIF) describes the clinical phenomenon of repeated failure of implantation after transfer of an in vitro generated embryo. Failure of implantation is usually defined as non-elevation of serum beta human chorionic gonadotrophin (beta-hCG) increase about 10–14 days following embryo transfer.

In the literature there is no unanimity regarding the number of failed embryo transfer cycles necessary for the definition of RIF. Tan et al. in 2005 reported considerable variations in the definition of RIF and reported a most common definition of ≥3 failed IVF cycles (range 2–6 cycles). Rinehard in 2007 attempted to find a concordant definition and concluded that each fertility center should establish their own definition of RIF based on their own data and postulated a definition of RIF for his own program by no elevation of beta-hCG within ≥8 transfers of 8 cell stage embryos or ≥5 transfers of blastocysts.

One problem in the definition of RIF is the lack of a “normal” implantation rate since patients undergoing IVF represent already a preselected infertile collective. The study of Paulson et al. reported stable implantation rates for up to four cycles for recipients in an oocyte donation program in which embryos originating from oocytes of fertile women are usually transferred.

IS THROMBOPHILIA CAUSING RIF?
Implantation of an embryo is believed to depend on sufficient microvascular blood supply. Therefore, microvascular clotting of vessels of the decidua during implantation due to hypercoagulability has been discussed to be the cause of RIF in patients with thrombophilia. Moreover, the existence
Is prophylactic anticoagulation using heparins and aspirin effective in the treatment of RIF?

Embryo implantation is a dynamic and complex process and depends on the interaction of multiple signals. Still, many signal cascades are not fully understood. Low-molecular-weight heparin (LMWH) is thought to improve conditions of implantation via prevention of clot formation during decidualization and placentation. Additionally, it was shown that LMWH affects expression of antiphospholipid antibodies is thought to be associated with thrombosis, habitual abortion, and pregnancy complications. The exact mechanisms, however, remain unclear.

A cause-effect relationship is usually proved by an experiment, either in vivo (randomized trial) or in an in vitro setting. Since it is not technically and ethically feasible to induce thrombophilia experimentally in women undergoing IVF, only observational data can be explored for associations, from which cause-effect inferences may (or may not) be drawn.

Several case-control studies investigated whether patients suffering from RIF have a higher prevalence of thrombophilia or antiphospholipid antibodies (Table 8.2).

In a meta-analysis and systematic review of Di Nisio et al., a higher prevalence for factor V Leiden (FVL) mutations (especially for homozygote women, suggesting a “dose effect”) in patients failing to achieve pregnancy in an IVF program was reported (odds ratio 3.08). This finding is shown homogeneously by n = 8 studies with a combined sample size of n = 1404 patients (I² = 0%; p < 0.0001). For the other investigated inherited thrombophilic factors (prothrombin mutation, MTHFR, protein C, protein S, antithrombin), Di Nisio et al. report no higher prevalence among the eight included studies.

Furthermore, a higher prevalence of antiphospholipid antibodies for patients with ART failure was also reported by Di Nisio et al. (odds ratio 3.33). However, the 20 included studies combining n = 3542 patients were significantly heterogeneous (I² = 75%), and accordingly, this finding should be taken with caution.

In summary, increased prevalence for thrombophilic factors has been observed in patients undergoing IVF treatment and recurrently failing to achieve pregnancy. However, numerous factors are associated with failure in an IVF program, and ideally, a multivariate analysis that also takes into account confounders such as age, body weight, duration of infertility, type of infertility, etc. should be performed on large data sets. A true population-based study considering fertile, subfertile, and treatment-resistant patients would enhance understanding of the prevalences of thrombophilic factors.

### Table 8.1 Thrombogenic risk factors and the associated increase in relative risk for thrombosis.

<table>
<thead>
<tr>
<th>Acquired Risk Factor</th>
<th>Increase in relative risk</th>
<th>Inherited Risk Factor</th>
<th>Increase in relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immobilization</td>
<td>~4–10-fold</td>
<td>Factor V mutation</td>
<td>~7-fold (heterozygous)</td>
</tr>
<tr>
<td>Smoking</td>
<td>~1.17-fold</td>
<td>Prothrombin mutation</td>
<td>~3-fold (heterozygous)</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>~4-fold</td>
<td>Protein S deficiency</td>
<td>2–20-fold</td>
</tr>
<tr>
<td>Obesity and metabolic syndrome</td>
<td>~2-fold in odds ratio</td>
<td>Protein C deficiency</td>
<td>10–20-fold</td>
</tr>
<tr>
<td>Antiphospholipid syndrome</td>
<td>~3-fold</td>
<td>Antithrombin III</td>
<td>10–100-fold</td>
</tr>
<tr>
<td>Cancer</td>
<td>~12-fold (depending on subtype)</td>
<td>Elevated factor VIII level (&gt;150%)</td>
<td>~4.8-fold</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Participants with RIF</th>
<th>Thrombophilic factor</th>
<th>Definition of RIF</th>
<th>Prevalence of thrombophilia in RIF group</th>
<th>Prevalence of thrombophilia in control group</th>
<th>P-value (prevalence thrombophilia in RIF vs. control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simur et al.</td>
<td>2008</td>
<td>n = 51</td>
<td>FVL, prothrombin, MTHFR</td>
<td>≥3 IVF failures</td>
<td>62.7%</td>
<td>53.9%</td>
<td>0.37</td>
</tr>
<tr>
<td>Azem et al.</td>
<td>2004</td>
<td>n = 45</td>
<td>Mutations of prothrombin, FVL, MTHFR, protein C, protein S antithrombin III deficiencies</td>
<td>≥4 IVF failures</td>
<td>26.7% (excluding homozygotic MTHFR)</td>
<td>9.1% (excluding homozygotic MTHFR)</td>
<td>0.003</td>
</tr>
<tr>
<td>Qublan et al.</td>
<td>2006</td>
<td>n = 90</td>
<td>FVL, MTHFR antiphospholipid syndrome</td>
<td>≥3 IVF failures</td>
<td>68.9%</td>
<td>25.6% and 25% (depending on subgroup)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Grandone et al.</td>
<td>2001</td>
<td>n = 18 (n = 8 with fetal loss)</td>
<td>FVL, prothrombin mutation, MTHFR, protein C and S, antithrombin</td>
<td>≥3 IVF failures</td>
<td>27.7%</td>
<td>0% and 6.0% (depending on subgroup)</td>
<td>not available</td>
</tr>
<tr>
<td>Bellver et al.</td>
<td>2008</td>
<td>n = 26</td>
<td>FVL, prothrombin mutation, MTHFR, protein C, protein S, antithrombin, APCR</td>
<td>≥2 good-quality embryo transfers</td>
<td>19.2%</td>
<td>9.4%</td>
<td>&quot;no significant difference&quot;</td>
</tr>
<tr>
<td>Vaquero et al.</td>
<td>2006</td>
<td>n = 59</td>
<td>FVL, prothrombin mutation, MTHFR, protein C, protein S, antithrombin, APCR</td>
<td>≥2 IVF failures</td>
<td>25%</td>
<td>20%</td>
<td>p = 0.31</td>
</tr>
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</table>

*Abbreviations:* APCR = activated protein C resistance; MTHFR = methylene tetrahydrofolate reductase; FVL = factor V Leiden; IVF = *in vitro* fertilization.
Conclusion

Thrombophilia has not been shown to cause RIF in patients undergoing IVF treatment, though observational case-control studies have reported a higher prevalence of thrombophilic factors in RIF patients compared with fertile patients. Routine screening of IVF patients for thrombophilia is currently not warranted. The use of LMWH to improve live birth rates in RIF patients has not been firmly established; however, risk of thrombosis during ovarian stimulation and pregnancy may necessitate the use of anticoagulants in patients at risk for thrombosis (e.g., patients with homozygous FVL mutation).

Figure 8.1 The systematic review of Akhtar et al.\textsuperscript{22} shows a forest plot of interventional studies on LMWH (low-molecular-weight heparin) treatment in ART. (From Akhtar MA et al. Cochrane Database Syst Rev. 2013 August 17;(8):CD009452, with permission.)

and activities of proteins as well, suggesting a broad influence on physiology.\textsuperscript{2} Therefore, LMWH might modulate decidualization in a way that enhances endometrial implantation competence rather than mere prevention of blood clotting. LMWH does not cross the placenta and is considered safe in pregnancy.\textsuperscript{24} The use of LMWH to increase live birth rates in ART patients was investigated by a meta-analysis from Akhtar et al.\textsuperscript{22} which included 386 patients from three randomized controlled trials. Control groups of the included studies received placebo or no treatment.

Pooling of results of all three studies shows evidence for a significant increase in live birth rates (odds ratio 1.77) for the LMWH-treated group (Figure 8.1). Quality of evidence, however, was rated very low because of high heterogeneity and sensitivity of the choice of the statistical approach used. Furthermore, the combined sample size was rather small. It should be concluded that LMWH treatment in the context of ART is not justified outside clinical studies.

Additionally, acetylsalicylic acid (aspirin) is thought to improve endometrial microcirculation by inhibition of platelet aggregation via thromboxan.\textsuperscript{25} It has been used to increase the chance of implantation after embryo transfer, reduce the risk of abortion and decrease the rate of preterm preeclampsia.\textsuperscript{26} It may counteract hypercoagulability by protection of the trophoblast after placenta tion has been established.\textsuperscript{27,28}

For patients with a history of RIF, interventional studies have investigated whether prophylactic anticoagulation using LMWH (in combination with aspirin) is effective to increase implantation and live birth rates (Table 8.3).

Moreover, a retrospective analysis of patients with RIF and thrombophilia who were treated with LMWH showed no difference in pregnancy rates for patients with LMWH versus no LMWH.\textsuperscript{30} For patients with history of RIF without thrombophilia two studies found a marginal nonstatistical significant increase in pregnancy rates for patients receiving LMWH and suggested further exploration in larger sample sizes,\textsuperscript{31,32} whereas one study failed to show any advantage for patients treated with LMWH.\textsuperscript{33}

Furthermore, since in the study of Paulson et al.,\textsuperscript{34} patients in oocyte donation programs with a previous history of RIF (n = 70 patients with n = 114 cycles in the oocyte donation program) achieved similar pregnancy and live birth rates as other patients, oocyte quality seems to be the most essential factor for the likelihood of embryo implantation rather than the use of LMWH.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Included patients</th>
<th>Design</th>
<th>Outcome</th>
<th>Intervention</th>
<th>Results</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qublan et al.²⁹</td>
<td>2008</td>
<td>n = 83</td>
<td>Prospective,</td>
<td>Implantation,</td>
<td>Enoxaparin 40 mg/day</td>
<td>Implantation rate: 20.9% (LMWH) vs. 6.1% (placebo); pregnancy rate: 31% (LMWH) vs. 9.6% (placebo); live birth rate: 23.8% (LMWH) vs. 2.8% (placebo)</td>
<td>Implantation rate: p &lt; 0.001; pregnancy rate: p &lt; 0.05; live birth rate: p &lt; 0.05</td>
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<td></td>
<td></td>
<td></td>
<td>randomized trial</td>
<td>pregnancy, and live birth rates</td>
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<td>Stern et al.²⁸</td>
<td>2003</td>
<td>n = 143</td>
<td>Randomized crossover</td>
<td>Implantation rate</td>
<td>Unfractionated heparin (5000 IU b.i.d.) and aspirin (100 mg daily)</td>
<td>Implantation rate: 6.8% (LMWH + ASS) vs. 8.5% (placebo); live birth rate: 6% (LMWH + ASS) vs. 7% (placebo)</td>
<td>Relative pregnancy rate = 0.65 (95% CI 0.33–1.29); live birth rate: 0.60 (95% CI 0.27–1.35)</td>
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**Abbreviations:** RIF = recurrent implantation failure; ANA = antinuclear antibody; APA = antiphospholipid antibodies; ASS = aspirin; LMWH = low-molecular-weight heparin; vs. = versus.
REFERENCES


Andrological causes of recurrent implantation failure

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INTRODUCTION

Recurrent implantation failure (RIF) is a term used in in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures; however, a uniform definition is still lacking. Based on a systematic review assessing the definitions of RIF used in literature, RIF is the failure of attachment of the embryo to the lining of the womb after the transfer of a cumulative number of at least four good-quality embryos, in a minimum of three consecutive fresh or frozen cycles, in a woman under the age of 40 years. RIF is the consequence of embryo- or uterine-related factors and should be distinguished from IVF failure, which also includes poor response to ovarian stimulation and absence of good-quality embryos. It has been difficult to determine the exact prevalence of RIF, because of the various definitions used to describe the condition as well as the different outcomes employed to assess implantation success (e.g., biochemical vs. clinical pregnancy). The probability of one embryo failing to implant is 70%; thus, according to the aforementioned definition, following the transfer of four embryos, the probabilities of all embryos failing to implant is $0.70^4 = 0.24$. Successful implantation requires trophoblastic growth, invasion into the endometrium, and stimulation of vascularization to provide its own blood supply. Multiple causes of implantation failure have been proposed, concerning principally the female partner (poor oocyte quality, thrombophilia, autoimmune disorders, and uterine anomalies). Male factor infertility has also been found to be associated with high order RIF; however, the pathophysiological basis of this association is still poorly understood. It has been suggested that sperm of poor quality leads to the formation of morphologically high-quality embryos, which may harbor inherent defects and fail to implant.

Investigations concerning the male partner remain limited and often restricted to sperm analysis. However, it is widely accepted that conventional semen analysis parameters do not accurately reflect sperm quality. Consequently, several sperm function tests, such as sperm penetration assay, sperm-zona pellucida binding test (hemizona assay), acrosomal reaction test, hyaluronan binding test, hypo-osmotic swelling test, and magnetic-activated cell sorting, have been employed in order to further assess and refine sperm quality, with questionable, however, clinical utility. In an attempt to improve the predictors of successful implantation, spermatozoa are also assessed for chromosome aneuploidy using fluorescence in situ hybridization (FISH) or, alternatively, for the degree of single- and double-stranded DNA breaks, known as DNA fragmentation (DF) analysis.

The aim of this review is to discuss and critically appraise established and potential andrological causes of RIF as well as the current management strategies.

AVAILABLE EVIDENCE ON SPERM QUALITY

Sperm analysis parameters

The routine semen analysis remains a hallmark in the evaluation of the infertile male. Apart from evaluating sperm concentration, motility, and morphology, indicators of patency and function
of the male genital tract are also assessed, including semen volume, liquefaction time, and pH. Moreover, biochemical markers reflecting the function of different segments of the genital tract, such as fructose, zinc, and α-glycosidase, can be measured in the seminal plasma.

There is published evidence that poor semen parameters, morphology in particular, result in low blastocyst formation rates after IVF, suggesting that sperm can influence human preimplantation embryo development. Loutradi et al. observed a negative relationship between semen quality and embryo development, even before the activation of the embryonic genome, suggesting that sperm can affect embryogenesis from a very early stage. Moreover, embryo development, pregnancy, and implantation rates have been found to be affected by the source of sperm (ejaculated, epididymal, or testicular) and the type of male factor infertility.

Many studies have also indicated that the direct quantitative assessment of sperm movement by computer-aided sperm analysis (CASA) reflects the fertilizing ability of human spermatozoa in vitro, under conditions where the conventional semen profile is of limited diagnostic value. In a retrospective analysis of the relationship between the CASA estimates and fertilization rates in IVF cycles, two parameters—namely curvilinear velocity and rapid sperm movement—provided reliable estimations of the fertilizing ability of human sperm. When cryopreserved donor semen is used, numerous sperm parameters have also been suggested as possible predicting factors of clinical pregnancy rate, such as prefreezing sperm motility, post-thaw sperm motility, forward progression, inseminating motile count, and total motile sperm counts.

Abnormal sperm morphology has been described as a useful determinant of male fertility, in vivo and in vitro, and has been related to increased levels of chromosomal abnormalities and a high degree of DF. However, such defects cannot be predicted by the morphological parameters of the embryos prior to transfer. Moreover, as suggested by some studies, this is not the case in couples undergoing ICSI, where teratozoospermia is not that closely related to the potential of embryonic development and cycle outcomes.

**Genetic factors**

**Sperm chromosomes and DNA fragmentation**

It is widely admitted that, in many cases, routine semen analysis only provides clues that may indicate the need to perform additional evaluation. Patients with a clinical background of RIF are at risk of harboring sperm chromosomal abnormalities (numerical or/and structural), the incidence of which are higher in oligoasthenoteratozoospermic patients. In particular, sperm aneuploidy and diploidy rates were assessed in male partners of ICSI couples with normal karyotypes (using dual- and triple-color FISH techniques for chromosomes 13, 18, 21, X, and Y) and an increased incidence of sex chromosome disomies was observed in couples with RIF. Male chromosomal structural aberrations, such as translocation, inversion, and pericentric inversion, are also of particular concern. The development of locus-specific and subtelomeric DNA probes has allowed the analysis of chromosome segregation in carriers of inversions (pericentric and paracentric), Robertsonian translocations, and reciprocal translocations. These studies confirmed that carriers of structural chromosomal reorganizations produce chromosomally unbalanced sperm. If these gametes fertilize an oocyte, the resulting embryos, depending on the chromosome regions implicated, can give rise to abortions or offspring affected by chromosomal abnormalities. In addition, centrosome anomalies resulting in chaotic mosaics were most likely of paternal origin. It has been also proposed that balanced parental translocations may be implicated in the pathogenesis of implantation failure in IVF, and that genetic evaluation should be considered as part of the investigation of these couples. On the other hand, another large study by Rubio et al. analyzed the results of 131 ICSI cycles in couples with repeated abortion or implantation failure, in which the increased frequency of aneuploidy in spermatozoa did not affect the success of assisted reproductive techniques (ART).
Multiple studies have reported the negative effect of high sperm DF on reproductive outcomes in ART. Sedó et al. found that sperm DF significantly affected embryo blastulation and implantation in ICSI patients who received donated eggs and showed that sperm DF might compromise the progression of embryo development, resulting in arrested embryos. An updated systematic review and meta-analysis concluded that there is sufficient evidence in the existing literature suggesting that sperm DNA damage has a negative effect on clinical pregnancy following IVF and/or ICSI treatment. However, current literature does not support the hypothesis that sperm DF is an important cause of RIF as well, or that sperm DNA integrity testing has value in such patients.

Y chromosome microdeletions

Microdeletions of the various azoospermia factor (AZF) regions on the long arm of the Y chromosome are another major genetic factor involved in male infertility. Deletions of the AZFa and AZFb regions are typically associated with azoospermia and absence of sperm during testicular sperm extraction (TESE) procedures. On the other hand, deletions of the AZFc region result in a less-severe defect of spermatogenesis, allowing successful TESE or even the presence of sperm in the ejaculate. It is reported that the clinical outcomes of ICSI for oligozoospermic/TESE patients with AZFc microdeletions are comparable to those of infertile patients with normal Y chromosomes, in contrast to previous studies that associated AZFc microdeletions with lower fertilization rate and poorer embryo quality. Thus, the correlation between the incidence of AZF microdeletions and ART results remains controversial and needs to be verified in a large cohort of infertile men.

Immunological maladaptation

Immunological maladaptation is a putative cause of implantation failure in humans. As maternal lymphocytes recognize fetal antigens, tolerance is necessary to prevent a graft versus host type reaction and consequently abortion. Regulatory T (T<sub>reg</sub>) cells are recruited to abate the stimulation of maternal lymphocytes by fetal antigens. Villous trophoblast cells express several minor histocompatibility and paternal antigens (H-Y). Seminal plasma is of relevance as it can induce paternal antigen-specific T<sub>reg</sub> cells in the female genital tract. Elimination of T<sub>reg</sub> cells during implantation or early pregnancy has been associated with implantation failure or fetal resorption in mice.

Sperm epigenetics

Oxidative stress

Accumulating evidence suggests that oxidative stress plays an important role in the pathophysiology of male factor infertility. A surrogate marker of assessing the magnitude of oxidative stress in the seminal plasma is the level of reactive oxygen species (ROS). The main sources of ROS in semen are leukocytes and morphologically abnormal sperm, although ROS can also be produced by precursor germ cells. Under physiologic conditions, sperm requires a small amount of ROS to regulate certain functions, such as sperm capacitation, acrosome reaction, and sperm-oocyte fusion. However, the excessive production of ROS might have deleterious effects on sperm structure and functionality. Sperm is particularly susceptible to ROS-induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids, whereas their cytoplasm contains low concentrations of scavenging enzymes; thus they have a limited capacity to repair their own DNA. A balance between the generation of ROS and the presence of antioxidants in the seminal plasma is required to avoid excessive oxidative stress. Moreover, ROS seem to have an effect on the sperm proteome, and differences in the levels of sperm protein expression under oxidative stress have been observed. These differences have been attributed to direct effects
of oxidative stress on gene transcription, as well as post-translational oxidation of amino acid residues that lead to the formation of protein-protein crosslinked aggregates.\textsuperscript{30}

**Proteomics**

Proteomic analyses have identified several sperm-specific proteins involved in pre- and postfertilization events, but their exact role in regulating embryonic development is still not elucidated. Proteomic alterations occurring during spermiogenesis have been proposed to alter sperm epigenetic signatures, ultimately resulting in abnormal embryo development.\textsuperscript{31} These proteomic alterations seem to be subtle, not affecting major features of sperm quality, but putatively influencing sperm genetic chromatin distribution. Specific proteomic studies aimed to investigate the correlation between sperm protein levels and ART results are scarce.

Thus far, only histones and protamines have been validated to be a part of nongenomic paternal contribution to the embryo. The replacement of most histones by protamines 1 and 2 during spermiogenesis facilitates a high order of chromatin packaging necessary for normal sperm function.\textsuperscript{32} A significant negative correlation between sperm motility and protamine-2 mRNA levels has been demonstrated.\textsuperscript{33} It has also been suggested that protamines are not only important for fertilization but may additionally have an impact on the correct initiation of gene expression in the early embryo.\textsuperscript{34} Moreover, incorrect histone to protamine exchange results in a prolonged presence of histones, which are known to exhibit a variety of epigenetic modifications that are transmitted through fertilization.\textsuperscript{35}

The cysteine-rich secretory proteins (CRISPs) are mainly expressed in the male reproductive tract and have been implicated in many aspects of male germ-cell biology, involving haploid germ-cell development, epididymal maturation, capacitation, motility, and the actual processes of fertilization.\textsuperscript{36} Several lines of evidence suggest a role for CRISPs in the interaction between the sperm and the oocyte at fertilization.\textsuperscript{37} However, the exact role of each CRISP protein is still under investigation and their role in RIF remains to be elucidated.

**FACTORS THAT AFFECT SPERM QUALITY**

**Paternal age**

Advanced paternal age has been associated with a decrease in sperm quality (particularly motility), increase in birth defects, epigenetic modifications and DNA mutations, along with chromosomal aneuploidies.\textsuperscript{38} Eventually, the incidence of autosomal dominant diseases, such as achondroplasia, polyposis coli, and Marfan syndrome, is reported to be increased among the offspring of older men.\textsuperscript{39} However, the evidence associating paternal age with IVF failure and RIF is equivocal.

According to several studies, advanced paternal age is believed to impact reproductive and fertility outcomes, including a decrease in ART success rate and an increase in the rate of preterm birth. In particular, age of the male partner $\geq 35$ years has been associated with decreased clinical pregnancy rates following artificial intrauterine insemination (IUI), even in the presence of normal semen analysis and favorable characteristics of the female partner.\textsuperscript{40} Similarly, a significant decline in IUI pregnancy rates per cycle in men aged $\geq 45$ years have been reported compared to men $<30$ years (9.3% vs. 12.3%).\textsuperscript{41} In a prospective study, it was reported that with increasing paternal age, the live birth rate of IVF or gamete intrafallopian transfer (GIFT) decreased.\textsuperscript{42} Similarly, assessment of the outcomes of 859 conventional IVF and 1632 ICSI cycles demonstrated that from the age of 51 years, paternal age negatively affects the rates of blastocyst formation (IVF) and clinical pregnancy (ICSI).\textsuperscript{43} Moreover, a study comparing 1495 women who reported spontaneous abortion in pregnancy with 12,633 women reporting live births in their previous pregnancy found that increasing paternal age is significantly associated with spontaneous abortion, independent of maternal age and multiple other factors such as maternal diabetes, maternal smoking, social class, and education.\textsuperscript{44}
Factors that affect sperm quality

On the other hand, there are studies that do not associate advanced paternal age (up to 64 years) with sperm characteristics or its ability to fertilize human eggs. Moreover, it has been reported that if a couple is able to produce and transfer a single thawed euploid embryo, no difference in IVF pregnancy outcomes is identified with increasing paternal age. To add further contradiction, there are studies claiming that embryo morphology during cleavage is not affected by male age and that male age is irrelevant for the outcome of ART procedures.

Lifestyle factors

Environmental threats, including lifestyle-related factors, may impair male reproductive health and have been linked to the controversial issue of deterioration of semen quality over the last century.

Smoking

Approximately 37% of men of reproductive age smoke cigarettes. Tobacco smoking may affect sperm development and function, with a negative effect on semen parameters. It has been reported that exposure to cigarette smoking was associated with reduced sperm count, motility, and morphology. Subgroup analyses indicated that the effect size was higher in infertile men than in the general population and in moderate/heavy smokers than in mild smokers. Moreover, preconception paternal smoking has been correlated with increased incidence of several types of cancer and birth defects in offspring suggesting that the smoke-induced genetic or epigenetic changes that arise in spermatzoa are transmitted to offspring.

Smoking has also been demonstrated to impact sperm DNA methylation patterns in a consistent manner. Even if altered DNA methylation is not directly passed on to offspring, these alterations might influence early embryonic gene expression or adjust reprogramming or early development in other ways. Experiments in rodents show that exposure of adult male mice to sidestream tobacco smoke significantly increases sperm DNA mutations and aberrations in sperm chromatin structure. In addition, smoking also alters sperm microRNA content, greatly increases the abundance of ROS in the seminal plasma, and causes oxidative DNA damage in sperm. Smokers are at increased risk of sperm defects, such as partially or fully inactive mitochondria and non-intact acrosomes, in addition to increases in sperm DF and changes to sperm proteome.

Alcohol

Ethanol can suppress reproductive function and sexual behavior in humans. Some studies suggest that alcohol exerts direct toxicity to the testes and causes fertility disturbances by reducing sperm count and motility, although others did not confirm these findings. Chronic ingestion of ethanol may cause defects in spermatogenesis and sperm motility, aberrant testicular and accessory gland morphology, reduced cauda epididymis sperm content, and impaired epididymal sperm maturation. Occasional alcohol intake does not seem to impair semen quality, whereas both seminal volume and sperm morphology are negatively affected by daily consumption.

Observations propose a potential association between preconception male alcohol exposure and altered epigenetic programming in sperm. Animal models of paternal alcohol exposure show alterations in the control of key enzymes regulating chromatin structure, as well as changes in the DNA methylation profiles of alcohol-exposed sperm, which have well-characterized impacts on both fetal and placental growth.

Genital heat stress

Epidemiological studies assessing occupational exposure to high temperatures underline the role of genital heat stress as a cause of impaired semen quality and of reversible infertility in men. Several sperm traits, such as sperm head features (morphometry and chromatin integrity), which likely play specific roles in IVF and embryonic development, are reported to be altered as a result of a thermal insult. Other investigators have demonstrated that scrotal heat stress can compromise
the DNA integrity of sperm and this may have clinical implications for patients undergoing IVF and ICSI. Daily habits such as sedentary lifestyle, insulating genital clothing, bicycling, and use of a sauna are believed to increase scrotal temperature and may exert detrimental effects on sperm quality; however, scrotal cooling has not been proved to improve semen quality.

Recreational drug use

Recreational drugs, including marijuana, cocaine, methamphetamines, and opioid narcotics, have been shown to negatively impact male fertility. A study in Denmark showed adverse associations between frequent marijuana use (more than once weekly) and semen quality among healthy young men, the association being even more pronounced among men abusing other recreational drugs as well. Studies on animal models have demonstrated that chronic cocaine administration in male rats results in seminiferous tubule degeneration, reduced pregnancy rates, and a decrease in mature spermatogenic forms.

Obesity

Recent data suggest that reproductive health may also be impacted by obesity. While it is generally accepted that female body mass index (BMI) impacts fecundity, the relationship of male infertility with BMI is less clear. Some studies have suggested that elevated male BMI can lead to impaired sperm production, whereas others have found no relationship between male BMI and semen parameters. Furthermore, adiposity may be better related to sperm production, when assessed not only by BMI but by assessing waist circumference as well. Overweight and obese men have been shown to have a higher incidence of low ejaculate volume, sperm concentration, and total sperm count.

There has been growing concern over whether the BMI of male partners is associated with ART outcomes. Some researchers suggest that increased BMI may cause decreases in embryo quality and pregnancy rates, while reports indicate that hyperinsulinemic men may exhibit a higher percentage of poorly compacted DNA in their sperm and less success in IVF. In other studies, IVF is distinguished from ICSI, as the authors demonstrated that overweight status of the male partner may cause impaired sperm-egg interaction after IVF, but not after ICSI. Similarly, in a Chinese study, increased BMI of the male partner was associated with decreased fertility rate after IVF cycles. However, male BMI was not shown to be significantly associated with late-stage embryonic development, pregnancy rates, or miscarriage rates, suggesting that specific sperm impairments can be repaired by postfertilization events in the oocyte.

Environmental factors

Hormone disruptors

Hormone disruptors, such as anabolic steroids, polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons, phthalates, bisphenol A, pesticides, and alkylphenols have been studied for their impact on male fertility in several species of animals and in humans. Growing evidence suggests that xenoestrogen bisphenol A (BPA) (a widespread environmental contaminant, employed in the production of certain plastics and epoxy resins) can bind to receptors on sperm and, thus, alter sperm function. It has been reported that high concentrations of BPA alter sperm function, fertilization, and embryonic development via regulation and/or phosphorylation of fertility-related proteins in sperm collected from experimental mice.

Phthalates are mainly used as plasticizers, substances added to plastics to increase their flexibility, transparency, and longevity. They are used in many consumer products, such as building materials, toys, food packaging, cosmetics, and medical devices. A review of studies on laboratory animals confirmed that phthalates cause diminished sperm count, increase the frequency of abnormal sperm, and damage DNA in germ cells, especially after chronic exposure and in cases of exposure of immature animals (both during gestation and post-term). Phthalates may also induce
mutations in male gametes, leading to increased pre- and postnatal mortality of the offspring. Interestingly, phthalates have been associated with diminished quality of semen in the F1 generation, while in males, but not in females, urinary concentrations of selected metabolites of phthalates and phthalate alternatives are associated with poor blastocyst quality.

Acrylamide and glycidamide (a reactive epoxide metabolite from acrylamide) are industrial chemicals that are used in several ways, such as the production of polyacrylamides for wastewater treatment, textiles, paper processing, and cosmetics. Acrylamide is also a product formed in certain foods prepared at high-temperature frying, baking, or roasting, such as fried potatoes, bakery products, and coffee, and has been associated with a decrease in sperm count, motility, and morphology. Acrylamide has been shown to induce disruption or breakage of chromosomes, whereas glycidamide has mutagenic effects. There no studies on the effects of these compounds on RIF but low-dose chronic exposure is proposed to cause mutations without affecting the fertilization capacity of sperm or leading to deaths in the offspring, therefore allowing these mutations to be inherited.

Heavy metals
It has been hypothesized that exposure to heavy metals may compromise male reproduction, as demonstrated by epidemiological and animal studies. Cadmium, mercury, lead, and arsenic levels may affect semen quality. When toxic metals were measured in seminal plasma collected from 30 men of IVF couples to evaluate their impact on semen quality and IVF outcomes, a negative association was suggested between mercury-adjusted cadmium levels and pregnancy rates. It has been also reported that increased seminal plasma lead levels adversely affect the fertility potential of sperm in IVF. However, no specific relevant implications with RIF have been reported yet.

Radiation
Some epidemiological studies suggest a possible link between the use of mobile phones and decreased semen quality parameters, but the results are not consistent. The common practice of storing mobile phones in close proximity to the testes can significantly impair motility, vitality, and the integrity of sperm DNA by the induction of ROS generation through the exposing of the reproductive system to relatively high levels of radiofrequency electromagnetic radiation (RF-EMR). A mechanistic model has been proposed according to which RF-EMR exposure leads to defective mitochondrial function, which in turn is associated with increased ROS production.

Regarding ionizing radiation, available data originate by experiments conducted on animals. When mature male mice were exposed to 2 or 4 Gy of $^{137}$Cs $\gamma$-rays, and their sperm were used for IVF, structural chromosomal aberrations, aneuploidy, and mosaicism were documented in early cleavage embryos. In the same study, it is proposed that it is reasonable to consider a heritable risk of mosaicism rather than aneuploidy in embryos derived from sperm after irradiation. An increased incidence of aneuploid blastomeres (31.6%) was also reported in eight-cell mouse embryos derived from the sperm of males exposed to 4 Gy $\gamma$-rays. No direct data exist on RIF.

Genital tract infections
Chronic inflammatory conditions of the genital tract, such as male accessory gland infections (MAGIs), are frequently encountered in male fertility problems. With regard to their impact on male reproductive function, epididymitis seems to be more relevant than inflammation/infection of the prostate and/or seminal vesicles. Chronic epididymitis may result in reduced sperm count and motility. Besides changes in the conventional sperm parameters, alterations in DNA integrity have also been observed. A reduction in natural and assisted cumulative pregnancy rate and an increase in miscarriage rate are related to the presence of human papillomavirus (HPV) at the sperm level. The exact mechanisms by which MAGI are able to impair implantation rates remain unclear, with increased oxidative stress or a direct effect of microorganisms on sperm being the most plausible.
Sperm must be properly prepared for IVF programs in order to control the fertilization rate and ensure that embryos are of high quality and have appropriate developmental abilities. García-Herreros et al. concluded that different sperm washing-selection methods commonly employed during the IVF process may lead to alterations in sperm morphometric characteristics, which might explain some of the variability of implantation rates in IVF.

**SUGGESTED MANAGEMENT**

Regarding idiopathic male infertility, a recent meta-analysis suggested a significant improvement in sperm DF and an increase in ART pregnancy rates with the use of antioxidants. The data are less robust regarding spontaneous conceptions or in couples with a history of recurrent embryo loss. Multiple randomized trials have investigated antioxidant supplementation for treatment of male infertility, with several demonstrating a positive effect on semen quality, especially sperm motility. Omega-3 polyunsaturated fatty acid supplementation has been found to improve the semen profile, while coenzyme Q10 was found to effectively improve sperm kinetic features. However, the existing data are still debatable and large-scale studies are required focusing on the impact of antioxidants on sperm parameters and their relationship with early embryo development.

Surgical repair has been suggested for men with clinically apparent varicocele, impaired semen parameters and infertility, while varicocelectomy has been shown to effectively improve sperm DF. In cases of idiopathic male fertility resulting in ICSI cycle failure with ejaculated sperm, an ICSI cycle with surgically retrieved testicular sperm is an alternative choice that can have marked improvement in outcome. A possible explanation for this observation is that the testis–blood barrier offers more protection from oxidation insults than the epididymal environment.

Despite the detrimental effects that smoking and excessive alcohol consumption have been demonstrated to exert in male fertility, there are no randomized studies showing that semen parameters or chance of pregnancy improve if the male partner quits smoking or reduces alcohol consumption. Nevertheless, with respect to the general positive health effects that such a lifestyle modification may have, avoiding smoking and alcohol consumption at least prior to and during the ART treatment should be encouraged.

The same values should be taken into consideration when approaching the obese infertile male patient. There is only one nonrandomized study, indicating that weight reduction leads to improvement of semen parameters, which, however, included only severely obese patients (BMI > 35 kg/m²). Nevertheless, weight loss is expected to improve reproductive hormone balance, while those patients with metabolic syndrome and hyperinsulinemia may benefit from metformin treatment and the use of nutritional supplements with antioxidant properties. Increased fruit and vegetable consumption should also be encouraged, as it has been associated with better IVF outcomes. There is also evidence for a favorable effect of vitamin D supplementation on semen quality, testosterone concentrations, and fertility outcomes.

Last but not least, for couples who struggle with RIF, it is essential that the male is examined for either chromosomal aneuploidy or chromosomal structural abnormalities. While it is difficult to assess the precise risk associated with increased levels of sperm aneuploidy, such information is crucial in order to counsel the couples about the associated genetic risks and their reproductive and testing options. In all cases, genetic counseling should include a three-generation pedigree, looking specifically for a familial history of infertility, recurrent pregnancy loss, birth defects, intellectual disability, and apparent genetic disease. For men with reciprocal chromosomal translocations, preimplantation genetic diagnosis (PGD) is recommended, as this has increased the live birth rate from 4.9% to more than 80% in some studies. Nevertheless, considering the significant cost of the procedure, the option of using donor sperm or adopting might be more appealing for some couples.
REFERENCES


INTRODUCTION
Implantation is a complex process that requires a healthy embryo, a receptive endometrium and their successful interaction. Factors that affect any of these can contribute to recurrent implantation failure (RIF).

RIF is an ill-defined sign with a multitude of reasons behind it. RIF patients make up a heterogeneous group of infertile couples. This is a methodological problem since it is not usually possible to perform large-scale studies with well-defined groups. Moreover, embryonic aneuploidy, the leading cause of implantation failure, causes a low signal-to-noise ratio, rendering the detection of other etiologic factors difficult. Therefore, most studies on RIF are of low quality and often conflict with each other. One frequently has to rely on studies involving general assisted reproductive technology (ART) patients, not necessarily on RIF. Some theories relate to recurrent pregnancy loss rather than RIF per se. These are major problems while counseling a couple with RIF.

Having said these, the couple suffering from RIF is confused and at times desperate. They need effective treatment here and now, before high quality evidence becomes available for every controversy. Therefore, it is important to present as evidence-based an approach as possible for investigating the problem in depth, instead of taking random shots at an indefinite target. The road map should be clear and discussed with the couple thoroughly from the beginning.

We will discuss possible causes of RIF and their diagnostic evaluation in the following order: genetic, anatomic, hematological and immunological, endocrine factors, and endometrial receptivity. The chapter will conclude with a proposed algorithm for assessment of a couple suffering from RIF.

GENETIC FACTORS
Parental karyotypes
Embryonic aneuploidy is the leading cause of miscarriages and implantation failure.1 People with balanced reciprocal translocations or Robertsonian translocations are functionally and phenotypically normal since they have a normal quantity of genetic material. However, segregation during gametogenesis may result in unbalanced oocytes or sperm and eventually lead to an increased incidence of chromosomally abnormal embryos.

Chromosomal abnormalities (CA) are more common in men with severe male factor infertility. Mau-Holzmann reported the frequency of CA to be 13.1% and 4.3% in infertile men with azoospermia and oligozoospermia, respectively.2 Stern et al. compared karyotype results of 293 women and 221 men who did not achieve clinical pregnancy after transfer of ≥10 embryos with 500 infertile couples referred for in vitro fertilization (IVF).3 Excluding Turner syndrome and Klinefelter syndrome, 13 of 514 (2.5%) RIF patients had CA, whereas 13 of 1000 (1.3%) controls had an abnormal karyotype. Translocations were significantly more prevalent in the RIF group compared with controls, both in females (3/293 vs. 1/500; p < 0.005) and males (4/221 vs. 2/500; p < 0.005). Raziel et al.
reported a strikingly high rate of CA of 15.4% in 65 couples with high-order implantation failure, defined as not achieving a clinical pregnancy after transfer of ≥15 embryos in ≥6 ART cycles. De Sutter et al. performed chromosomal analysis on 317 women and 298 men who had a history of ≥3 consecutive ART failures. A total of 13 (2.1%) patients were diagnosed with CA, eight females (2.5%) and five males (1.7%). The prevalence of CA was increased in the female RIF population compared with female newborns (0.78%) and normo-ovulatory women starting ART (0.58%). Male patients with RIF had a similar prevalence of CA when compared with male newborns. It is important to note that patients with severe male factor infertility were excluded from the study, so the prevalence of CA in male RIF patients is likely underestimated in this study.

Apparently, CA are more common in couples with a history of RIF, and they should be offered karyotype testing. While only 2%–3% of the patients are likely to have abnormal results and no curative therapy is possible, this information can be helpful when counseling the couple about their prospects and offering preimplantation genetic diagnosis or gamete/embryo donation to those who may benefit from these options.

**Sperm DNA integrity**

Lately, sperm DNA integrity tests have drawn attention as a parameter of male reproductive potential. Various methods to test DNA integrity are available, such as the sperm chromatin structure assay (SCSA), the deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, the single-cell gel electrophoresis assay (comet assay), and the sperm chromatin dispersion (SCD) test. These methods do not test for specific DNA sequences. Instead, they give an estimate of the general condition of DNA.

The latest meta-analysis on the relationship between sperm DNA integrity and ART outcomes included six studies and 998 patients. Overall, low DNA damage was associated with higher live birth rates (RR 1.17, 95% CI 1.07–1.28; p = 0.0005). However, it is noteworthy that DNA damage threshold values used in the included studies varied between 10% and 50%, and the tests used, as well as the patient populations, were heterogeneous. Moreover, when only the studies that accounted for female factors were analyzed (n = 202), live birth rates were similar in men with high and low sperm DNA fragmentation using ICSI (RR 1.08, 95% CI 0.39–2.96).

Bronet et al. prospectively analyzed embryo aneuploidy rates and sperm DNA fragmentation in 38 couples undergoing preimplantation genetic screening (PGS) due to recurrent miscarriage (n = 30) and RIF (n = 8). There was no correlation between aneuploidy rate and sperm DNA fragmentation (R² = 0.02, p = 0.37; R² = 0.04, p = 0.18, for fresh and processed sperm samples, respectively).

In conclusion, there is inadequate evidence to promote sperm DNA integrity tests in patients with RIF. Even with the assumption that the test has predictive value, it is not known if use of antioxidants or other medications improves reproductive outcomes. There is not much to offer to the patient with a high DNA fragmentation rate except for lifestyle changes such as switching to a healthy diet or quitting smoking, which should be suggested regardless of sperm DNA fragmentation status.

**ANATOMICAL FACTORS**

**Congenital uterine anomalies**

In a recent meta-analysis, Venetis et al. reported that except for septate uterus (RR 0.86, 95% CI 0.77–0.96; three studies), chance of spontaneous conception was not decreased in women with congenital uterine anomalies (CUA), namely arcuate, didelphys, unicornuate, and bicornuate uteri. Neither individual nor combined data for different CUA types (RR 0.66, 95% CI 0.37–1.19; four studies) showed a significant difference between ART outcomes in women with or without CUA.

Arcuate uterus is the mildest of congenital uterine anomalies. In their practice committee report, the American Society of Reproductive Medicine (ASRM) has declared it as a clinically irrelevant
variant of the normal. Surgical correction of arcuate uterus is not advised to improve ART success or obstetric outcomes.\textsuperscript{10} Septate uterus is the most common congenital uterine anomaly with a prevalence around 1%.\textsuperscript{11} While its role in first- and second-trimester abortions and other adverse obstetric outcomes such as malpresentation and premature rupture of membranes are shown in a number of studies, its effect on natural and assisted conception is uncertain.\textsuperscript{9} In a prospective study, 44 women with uterine septum and otherwise unexplained infertility, who underwent septum resection, were followed for a year without any interventions.\textsuperscript{12} Their pregnancy rates were compared with those of 132 women with unexplained fertility who were also managed expectantly. Pregnancy rates were 38.6\% versus 20.4\%, and live birth rates were 34.1\% and 18.9\% in the septum and the control groups, respectively, signifying a statistically significant benefit. Unfortunately, the available evidence supporting metroplasty in patients with uterine septum is from nonrandomized studies with small sample sizes.\textsuperscript{13} Yet, given its clear adverse effects on obstetrics and controversial effect on ART outcome, and the observed benefit on miscarriage rates, it is plausible to recommend hysteroscopic resection to women with RIF.

Major CUA such as uterus didelphys, unicorunate, and bicornuate are usually spotted in patients with RIF while undergoing fertility treatments. However, these anomalies are associated with adverse pregnancy outcomes rather than implantation failure.\textsuperscript{9}

**Acquired anatomical factors**

**Endometrial polyps**

Three nonrandomized studies showed improved spontaneous pregnancy rates after polypectomy.\textsuperscript{14-16} In an RCT involving women undergoing intrauterine insemination cycles, hysteroscopic removal of polyps, with an average diameter of 9 mm, resulted in significantly increased pregnancy rates.\textsuperscript{17}

However, there is no evidence supporting a positive effect of polypectomy in IVF patients. There are only retrospective cohort studies. Of 83 patients with endometrial polyps <2 cm in diameter, 49 had their embryos transferred without any intervention, while 34 had embryo transfer after hysteroscopic polypectomy. Clinical pregnancy rates were not significantly different.\textsuperscript{18} Another study on the relationship between polypectomy and pregnancy rates did not show improved pregnancy rates after removal of polyps up to 1.5 cm.\textsuperscript{19}

Despite the lack of evidence for patients with a history of RIF, it could be plausible to remove endometrial polyps in these patients since even a marginal benefit can be what the couple needs.

**Fibroids**

While most women with fibroids are fertile, fibroids are more common in the infertile population.\textsuperscript{20} Abnormal vascularization, increased uterine contractility, chronic inflammation, altered HOXA-10 and cytokine expression in the endometrium are proposed mechanisms that may impair embryo implantation.\textsuperscript{21,22}

A recent meta-analysis reported significantly lower implantation (RR 0.28), clinical pregnancy (RR 0.36), and live birth rates (RR 0.32) and higher spontaneous abortion rates (RR 1.68) in women with submucous fibroids.\textsuperscript{23} On the other hand, the effect of intramural fibroids on fertility and their management is less clear. Recent studies point to the potential detrimental effect of intramural fibroids on fertility.\textsuperscript{23} In a meta-analysis on the effect of non-cavity-distorting intramural fibroids on the outcome of IVF treatment, data from 11 studies including a total of 1626 patient with fibroids and 2355 without were pooled. There was a relative reduction of 21\% in live birth rates in women with intramural fibroids that do not distort the endometrial cavity (RR 0.79, 95\% CI 0.70–0.88 \(p < 0.0001\)).\textsuperscript{24}

Casini et al. prospectively evaluated 181 infertile patients with single fibroids <4 cm and no other causes of infertility.\textsuperscript{25} They grouped patients according to location of their fibroids, randomized
them into myomectomy and follow-up subgroups, and followed them for spontaneous pregnancy for a year. Clinical pregnancy rates were significantly improved with surgery in patients with submucous and submucous-intramural fibroids (43.3% vs. 27.2%, $p < 0.05$ and 36.4% vs. 15%, $p < 0.05$, respectively), while the improvement was not significant in patients with intramural and intramural-subserous fibroids, despite a trend toward benefit (56.5% vs. 40.9% and 35.3% vs. 21.4%, respectively). Some researchers tried to determine a threshold fibroid diameter above which fertility is significantly affected. While being far from conclusive, most of them suggest a cutoff value of 3–5 cm.26–30

Nevertheless, there is no solid evidence to advocate myomectomy for these patients since it is not clear whether it restores fertility.23–25 When the doubtful benefit is compared with the cost, disadvantages, and risks of such a major surgery, including the delay in ART treatment and the small but present risk of uterine rupture during pregnancy and labor, most clinicians proceed without myomectomy at the initial cycles. However, with each unsuccessful cycle, this option is considered more seriously to dismiss any factor that will decrease the chance of implantation. In this line of thinking, patients with RIF should carefully be evaluated for submucous-intramural fibroids, and their removal can be discussed as a weighty option.

Adenomyosis

In a meta-analysis, clinical pregnancy and live birth rates per cycle were significantly decreased in women with adenomyosis (OR 0.73, 95% CI 0.6–0.9 and OR 0.59, 95% CI 0.42–0.82, respectively), while miscarriage rates were increased (OR 2.2, 95% CI 1.53–3.15).31 Mavrelos et al. studied 375 women undergoing IVF and evaluated them using three-dimensional ultrasound (3D US) between the third and sixth days of their menstrual cycle. Of these, 19.2% were diagnosed with adenomyosis, and they showed significantly lower clinical pregnancy rates than those without the disease (29.2% vs. 42.6%; $p = 0.044$; RR 0.68, 95% CI 0.47–1.0).32 Despite the fact that patients with adenomyosis were older and had lower ovarian reserve, logistic regression analysis showed adenomyosis to be an independent factor from age and anti-Müllerian hormone (AMH) in patients who had at least four of the seven ultrasound signs of adenomyosis. Moreover, clinical pregnancy rates dropped gradually as more severe disease was discovered, further supporting a causal relationship.

There is no consensus on management during IVF, especially of the diffuse forms. Surgery, especially in focal disease, and a suppression period with gonadotropin-releasing hormone (GnRH) agonists are the two treatment options, with unproven effectiveness.

Adenomyosis should be in the checklist of the clinician since now it is considered as a possibly important factor hindering IVF success.

Intrauterine adhesions

Two hundred ten women with normal hysterosalpingography (HSG), who had underwent $\geq 2$ failed IVF cycles in which $\geq 2$ good-quality embryos were transferred, were evaluated with office hysteroscopy, and 8.5% of them were found to have flimsy or mild endometrial adhesions, while 2.3% had cervical adhesions.33 Most patients with intrauterine adhesions benefit from hysteroscopic treatment, reaching a 63% pregnancy rate in a pool of 1542 patients.34 Still, severe adhesions may recur and result in lower live birth rates than mild and moderate forms (31.9%, 81.3%, and 66%, respectively).35

Patients with RIF, especially if they have a history of uterine procedures, should be carefully evaluated for intrauterine adhesions as they may be easily missed, and their treatment provides successful results in expert hands, especially in mild and moderate forms.

Hydrosalpinx

A meta-analysis of 5592 IVF patients with tubal factor infertility, of which 1004 had hydrosalpinx, showed pregnancy rates of 19.7% and 31.2% (OR 0.64, 95% CI 0.56–0.74) for patients with
and without hydrosalpinx, respectively. More dramatically, a meta-analysis of 5569 cycles that included a subgroup of 1144 cycles from patients with hydrosalpinx reported 50% lower clinical pregnancy rates for patients with hydrosalpinx. Finally, an RCT by Strandell et al., involving IVF patients with visible hydrosalpinx in ultrasonography, showed that laparoscopic salpingectomy doubled the chance of delivery compared with no intervention.

Patients with RIF should be carefully evaluated for signs of hydrosalpinx in ultrasonography, especially if they have undergone any abdominal operations or were diagnosed with a sexually transmitted disease since their last evaluation with ultrasound or HSG. Despite the absence of evidence, occult hydrosalpinx, which is not large enough to be visualized by ultrasonography, can be expected to hamper chances of ART success. Thus, we recommend ruling out occult hydrosalpinx in a woman suffering RIF.

Assessment of uterine factor and hydrosalpinx

Despite the fact that patients with RIF have undergone many examinations during their treatment cycles, a thorough gynecological examination should be done with a fresh, unconditioned look to rule out any problems that may lower the chance of implantation in RIF patients.

Three-dimensional (3D) transvaginal US has the advantage of obtaining a coronal view and providing accurate and reproducible information about both external and internal contours of the uterus, ideally when endometrium is 5 mm or thicker. Three-D US showed 100% specificity and sensitivity for diagnosing CUA in two different studies, and its concordance with specificity and sensitivity of laparoscopy and hysteroscopy was reported to be 100% and 96%, respectively. A study of 54 women with infertility comparing 3D US and HSG reported 3D US to have a sensitivity of 100% and HSG 66.7% for correctly diagnosing and grading intrauterine adhesions. HSG failed in diagnosing lower uterine segment adhesions mostly, mistaking them for complete cavity obstructions.

The preferred method of examination should be 3D transvaginal US, a very sensitive and specific tool that can successfully diagnose almost all of the uterine pathologies. In cases in which the suspected pathology’s relationship with the endometrial cavity needs further investigation, it can be combined with saline infusion sonography (SIS) and provide comparable results to hysteroscopy, the accepted golden standard. SIS improves diagnostic performance of both 2D and 3D US. Sylvestre et al. documented a sensitivity of 100% and positive predictive value (PPV) of 92% for 3D SIS when compared to hysteroscopy. El-Sherbiny compared the accuracy of 2D SIS and 3D SIS in 120 patients undergoing hysteroscopy. Sensitivity, specificity, and PPV and negative predictive values (NPV) of 2D SIS for intrauterine adhesions were 50%, 98.1%, 75%, and 94.6%, respectively, while they were 75%, 100%, 100%, and 97.3%, for 3D SIS. Interestingly, in another recent study, 3D SIS diagnosed synechia in only 50% of the hysteroscopically proven cases in a study with 16 Asherman syndrome patients. However, it should be noted that US examinations are user-dependent and give the best results in experienced hands.

Bermejo et al. evaluated 65 patients with possible CUA using both 3D US and magnetic resonance imaging (MRI) and demonstrated a high degree of concordance between the two modalities (kappa index = 0.88). Likewise, we reported 100% categorical agreement between 3D US and MRI in not only diagnosis but also measurements of uterine septa. As 3D US provides comparable results, the more expensive and less practical MRI should be reserved for complex congenital uterine pathologies, some complex multiple fibroid cases, cases where adenomyosis diagnosis should be confirmed, and for some patients who cannot undergo transvaginal US examinations and for whom transabdominal US is suboptimal.

Since HSG does not provide information about serosal surface and is not 100% sensitive for diagnosing endometrial polyps and submucous fibroids, it should be performed whenever hydrosalpinx is suspected, when other pathology is ruled out with 3D US-SIS. However, when hydrosalpinx is clearly visible in US, it is not mandatory. Since 3D SIS provides comparable results, hysteroscopy is used more for treatment and less for diagnostic purposes.
It is interesting to note that accuracy of diagnostic methods for intrauterine adhesions varies considerably between the studies. This may be due to the fact that it is technically more difficult to diagnose adhesions, and operator experience and device quality may play a role.52 The 2017 practice guidelines of the American Association of Gynecological Laparoscopists (AAGL), in collaboration with European Society for Gynecological Endoscopy (ESGE), suggest performing hysteroscopy, if available, since it is the most accurate method for diagnosis of intrauterine adhesions.53 SIS and HSG are declared as the reasonable alternatives. On the other hand, some authors point out that 3D SIS provides comparable results with hysteroscopy, and therefore it could be used for diagnosis and hysteroscopy for indeterminate or suspicious cases.52,54,55

Whether diagnostic hysteroscopy benefits the subgroup of RIF patients was investigated in a multicenter RCT called TROPHY.56 This study recruited 702 women younger than 38 years with normal ultrasound findings who had 2–4 failed IVF cycles and randomized the women into outpatient hysteroscopy before IVF or starting IVF right away. Eighty-five women in the hysteroscopy group were found to have cervical or uterine abnormalities, mostly minor. Still, 15 of the 34 uterine cavity abnormalities were surgically corrected. The live birth rates of both groups were the same, 29% (RR 1.0, 95% CI 0.79–1.25; \( p = 0.96 \)). It was concluded that outpatient hysteroscopy before IVF did not increase the live birth chance of women with a history of 2–4 failed cycles who had normal ultrasound findings.

Therefore, it seems reasonable to perform a SIS and perform other diagnostic methods when needed. Hysteroscopy should be reserved for patients who are suspected or known to have uterine pathologies that can benefit from surgical correction.

**HEMATOLOGICAL AND IMMUNOLOGICAL FACTORS**

In a survey performed in the United Kingdom, 75% of the 44 participating centers reported testing for lupus anticoagulant/anticardiolipin antibodies in patients with RIF, making it the most popular test, surpassing parental karyotype testing and hysteroscopy.57 Congenital thrombophilias was the fourth most investigated condition with 59%. Despite the widespread use of these tests, the evidence on the effect of thrombophilias on ART success is conflicting. Moreover, even if there is causal relationship between the two, whether or not treatment with anticoagulants improves the outcomes is unknown.

**Congenital thrombophilias**

Congenital thrombophilias are genetic conditions that predispose the affected person to venous thromboembolism. The most well-known are factor V Leiden mutation (FVL), prothrombin gene mutation G20210A (P2), methylene tetrahydrofolate reductase C667T mutation (MTHFR), and proteins C and S and antithrombin (AT) deficiencies.58 Some of these conditions may be associated with late pregnancy complications such as preeclampsia, growth restriction, and second- or third-trimester fetal loss, but not first-trimester loss.59

Most of the studies on the relationship of RIF and congenital thrombophilia are case reports or cohort studies. A meta-analysis of available case-control and cohort studies did not find any significant association between IVF failure and P2 or deficiencies of proteins C or S or AT.60 FVL was shown to increase the ART failure three times according to the analysis of eight case-control studies (OR 3.08, 95% CI 1.77–5.36); however, the association was insignificant when the only three cohort studies on the subject were analyzed (RR 0.62, 95% CI 0.35–1.08).

In a meta-analysis, a subgroup analysis of eight case-control studies on the relationship between P2 mutation and ART failure did not show any significant difference when homozygote or heterozygote patients were compared with controls (OR 1.48, 95% CI 0.71–3.06).61 Evaluation of seven studies showed that patients with either homozygote or heterozygote mutations had similar ART failure rates with normal controls (OR 1.31, 95% CI 0.95–1.80). According to the analysis of three studies on protein C, protein S, and AT deficiencies, the incidence of these conditions was similar
in women with and without a history of ART failure (OR 1.68, 95% CI 0.17–16.49), (OR 1.58, 95% CI 0.45–5.48), (OR 2.09, 95% CI 0.39–11.28), respectively.62–64

In conclusion, available evidence on the effect of congenital thrombophilias on RIF is based on case-control or cohort studies, and the pooled data implies that routine testing for these conditions in patients with RIF is not indicated.

**Acquired thrombophilias and antiphospholipid antibodies**

A variety of medical conditions may result in acquired thrombophilia, such as antiphospholipid syndrome (APS), paroxysmal nocturnal hemoglobinuria, polycythemia vera, essential thrombocytosis, heparin-induced thrombocytopenia, or increased serum estrogen levels.58 Most either necessitate specific medical attention or are transitory conditions. On the other hand, APS is a chronic thrombophilic condition that may have been undiagnosed in patients with RIF.

Antiphospholipid antibody (APA) positivity has been associated with RPL by many clinicians and is perceived as a potential cause of RIF. As mentioned previously, 75% of the fertility centers in the United Kingdom screen their patients with RIF for APAs, namely lupus anticoagulant, antiphospholipid IgM and IgG, and anti-β2 glycoprotein-I IgM and IgG.57 In 2013, the Obstetric Task Force of the 14th International Congress on Antiphospholipid Antibodies performed a literature review of the studies examining the relationship between APA and RPL.65 Of the 46 studies identified, 27 reported an association. It was noted that majority of the studies were small in size, used arbitrary cutoff values for APA positivity, and documented APA positivity on a single occasion. However, the updated Sapporo criteria require that the APA levels should be above 40 GPL or MPL, or three standard deviations of healthy controls on ≥2 occasions, at least 12 weeks apart.66 Therefore, the task force declared the association between APA and RPL to be inconclusive. How reasonable is applying the same line of thinking to RIF, a different condition than RPL, is questionable.

A meta-analysis by Di Nisio et al. on the effect of APA on ART success yielded conflicting results.61 When only the case-control studies were examined (20 studies including 3542 patients), positivity of at least one APA showed a three-fold increase in ART failure risk (OR 3.33, 95% CI 1.77–6.26). However, analysis of nine cohort studies with a total of 1556 patients on the effect of APA positivity and ART outcome showed no significant difference in pregnancy test results (RR 0.95, 95% CI 0.76–1.19). Clinical pregnancy and live birth rates were similar as well. This discrepancy between the two analyses can be attributed to the heterogeneity of the studies and use of inappropriate control groups, since healthy controls with spontaneous pregnancies may not reflect the effect of infertility or the ART procedures.

In summary, the current evidence on the association of APA positivity and ART outcomes or RIF is weak due to problems with heterogeneity, methodology, and lack of power. Moreover, even if there is an association between APA positivity and ART outcomes, more research on treatment options and their effects is needed. Therefore, screening for APA is highly controversial and, in our opinion, should not be offered routinely outside a research setting or in the absence of other indications than RIF.

**Other immunologic factors**

A variety of immunologic mechanisms, for example, increased peripheric natural killer (NK) cell count, Tₕ/L/Tₕ,2 ratio, have been proposed as etiologic factors for RIF. Resultantly, a wide array of tests and treatments are being performed worldwide. Even though it could be naïve to assume that the immune system does not play a role in embryo implantation and viability of pregnancy, several conditions must be met before any immunological test or treatment is offered in clinical practice. First, a pathologic process hampering implantation has to be clearly defined. Second, a reliable and reproducible diagnostic algorithm enabling the identification of couples with this particular process needs to be developed. Last, an effective treatment proved by credible trials has to be defined. Unfortunately, none of these are done yet. Thus, we regard it appropriate to offer any immunologic
tests only in the context of properly designed, ethically sound and approved research projects, without incurring any additional costs to the couples.

ENDOCRINE FACTORS

Thyroid function and autoimmunity

The Endocrine Society suggests testing for thyroid peroxidase antibodies (anti-TPO) if thyroid stimulating hormone (TSH) is above 2.5 mIU/L repeatedly and starts treatment if antibodies are positive.57

The possible role of thyroid autoimmunity (TAI) in ART outcomes has been a subject of debate as well. Conflicting results from mostly small-sized studies have been published on the relationship between TAI and ART. A 2016 meta-analysis pooled 299 TAI-positive patients and 1931 TAI-negative patients from nine cohort studies and concluded that women with TAI had a lower live birth rate after ART compared with those without TAI (OR 0.73, 95% CI 0.54–0.99; p = 0.04).68,69 However, almost simultaneously, Unuane et al. published a large retrospective cohort study comparing cumulative delivery rates after six cycles in euthyroid patients with and without TAI.70 Their study included 333 TAI-positive patients and 2019 TAI-negative patients, both arms enrolling more patients than those pooled in the aforementioned meta-analysis. With a cumulative delivery rate of 47% in both groups, Unuane concluded that TAI status does not affect cumulative delivery rates in IVF/ICSI patients. It is noteworthy that the results were similar when a subgroup analysis according to TSH thresholds of 2.5 and 5 mIU/L was performed.

RIF patients have a complicated history; therefore it is easy to overlook a simple biochemical test like TSH. Even if it has been done, it may be dating a few years back, probably to the beginning of the treatment. Obtaining a current TSH level before starting a new treatment would be prudent in a patient with RIF, since it is an inexpensive, simple test that is able to detect an easily treatable condition. Measuring free thyroxine (fT4) levels or thyroid antibodies can be saved for patients with TSH levels above 2.5 mIU/L.

Blood glucose

As impaired glucose tolerance may result in adverse pregnancy outcomes, they may have a role in RIF as well. As a practical and relatively cheap screening tool, hemoglobin A1c can be tested in these patients. Women with risk factors, such as obesity, polycystic ovarian syndrome, glucose tolerance, or a family history of diabetes mellitus can be offered oral glucose-tolerance tests.

Vitamin D

A recent meta-analysis reported that vitamin D replete ART patients had a significantly higher live birth rate (seven studies including 2026 patients, OR 1.33, 95% CI 1.08–1.65) and clinical pregnancy rate (11 studies including 2700 patients, OR 1.46, 95% CI 1.05–2.02).71

The possible mechanism by which vitamin D affects implantation and early pregnancy is unclear. To the best of our knowledge, no research has been published regarding vitamin D levels in patients with RIF. Therefore, it is not possible to declare a firm stance on its screening. Yet, in light of the current research and due to the fact that it is a relatively easy and cheap test, vitamin D levels can be tested and supplementation can be prescribed if they are below the 30 ng/mL level, which is the threshold declared by the Endocrine Society.72

ENDOMETRIAL RECEPTIVITY

Only 65% of euploid blastocysts successfully implant.73 This implies that in addition to genetic defects at the subchromosomal level, other factors, possibly endometrial receptivity or its crosstalk with the embryo, can be dysfunctional.

It is suggested that the window of implantation (WOI) is not constant for all women, and patients whose WOI has shifted may experience asynchrony between the endometrium and the embryo, resulting in RIF.74
A commercial test called endometrial receptivity array (ERA) has been developed to analyze the endometrial transcriptome in natural or hormone replacement cycles using an array of 238 genes. According to the combination of genes transcribed in the sample acquired from the uterus with a pipelle catheter on a specific date in the cycle, the result is given as receptive, meaning endometrium is synchronous with the WOI, or non-receptive, meaning there is discrepancy between the two. In the latter case, another sample is acquired in the next cycle at the presumed receptive date until the receptive period is determined. A prospective multicenter trial compared 85 RIF patients with 25 women with <2 failed cycles. Seventy-four percent of RIF patients and 88.1% of the controls were reported to have receptive endometrium. When personalized embryo transfers (i.e., the day of embryo transfer changed in relation to the duration of endometrial progesterone exposure based on ERA results) were performed in eight women in the non-receptive group, pregnancy rates were similar to the general IVF population, 50% versus 48%, respectively. The authors concluded that in RIF patients, ERA provides pregnancy rates comparable to those who had a maximum of one failed ART cycle.

However, the fact that only a small percentage of patients are found to be non-receptive and almost all the clinical studies are coming from a single group raises questions about its practicality and validity.

In a recent independent study from Japan, ERA was performed on 50 patients with RIF and 24% were found non-receptive. Pregnancy rates per patient were 58.8% and 50% for the initially receptive and non-receptive groups, respectively (p = 0.89). Likewise, implantation rates (32.8% vs. 31.6%, respectively) and take-home baby rates (23.7% vs. 16.7%, respectively) showed no statistical significance.

More recently, Cho et al. reported a case of a 44-year-old woman with two failed donor blastocyst transfers who had four ERA tests in a 4-month period. Significant variability was present in the first three tests, as none of the tests overlapped in the WOI timeframe, despite the fact that her weight or the hormone replacement protocol were the same in all cycles. The authors conclude that consistency and reproducibility of the ERA test should be validated with independent studies.

An interesting and promising study was performed in 2016 by Koot et al. on 43 RIF patients and 72 controls, consisting of readily conceived women with ICSI due to severe male infertility. The authors suggested that the only problem is not the endocrine shift in WOI, but probably intrinsic molecular endometrial defects. Using mid-luteal endometrial biopsy samples, they were able to identify a 303-gene transcriptome signature predictive of RIF. Sensitivity, specificity, PPV, and NPV of the test in the signature development set were 90.3%, 94%, 90.3%, and 94%, respectively. However, in an independent validation set of 34 samples, the transcriptome signature had 58% sensitivity but 100% PPV for RIF. If confirmed, this could be a very useful tool to inform couples of their future prospects.

While, the latter is not commercially available yet, we think ERA is more appropriate for the research setting than routine clinical practice at the moment.

**CONCLUSIONS AND SUGGESTED ALGORITHM**

Investigating the etiology of RIF somewhat resembles sailing in uncharted waters. The evidence behind a causal relationship between suggested pathologies and RIF is, in most cases, low quality. Likewise, effectiveness of proposed treatments is poorly supported by evidence. With all these uncertainties, a clinician’s duty is to honestly share their professional opinion on controversies, while carefully avoiding undue despair as well as raising false hope with tests or treatments of unproven effectiveness. The majority of the possible reasons behind RIF should have been examined even before starting the first ART cycle; however, after every failed ART cycle, one should always check whether necessary investigation has been completely done, noting anything that could have changed the couple’s condition since the latest test has happened in between cycles. A template can be used as a framework for a clinic’s own protocol for assessment of couples with RIF (Table 10.1).
### Table 10.1  
**Etiologic factors and diagnostic tests for investigation of recurrent implantation failure.**

<table>
<thead>
<tr>
<th>Etiologic factor</th>
<th>Proposed test</th>
<th>Tests with unproven value</th>
<th>Tests that are unnecessary in the absence of another indication</th>
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<td>• Peripheric NK count</td>
<td>• APS screening with Sapporo criteria&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>TSH</td>
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<td>Vitamin D</td>
<td>• Other immunologic tests</td>
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<td>Endometrial receptivity</td>
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**Abbreviations:**  
- APS, antiphospholipid antibody; SIS, saline infusion sonography.  
- <sup>a</sup> Magnetic resonance imaging for complex congenital uterine anomalies or adenomyosis.  
- <sup>b</sup> If history or ultrasound imaging suggests intrauterine adhesions.  
- <sup>c</sup> In the presence of recurrent pregnancy loss.  
- <sup>d</sup> In the presence of personal or family history of thrombosis.

### REFERENCES


Optimizing embryo culture for recurrent implantation failure

KATERINA CHATZIMELETIOU

The human preimplantation embryo is exposed to specific nutrients within the reproductive tract. Analysis of the fluids within the fallopian tube and the uterus have revealed that glucose levels are at their lowest (0.5 mM) and pyruvate (0.32 mM) and lactate (10.5 mM) levels at their highest in the fallopian tube at the time when the fertilized oocyte and early-cleavage-stage embryo is present, while in the uterus, glucose is present at a much higher level (3.15 mM), whereas pyruvate (0.1 mM) and lactate (5.87 mM) are significantly lower than in the fallopian tube. This nutrient availability reflects the energy requirements of the developing preimplantation embryo and is mirrored in the culture media used for in vitro fertilization (IVF). The early-cleavage-stage embryo is characterized by pyruvate uptake, low levels of oxidative metabolism and low oxygen consumption, whereas at the blastocyst stage, it exhibits both high levels of glycolysis and high oxygen consumption. Sequential media (cleavage and blastocyst stage media) were therefore developed to maintain the embryo’s metabolic needs in culture up to day three and from day three through day five postfertilization, respectively. However, single-step continuous media have lately been introduced, aiming to provide an uninterrupted culture from day one through day five and enabling the embryo to choose and consume the ingredients it requires at each specific stage of preimplantation development. To date, no statistically significant differences have been observed between sequential and one-step media with regard to implantation and pregnancy rates as well as among different media that belong to the one-step or the sequential category.

EXTENDED CULTURE

Extended culture to the blastocyst stage enables better embryo selection and can lead to increased implantation rates and reduced need for high-order multiple pregnancy (HOMP) reduction. A prospective, nonrandomized analysis was performed in 276 IVF patients who failed to conceive after at least two early embryo transfers of at least two grade 1–2 embryos per cycle. For their next attempt, these couples chose between day-two embryo transfer (D2 group; n = 147) and day-five/six blastocyst transfer (D5/D6 group; n = 129) before starting their following attempt. The results showed that the live birth rates per cycle (27.9% vs. 19.7%) and implantation rates per cycle (25.4% vs. 12.4%) were higher in the D5/D6 group compared with the D2 group. To evaluate the efficacy of blastocyst transfer among patients with at least three previous cleavage-stage failed embryo transfers and to compare implantation and pregnancy rates of blastocysts according to the day of transfer (day five or day six), Barrenextea et al. performed a retrospective clinical study. They concluded that blastocysts transferred on day five implanted almost five times the rate of those transferred on day six (23% vs. 5%). Pregnancy rates were triple as high among the 73 day-five patients compared with the 63 day-six transfer patients (38% vs. 11%).

HYALURONIC ACID-ENRICHED TRANSFER MEDIUM

Adjunct treatments currently being offered in IVF laboratories, aiming to enhance embryo quality and implantation potential, include embryo glue and adherence compounds. EmbryoGlue is a hyaluronic acid-enriched embryo transfer (ET) medium developed to improve implantation of embryos in in vitro fertilization-ET cycles (IVF-ET). The exact mechanism of action of the
hyaluronic acid on implantation is still unclear. Hyaluronic acid has been shown to increase cell–cell adhesion and cell–matrix adhesion,21 and in turn may play a key role during the initial stages of apposition and attachment of the blastocyst and endometrium.22 Its functions also include regulation of protein secretion, gene expression, cell proliferation, and differentiation.23,24 Hyaluronic acid can promote angiogenesis by both its degradation products25 and by interaction with epidermal growth factor (EGF),26 while its physical properties include producing a viscous solution that may facilitate the embryo transfer process and prohibit the expulsion of embryos from the uterus.22 It has also been suggested that it confers some degree of viral protection and anti-immunogenic properties that inhibit embryo rejection.27

In a prospective case-control study, embryos were transferred into 50 µL of EmbryoGlue for 10 min prior to transfer in the uterus, while in the control group embryos were transferred to conventional blastocyst culture medium.20 The clinical pregnancy rate in the study group was 7% higher than the control group, but not statistically significant. A significant difference in clinical pregnancy rate was, however, observed with the EmbryoGlue in patients with previous IVF failure (p = 0.04) as 50% of patients with previous IVF failure had successful implantation, but none of the patients with previous implantation failure, in the control group, achieved a pregnancy.20 Valojerdi et al., 200628 also reported in a prospective, randomized trial that clinical pregnancy rate in patients with tubal factor, and implantation rate (IR) in patients with the tubal factor infertility and those with recurrent implantation failure (RIF), increased significantly in patients whose embryos were treated with EmbryoGlue compared with those in the control group. In particular, the clinical pregnancy rate (PR) increased significantly in the patients with tubal factor (38% vs. 18.8%, p < 0.05), whereas the implantation rate increased significantly in the patients with the tubal factor (20.5% vs. 8.9%, p < 0.05) and in patients with recurrent implantation failures (16% vs. 11%, p < 0.05). In the EmbryoGlue group, the rates of live birth/embryonic sac and triplet deliveries increased significantly compared with those in the control group (74.5% vs. 62.5% for the former and 9.5% vs. 1.4% for the latter, p < 0.05).28

In a retrospective analysis the effect of hyaluronic acid-enriched transfer medium (HETM) on frozen-thawed embryo transfer (FET), outcomes were assessed.29 HETM was used for 347 cycles of 342 patients and standard medium for 1374 cycles of 1290 patients. Overall, FET outcomes were similar between the groups. For patients undergoing their first FET attempt, the IR (24.3% vs. 31.6%, p = 0.042) and clinical PR (34.3% vs. 50.1%, p = 0.004) were lower in the HETM group. For patients undergoing their second FET attempt, pregnancy outcomes were similar between the groups. For patients undergoing their third or more FET attempt, HETM was associated with a higher IR (33.3% vs. 16.4%, p < 0.001) and higher PR (52.2% vs. 27.4%, p < 0.001).29

SUPPLEMENTATION OF CULTURE MEDIUM WITH EPIDERMAL GROWTH FACTOR, 4-HYDROXYESTRADIOL, USE OF MITOCHONDRIAL NUTRIENTS

Poor embryo development and RIF is sometimes attributed to poor/impaired mitochondrial function and reduced energy availability.30 Injection of mitochondria-rich cytoplasm from donor oocytes has been suggested to improve oocyte and embryo quality and increase implantation and pregnancy rates.31 Administration of mitochondrial nutrients like coenzyme Q10 (CoQ10) has been shown to exert a beneficial effect to the function of oocyte mitochondria and reverse the oocyte age-related mitochondrial dysfunction and improve embryo development.32–34 In a prospective, randomized, double-blind, controlled study, the use of CoQ10 improved pregnancy rates in patients with poor embryo development and RIF.34 In animal studies, supplementation of medium with prolactin (PRL), epidermal growth factor (EGF), and 4-hydroxyestradiol (4-OH-E2) prior to embryo transfer has also been shown to improve implantation potential.35

COCULTURE OF EMBRYOS ON HOMOLOGOUS ENDOMETRIAL CELLS

Coculture of embryos on homologous endometrial cells has been performed in order to increase implantation rates in patients with repeated implantation failures.36–39 A retrospective comparison
of pregnancy rates between IVF-ET with coculture and standard culture methods was reported by Jayot et al. The authors concluded that the overall pregnancy rate for these patients was 21% per transfer versus 8% in previous IVF-ET cycles, while a higher percentage (28%) was obtained for women <39 years of age or on transfer of at least one morula (32.5% pregnancy per transfer). Eyheremendy et al. also showed an increase in pregnancy rates from 5% in the conventional IVF-ET cycles to 57% in the IVF-ET cycles with the endometrial cell coculture.

TIME-LAPSE SYSTEMS

Embryo scoring has been traditionally performed under a light microscope following removal of embryos from a conventional incubator for quality assessment by an embryologist. In recent years, time-lapse systems have been introduced, which can provide digital images of embryos at frequent time intervals, allowing continuous monitoring of embryo development and enabling the embryologists to evaluate the quality of the embryos without having to physically remove them from the incubator. The potential advantages of a time-lapse system (TLS) include (a) the ability to maintain a stable culture environment, therefore limiting the exposure of embryos to exogenous stress changes in gas composition, temperature, and movement and (b) an advanced scoring system that allows better embryo selection. In a double-blind, controlled study, Rubio et al. examined the effect of embryo incubation in a TLS combined with the use of a multivariable scoring system for embryo selection on implantation and ongoing pregnancy rate. In this study, the patients were randomized to the TLS group and a control group that had their embryos developed in a conventional standard incubator (SI) and assessed only by conventional morphologic criteria. The results showed that both the implantation rate and the ongoing pregnancy rate were significantly higher in the TLS group. In particular, the ongoing pregnancy rate was statistically significantly increased at 51.4% (95% CI 46.7–56.0) for the TLS group compared with 41.7% (95% CI 36.9–46.5) for the SI group. For pregnancy rate, differences were not statistically significant at 61.6% (95% CI 56.9–66.0) versus 56.3% (95% CI 51.4–61.0). The results per transfer were similar: statistically significant differences in ongoing pregnancy rate of 54.5% (95% CI 49.6–59.2) versus 45.3% (95% CI 40.3–50.4) and not statistically significant for pregnancy rate at 65.2% (95% CI 60.6–69.8) versus 61.1% (95% CI 56.2–66.1). Early pregnancy loss was statistically significantly decreased for the TLS group with 16.6% (95% CI 12.6–21.4) versus 25.8% (95% CI 20.6–31.9). The implantation rate was statistically significantly increased at 44.9% (95% CI 41.4–48.4) versus 37.1% (95% CI 33.6–40.7). However, Armstrong et al. concluded that there is insufficient evidence of differences in live birth, miscarriage, stillbirth, or clinical pregnancy to choose between TLS, with or without embryo-selection software, and conventional incubation. To date, no randomized controlled trials have been published evaluating the utility of TLS to improve outcomes among patients with RIF.

CONCLUSIONS

Any new technology aiming to improve implantation, clinical pregnancy, and live birth rates should, in most cases, first be tested in an appropriate animal model, then in clinical trials, to ensure safety, and finally in a randomized controlled trial (RCT) to provide high-quality evidence that the procedure is safe and effective. Improved embryo selection and uterine receptivity may explain the additional benefit of embryo transfer at the blastocyst stage for couples with repeated implantation failures. It is difficult, however, to conclude a favorable role of Hyaluronic acid-enriched media or coculture systems in patients with recurrent implantation failure.

REFERENCES


Optimizing endometrial receptivity for patients with recurrent implantation failure

The role of progesterone on the day of triggering final oocyte maturation

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INTRODUCTION

Recurrent implantation failure (RIF) as a clinical diagnosis refers to a group of women that repeatedly fail to conceive after assisted reproductive technology despite the transfer of a number of embryos. Notwithstanding the obvious heterogeneity in the composition of this diagnostic group, the common symptom is the inability of multiple embryos to implant.1

Despite the intense research efforts of the scientific community and the great advances that have been achieved, the exact process of implantation is still largely considered “a black box”. What seems to be clear is that implantation usually requires not just a capable embryo but also a receptive endometrium2 and a successful “crosstalk” between the two.3 Therefore, women affected by RIF are likely to have compromised endometrial receptivity for a number of reasons.1 Understanding the physiological basis of endometrial receptivity is important and can lead to a better understanding of why some women have a higher propensity to be affected by RIF than others.

PROGESTERONE AND ENDOMETRIAL RECEPTIVITY

The endometrium is a dynamic, hormonally responsive tissue that undergoes molecular, histopathological, and immunological changes throughout the menstrual cycle.4 The purpose of these changes is its preparation for the potential implantation of a hatched blastocyst. Histopathological analysis of luteal phase endometria shows secretory transformation of the glandular epithelium and stromal edema.5 This is followed by a predecidual reaction around the terminal portion of the spiral arteries and the luminal epithelium.4 Decidualization, the differentiation of stromal fibroblasts to “epitheliod” cells, is a key preparatory step for the establishment and maintenance of pregnancy.6 Furthermore, gene expression studies have identified specific signatures associated with the transformation of the endometrium to one that has the capacity to permit embryo implantation.7,8

Importantly, it is universally accepted that exposure of the endometrium to progesterone is fundamental for the attainment of a receptive status. Another key observation is the importance of timing, since the endometrium becomes receptive for only a specific period of time during the mid-luteal phase. The pioneering work by Psychoyos et al. demonstrated, using electron microscopy, that under the effect of progesterone, the endometrium undergoes specific changes that lead to the appearance of pinopodes.9 These structures were suggested to determine the time during a woman's cycle when her endometrium is receptive, named the implantation window,10 the duration of which...
was suggested to be no more than 48 hours. The same group produced evidence strongly supporting that after ovarian stimulation, this implantation window could be shifted, usually arriving 1–2 days earlier. The strongest predictor of the premature changes observed in these endometria was serum progesterone concentration in the late follicular phase.\textsuperscript{11,12}

**PROGESTERONE CONCENTRATION DURING OVARIAN STIMULATION**

In women, the main sources of progesterone are (a) the ovary, (b) the adrenals, and (c) the placenta. Despite the fact that progesterone is considered a hormone that dominates the luteal phase due to the increased production by the corpus luteum, it is also produced during the follicular phase.

In the early follicular phase, it has been shown that the main source of progesterone is the adrenals.\textsuperscript{13–15} It has also been demonstrated that, during ovarian stimulation with gonadotrophins for \textit{in vitro} fertilization (IVF) and especially during the late follicular phase, serum progesterone frequently increases.\textsuperscript{16,17}

This increase in serum progesterone was initially considered to be consistent with premature luteinization, which requires a luteinizing hormone (LH) rise or surge, which, in turn, stimulates the theca interna to produce progesterone and/or also luteinizes the existing follicles. However, through a number of studies, it was clearly shown that even in cases where LH is suppressed using gonadotrophin releasing hormone (GnRH) analogues or the oral contraceptive pill, a rise in serum progesterone is still observed.\textsuperscript{13–15} Although this led initially to the conclusion that the adrenal glands were the main source of the progesterone rise during the follicular phase, a subsequent study in which the adrenals were suppressed using dexamethasone strongly suggested that the progesterone rise is actually associated with the exogenous administration of gonadotrophins.\textsuperscript{18}

Today, the prevailing theory is that this progesterone rise is actually a side effect of ovarian stimulation and multifollicular growth.\textsuperscript{19,20} This has been deducted through the analysis of multiple observational studies that support that the progesterone rise is associated with the serum estradiol concentration during the late follicular phase as well as the number of oocytes retrieved.\textsuperscript{17} Hence, for the majority of patients undergoing ovarian stimulation, the increase in the serum progesterone concentration seems to be a result of the production of smaller quantities of progesterone by the granulosa cells of growing follicles. This notion has recently been backed up by \textit{in vitro} experiments that suggested that follicle-stimulating hormone (FSH) can actively promote the synthesis of progesterone by granulosa cells by upregulating the expression and increasing the enzymatic activity of 3\textbeta\textsuperscript{-}hydroxysteroid-dehydrogenase (3\textbeta\textsuperscript{-}HSD), which converts pregnenolone to progesterone.\textsuperscript{21}

**THE PROGNOSTIC ROLE OF PROGESTERONE CONCENTRATION ON THE DAY OF TRIGGERING FINAL OOCYTE MATURATION FOR THE ESTABLISHMENT OF PREGNANCY AFTER IVF**

The prognostic role of progesterone concentration during the late follicular phase for the establishment of pregnancy had already been suggested in the first few years of ovarian stimulation. More specifically, in 1989 during the 45th Annual Meeting of the American Fertility Society, Schoolcraft et al. reported that a serum concentration of progesterone greater than 0.9 ng/mL on the day of triggering of final oocyte maturation with human chorionic gonadotrophin (hCG) was associated with significantly lower pregnancy rates.\textsuperscript{22} Not surprisingly, this study motivated multiple researchers from different groups to examine the predictive role of serum progesterone on the day of hCG for the achievement of pregnancy. A plethora of studies was published within the next 15 years with some confirming a negative association between progesterone elevation and pregnancy rates\textsuperscript{23–33} while others rebutted such a finding.\textsuperscript{34–44} Amidst this controversy, a systematic review and meta-analysis published in 2007 attempted to provide an answer on the aforementioned clinical question. By systematically reviewing all the relevant studies until 2005, the authors concluded that there was no evidence to support a detrimental effect of higher progesterone values on the
achievement of pregnancy (odds ratio [OR] 0.75, 95% CI 0.53–1.06). Following that systematic review and meta-analysis, a number of large studies were published focusing on the same research question. In 2010, Bosch et al. published a seminal article in which, by examining more than 4000 review and meta-analysis, a number of large studies were published focusing on the same research with serum progesterone \( \geq 0.8–1.1 \) ng/mL. They analyzed multiple cutoffs used in the original studies: 0.4–0.6, 0.8–1.1, 1.2–1.4, 1.5–1.75, and 1.9–3.0 ng/mL. The negative effect of progesterone elevation was already present from serum progesterone cutoffs of all the available relevant studies until August 2012 (n = 63; 55,199 cycles) and provided conclusive evidence of the negative effect of elevated progesterone on the day of triggering final oocyte maturation for the achievement of pregnancy after a fresh embryo transfer (ET). They analyzed multiple cutoffs used in the original studies: 0.4–0.6, 0.8–1.1, 1.2–1.4, 1.5–1.75, and 1.9–3.0 ng/mL. The negative effect of progesterone elevation was already present from serum progesterone cutoffs of 0.8–1.1 ng/mL (Figure 12.1). This effect could be significant since, as it was shown in patients with serum progesterone \( \geq 1.5 \) ng/mL (the most widely used cutoff), pregnancy rates are reduced by approximately 10% (Figure 12.2) as compared with cycles with serum progesterone <1.5 ng/mL. Furthermore, this systematic review highlighted that progesterone elevation was not as rare as initially thought and that depending on the cutoff used, it could be affecting 33.5% (95% CI 26.7–41.0) of the cycles.

Figure 12.1 Forest plots of odds ratios (ORs) for pregnancy achievement in women with progesterone elevation (PE) when compared with those without PE (all studies analyzed). CI, confidence interval. (From Venetis CA et al. Hum Reprod Update. 2013;19(5):433–57, with permission.)
The same meta-analysis suggested that a detrimental effect of serum progesterone on the day of hCG was not present when the embryo created during a cycle with progesterone elevation was transferred to an endometrium that had not been exposed to increased progesterone levels. More specifically, by synthesizing data from the studies that had been performed in donor-recipient cycles or in frozen-thawed embryo transfer (FET) cycles, it became evident that an association was not present between the presence or not of progesterone elevation during the stimulation cycle and the pregnancy rates in the recipients or after a FET cycle (Figure 12.3). This once again confirmed the theory that the effect of elevated progesterone on the day of hCG is exerted through the advancement of endometrial maturation and the deterioration of endometrial receptivity. This had been already demonstrated through conventional histopathological analysis in 1997\textsuperscript{46} and it was also confirmed through gene expression analyses more recently.\textsuperscript{47,48}

Currently, it is widely accepted that serum progesterone concentration has a negative effect on the achievement of pregnancy after a fresh ET, while it does not appear to affect the chances of conception after a FET cycle. This negative effect is present regardless of the magnitude of ovarian response, although it has been suggested that better-quality embryos, usually associated with younger patients with larger oocyte yields, might be able to somehow mitigate it.\textsuperscript{49}

\textbf{Figure 12.2} Graphical representation of the transformation of the OR 0.64 (95% CI 0.54–0.76) to (a) absolute pregnancy rate reduction with 95% CIs (dotted lines) and (b) Number needed to harm (NNH) with 95% CIs (dotted lines), according to a range of baseline pregnancy risks. Based on these calculations, graph (c) depicts the expected pregnancy rate with 95% CIs (dotted lines) in a population with 40% baseline pregnancy rate (i.e., pregnancy rate in the non progesterone elevation [PE] patients) according to a range of PE rates in that population. (From Venetis CA et al. \textit{Hum Reprod Update}. 2013;19(5):433–57, with permission.)
THE POTENTIAL ROLE OF PROGESTERONE ON THE DAY OF TRIGGERING FINAL OOCYTE MATURATION IN WOMEN WITH RIF AFTER IVF

Considering the importance of late follicular serum progesterone for the achievement of pregnancy after a fresh ET, it would be relevant to explore the degree to which abnormally elevated progesterone contributes to RIF for some patients.

This research question was examined in a recently published study by Liu et al. In this retrospective, observational, single-center study, the authors analyzed 6673 consecutive cycles performed during 2007–2012 that resulted in a fresh ET. These cycles included women ≤40 years of age and with up to 20 oocytes retrieved. The authors hypothesized that if progesterone elevation is a significant contributory factor of RIF, its incidence will be higher in cases with increased numbers of failed IVF cycles. To test this hypothesis, the authors divided their sample in three groups. Group I included patients who had never had IVF/ET before, group II included those who had one previous IVF/ET failure and group III included patients with ≥2 previous failed IVF/ET cycles. The authors defined progesterone elevation as serum progesterone >6 nmol/L, based on previous studies. Using this cutoff, 1672/6673 (25.1%) cycles exhibited progesterone elevation. The authors used stepwise regression and identified age, estradiol concentration on the day of hCG, number of oocytes retrieved, and total dose of FSH as predictors of progesterone elevation. Subsequently, using random number generators, the authors matched groups I, II, and III for the aforementioned potential confounders. When evaluating the prevalence of progesterone elevation between the three different groups, it was evident that women with ≥2 previous failed

**Figure 12.3** Pooled odds ratio (OR) for achievement of pregnancy in women: (a) Forest plots of ORs for achievement of pregnancy in women undergoing frozen-thawed embryo transfer after a fresh cycle with or without progesterone elevation (PE) (per PE threshold group) and (b) Forest plots of ORs for achievement of pregnancy in women undergoing embryo transfer with donated oocytes from women who did or did not experience progesterone elevation (PE) (per PE threshold group). (From Venetis CA et al. *Hum Reprod Update*. 2013;19(5):433–57, with permission.)
IVF/ETs had a significantly higher prevalence of elevated progesterone compared with women with one failed IVF/ET or no failed IVF/ETs. This finding was also present when their sample was divided into poor responders (≤4 oocytes retrieved) and moderate responders (5–19 oocytes retrieved) (Table 12.1). Similarly, women with one failed IVF/ET had significantly higher incidence of elevated progesterone compared to women with no failed IVF/ET. This finding was confirmed when the analysis was repeated in subgroups of poor (≤4 oocytes retrieved) and moderate (5–19 oocytes retrieved) responders (Table 12.1).

Based on these results, the authors of this large retrospective analysis concluded that there is an association of RIF with elevated progesterone on the day of hCG. This implies that certain patients have a propensity to exhibit elevated progesterone at the end of their ovarian stimulation which in turn reduces the chance of pregnancy and, hence, might lead to multiple implantation failures.

Recently, Venetis et al. performed a retrospective study in 1702 IVF antagonist cycles in a single center, aiming to find baseline predictors of progesterone elevation (>1.5 ng/mL). The researchers showed that female age, FSH, and serum progesterone on day two of their menstrual cycle were the main prestimulation predictors of progesterone elevation. In the same study, it was demonstrated that duration of stimulation, the number of follicles ≥11 mm in mean diameter, and serum estradiol on the day of triggering final oocyte maturation were also strong predictors of progesterone elevation, confirming that progesterone elevation is associated with the intensity of ovarian stimulation. For that reason, the researchers, using multivariable regression analysis, attempted to find independent predictors of progesterone elevation after controlling for ovarian-stimulation characteristics. Interestingly, only basal progesterone was an independent predictor. More specifically, the higher the serum progesterone on day two of the menstrual cycle, the higher the probability of progesterone elevation (Figure 12.4). Based on our knowledge of the sources of progesterone during the early menstrual cycle, the most likely explanation is that these patients have a higher production of progesterone from their adrenals. When this is combined with the production by their developing follicles in the mid and late follicular phase, the total serum progesterone concentration is increased, and this detrimentally affects the chances of pregnancy after a fresh ET. This theory seems to be corroborated by the fact that Venetis et al. found that women with a history of progesterone elevation in a previous cycle had ~6 times higher odds of exhibiting progesterone elevation in the current cycle, independent of the intensity of ovarian stimulation.

Table 12.1 Prevalence of elevated progesterone (>6 nmol/L) on the day of triggering final oocyte maturation according to the number of previous failed IVF cycles.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>No previous failed IVF/ET cycle</td>
<td>1 previous failed IVF/ET cycle</td>
<td>≥2 previous failed IVF/ET cycle</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td><strong>p</strong></td>
<td><strong>p</strong></td>
</tr>
<tr>
<td>Unmatched sample</td>
<td>Matched sample</td>
<td>Poor response</td>
</tr>
<tr>
<td>1489/6102</td>
<td>300/1784</td>
<td>49/551</td>
</tr>
<tr>
<td>24.4%</td>
<td>16.8%</td>
<td>8.9%</td>
</tr>
<tr>
<td>150/489</td>
<td>100/315</td>
<td>15/90</td>
</tr>
<tr>
<td>30.7%</td>
<td>31.7%</td>
<td>16.7%</td>
</tr>
<tr>
<td>16/82</td>
<td>25/63</td>
<td>5/21</td>
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<td>40.2%</td>
<td>39.7%</td>
<td>23.8%</td>
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matched for age, estradiol level on the day of hCG administration, and number of oocytes retrieved.
The findings of these studies do suggest that elevated progesterone on the day of triggering final oocyte maturation could be contributing significantly to the phenomenon of RIF and that its proper management could improve the chances of these patients conceiving.

MANAGEMENT OF PROGESTERONE ELEVATION
Mitigation of the negative effects of progesterone elevation can be achieved either with primary prevention or active management in patients who exhibit progesterone elevation.

Primary prevention of progesterone elevation
Milder ovarian stimulation
As it has been convincingly shown that the probability of progesterone elevation is increased with a larger oocyte yield, it has been hypothesized that actively pursuing the development and retrieval of a smaller number of oocytes would reduce the chance of progesterone elevation. Although this approach does make sense physiologically, it should be stressed that it has not been tested in the context of a randomized controlled trial (RCT), and hence the net benefit, if any, of such a strategy in terms of pregnancy rates is unknown. Mild stimulation has in the past been suggested to have a beneficial effect on pregnancy rates, however, more recent data suggest that the optimal number of oocytes retrieved in terms of live birth rates is around 15. Furthermore, other studies suggest that a larger oocyte yield is associated with more euploid embryos available for transfer. Hence, aiming for a lower number of oocytes might reduce the chance of progesterone elevation but at the same time could also reduce the number of euploid embryos.

Reducing the steroidogenic drive on the granulosa cells of developing follicles during the late follicular phase
An alternative strategy that has also been proposed is reducing the steroidogenic drive on the granulosa cells of the developing follicles around the end of the follicular phase. Since progesterone elevation is considered to be mainly due to small amounts of progesterone produced by the granulosa cells of multiple follicles under the action of gonadotrophins, reducing the concentration of
gonadotrophins might have a beneficial effect. This was indirectly shown in a recent RCT in which it was demonstrated that performing ovarian stimulation in poor responders for the first seven days with a single dose of 150 µg of corifollitropin alfa (CFA) led to significantly lower serum concentrations of progesterone on day eight of stimulation when compared with patients who were stimulated with a daily dose of 450 IU of follitropin beta despite the fact that the number of developing follicles was similar between the two groups.\(^{58}\) This could be attributed to the unique pharmacokinetic profile of a single CFA injection which is characterized by an initially high concentration of CFA followed by a gradual decrease, similarly to a step-down protocol. This finding was recently confirmed in a reanalysis of two large RCTs on the use of CFA (ENGAGE and PURSUE) which demonstrated that the incidence of progesterone elevation in patients who were triggered after a single dose of CFA (i.e., on day eight) was significantly lower compared with those who were stimulated with daily recombinant FSH and triggered also on day eight.\(^{59}\)

Similarly, it has also been shown that increased presence of LH activity, especially during the late follicular phase can also lead to an increase in serum progesterone,\(^{60–62}\) most likely through the potent stimulatory effect of LH activity on granulosa cells of large follicles that have attained LH receptors. On the basis of these observations, one could hypothesize that reducing the dose of FSH and/or LH during the last few days of stimulation would reduce the probability of progesterone elevation. However, it should be stressed that such a strategy has not been properly tested in the context of an RCT, and hence it has not satisfactorily been shown to be an effective option.

Earlier administration of hCG and oocyte retrieval

This is an approach that involves close monitoring of serum progesterone and administration of hCG to trigger final oocyte maturation before serum progesterone reaches a level that is detrimental for endometrial receptivity. This strategy was initially proposed by Harada et al.,\(^{17}\) who demonstrated that an earlier administration of hCG was associated with higher implantation rates. Interestingly, in a study by Kolibianakis et al. which aimed to determine the best timing for triggering final oocyte maturation, it was shown that an hCG administration as soon as at least three follicles had a mean diameter of 17 mm (early hCG) was associated with higher ongoing pregnancy rates compared with triggering final oocyte maturation 48 hours later (late hCG).\(^{63}\) This difference could be explained by the fact that the early hCG group had significantly lower mean progesterone concentration (1.1 ng/mL) compared with the late hCG group (1.5 ng/mL). Therefore, this proposed strategy is only supported by observational data, and at the same time, it requires intense monitoring. Finally, this strategy is based on the assumption that there is a single cutoff above which there is a detrimental effect on endometrial receptivity; however, more recent data are suggesting that this is not likely to be true.\(^{17,49}\)

Corticosteroid administration

Another strategy that has been proposed for prevention of elevated progesterone on the day of hCG administration involves the administration of corticosteroids in order to suppress the production of progesterone by the adrenals. In a prospective, controlled study (n = 120), Fanchin et al.\(^{18}\) administered dexamethasone 1 mg/day from the time of pituitary suppression until the time of hCG administration in 60 patients, while the remaining 60 did not receive any dexamethasone. Although the net increase in serum progesterone between the start and the end of ovarian stimulation was similar between the two groups, the group that received dexamethasone had a lower mean serum progesterone concentration on the day of hCG. Furthermore, this group had a significantly higher ongoing pregnancy rate compared with the patients who did not receive dexamethasone (35% vs. 17%). The administration of dexamethasone for the prevention of elevated progesterone was also shown in a subsequent study by Eldar-Geva et al.\(^{14}\) In this study, none of the patients who received daily dexamethasone during ovarian stimulation exhibited elevated progesterone (>3 nmol/L) on the day of hCG compared with 13.9% of patients who did not receive dexamethasone.
In summary, based on the data provided by these two studies, corticosteroid administration during the follicular phase has also been suggested as a way of reducing the likelihood of elevated progesterone. However, in the absence of randomized data and, most importantly, data regarding the safety of this strategy for the woman and potentially her fetus, this option should only be used in the context of properly designed clinical studies.

**MANAGEMENT OF PATIENTS WITH ELEVATED PROGESTERONE**

**Replacement of high-quality embryos**

When elevated progesterone does occur, though, the options are limited. Proceeding with a fresh ET is of course an option, since progesterone elevation has been shown to reduce, but not completely eliminate, the possibility of a pregnancy. In that regard, it has been suggested that replacing embryos with higher implantation potential can somehow reduce the negative effect of elevated progesterone on pregnancy rates. Based on multiple reports, it does appear that younger patients with more oocytes retrieved and hence better-quality embryos are less affected by the detrimental effect of elevated progesterone, while cycles with embryos of poor quality are more profoundly affected. This means that replacing an embryo of high quality during a cycle with elevated progesterone will still lead to acceptable pregnancy rates, although most likely these rates will be lower than what could be achieved in the absence of elevated progesterone.

**Freeze-all embryos**

Freezing all available embryos and deferring embryo transfer is probably the most popular method of managing patients with elevated progesterone. This is based on the fact that, as discussed, the negative effect of elevated progesterone has been shown to be exerted on the endometrium, and it does not appear to have an effect on the quality of the oocytes and the resulting embryos. At the same time, highly efficacious cryopreservation protocols ensure high survival rates without compromising the implantation potential of the cryopreserved embryos.

The freeze-all policy has been suggested previously to be beneficial for women with implantation failure. Firstly, in 2014, a retrospective study in 269 patients with previous implantation failure of a blastocyst in a fresh cycle, compared pregnancy rates between those patients who decided to have another fresh embryo transfer following their next oocyte retrieval (n = 163) and those who opted to freeze-all embryos and undergo a FET cycle (n = 109). Using multivariate regression analysis, the authors showed that FET is associated with 3.8 times higher odds of live birth compared with a fresh embryo transfer.

In a more recent prospective study, Magdi et al., compared the freeze-all policy (n = 81) with fresh embryo transfer (n = 90) in women who had at least three previous failed embryo transfers with ≥4 high-quality embryos transferred in total. Patients were assigned to these groups quasi-randomly using alternate allocation. The mean serum concentration of progesterone on the day of hCG was not significantly different between the two groups (1.10 vs. 1.07 ng/mL). However, there was a significant difference (p = 0.005) in the probability of ongoing pregnancy between women in the freeze-all group (40.7%) and women in the fresh transfer group (21.1%). Based on these results, the authors concluded that the freeze-all policy seems to be beneficial for RIF patients.

Finally, one small RCT (n = 100) has specifically looked into the value of the freeze-all policy in patients with elevated progesterone (≥6 nmol/L) on the day of trigger. These patients were randomized to a fresh day 5/6 transfer (n = 50) or to a freeze-all and subsequent FET (n = 50). Patients who had a fresh embryo transfer had an 18.4% live birth rate per embryo transfer, while those who froze all their embryos and subsequently had an FET had a live birth rate per embryo transfer of 31.0%. However, this difference in live birth rates, although potentially clinically relevant, was not statistically significant, and due to the small sample of this study and other methodological concerns, no robust conclusions could be drawn.
CONCLUSIONS

Elevated progesterone on the day of triggering final oocyte maturation after ovarian stimulation is not a rare phenomenon and seems to be more frequent in women with RIF after IVF. Identifying this issue and managing it appropriately can improve the chances of pregnancy for these patients.

REFERENCES


The role of late follicular phase luteinizing hormone and estradiol on embryo implantation

Implications for recurrent implantation failure

JULIA K. BOSDOU, CHRISTOS A. VENETIS, and EFSTRATIOS M. KOLIBIANAKIS

INTRODUCTION

Endometrial receptivity and embryo quality are two major factors that contribute to implantation success. During ovarian stimulation for in vitro fertilization (IVF), the development of multiple follicles leads to abnormal hormonal levels. This is true for estradiol (E2), which rises up to 10 times or more compared with a natural cycle, and for progesterone, the levels of which also increase as a consequence of multiple follicular development, as well as for luteinizing hormone (LH), the levels of which are variably suppressed by the use of gonadotropin-releasing hormone (GnRH) analogues. If the abnormal hormonal environment during ovarian stimulation for IVF adversely affects the probability of implantation, then this may contribute to implantation failure. If hormonal levels, however, are not monitored during ovarian stimulation for multifollicular development, then implantation failure might be falsely perceived as unexplained.

OVARIAN STIMULATION AND EMBRYO IMPLANTATION

During implantation, the endometrium undergoes a transformation period, regulated by ovarian steroids, during which it acquires appropriate morphological structure and function. Implantation requires a number of functional molecules such as hormones, cytokines, and growth factors, which are actively involved in regulating trophoblast differentiation and invasion. Progesterone and estrogen are considered as the primary hormonal modulators of endometrial development, supporting embryo implantation. This is confirmed by the presence of their receptors in stromal and epithelial cells during implantation. After binding to their receptors, steroids have been suggested to promote uterine proliferation and differentiation through the production of local cytokines and growth factors.

Indeed, histologically abnormal endometrium is present on the day of oocyte retrieval in all IVF cycles using GnRH analogues and gonadotropins. This abnormality is demonstrated by a difference observed between the histological and the chronological dating of the endometrium,
referred to as an endometrial advancement. It has been shown that endometrial advancement of more than three days is significantly associated with a diminished probability of pregnancy.\textsuperscript{13,14} Moreover, gene expression analysis of the endometrium of stimulated cycles on the day of oocyte retrieval showed the presence of an altered gene expression in women with histologically advanced endometrial maturation exceeding three days.\textsuperscript{15}

Regarding LH, it is not clear whether there is an association between the probability of pregnancy and its variably suppressed levels during ovarian stimulation as a result of pituitary downregulation.\textsuperscript{8,16}

On the other hand, the effect of the abnormal hormonal environment to the developing follicles and eventually to oocyte and embryo quality is still a matter of debate.\textsuperscript{17} The use of exogenous gonadotropins for ovarian stimulation has been reported to induce chromosomal abnormalities such as aneuploidy in the oocyte as well as to affect embryo development.\textsuperscript{18,19} Reducing the intensity of ovarian stimulation by using milder ovarian stimulation or even natural IVF cycles has been suggested to reduce the proportion of aneuploidy in embryos.\textsuperscript{20}

In contrast, it has also been suggested that similar aneuploidy rates with no differences in embryo quality and types of chromosomal abnormalities are present between unstimulated and stimulated IVF cycles in the same young egg donors.\textsuperscript{21} Moreover, it has been shown that the degree of exposure to exogenous gonadotropins in patients with normal response to ovarian stimulation undergoing IVF is not associated with the likelihood of aneuploidy.\textsuperscript{22}

Overall, it appears that ovarian stimulation may affect embryo implantation either by altering endometrial receptivity and/or oocyte/embryo quality; however, certain questions remain unanswered. It is not clear, for instance, whether an effect of ovarian stimulation is predominantly due to a single abnormal hormonal level or due to a combination of different abnormal hormonal levels, at a specific moment or for a certain period during ovarian stimulation.

This chapter will focus on the role of LH and E2 during the late follicular phase of ovarian stimulation for IVF on the probability of implantation and its implications on recurrent implantation failure (RIF).

**ENDOGENOUS LH ON THE LATE FOLLICULAR PHASE AND EMBRYO IMPLANTATION**

The role of endogenous LH during ovarian stimulation for IVF has been in the focus of research well before the introduction of GnRH analogues in assisted reproductive technologies.\textsuperscript{24,25} GnRH analogue use increased the interest in this association, since inhibition of premature LH surge by GnRH agonists or antagonists is associated with suppressed LH concentrations.\textsuperscript{26,27} The degree of LH suppression following administration of GnRH analogues varies depending on the type, dose, and protocol of the analogue used.

On the other hand, it has been suggested that 0.15%–15% of all LH receptors are occupied with LH concentrations of 1–10 IU/L.\textsuperscript{28} Since the percentage of LH receptors that need to be occupied for a maximum steroidogenic response to be yielded \textit{in vitro} is 15%–20,\textsuperscript{29} it is then possible that higher LH concentrations could result in a more efficient stimulation of LH receptors in the developing follicle.\textsuperscript{30}

Several studies have suggested an adverse effect of deeply suppressed LH levels on the probability of pregnancy.\textsuperscript{16,31} However, evidence for absence of such an effect during ovarian stimulation for IVF is also present.\textsuperscript{8} In a relevant systematic review, a negative association between low endogenous LH levels during ovarian stimulation for IVF using GnRH analogues and the probability of ongoing pregnancy beyond 12 weeks was not observed. Similarly, in an individual patient data meta-analysis of six randomized controlled trials (RCTs), including 1764 patients undergoing ovarian stimulation with recombinant follicle-stimulating hormone (rFSH) and GnRH antagonist for IVF/intracytoplasmic sperm injection (ICSI), no association between the endogenous LH concentrations and pregnancy achievement was present.\textsuperscript{32}

In a recent study in patients stimulated with rFSH and GnRH antagonists for IVF, the association of serum LH levels during ovarian stimulation with cycle outcome was evaluated. Implantation rate and clinical pregnancy rate were not significantly associated with serum LH levels.\textsuperscript{33}
Based on the currently available evidence, it appears that no association can be supported between low endogenous LH levels and pregnancy achievement in GnRH analogue downregulated cycles. Thus, its involvement in RIF appears unlikely, while its assessment does not offer useful information other than the confirmation of adequate pituitary suppression.

ENDOGENOUS E2 ON THE LATE FOLLICULAR PHASE AND EMBRYO IMPLANTATION

The endogenous E2 concentration is the most frequently used marker for assessing follicular development, since E2 levels are associated with the number and size of developing follicles. However, due to the fact that E2 levels during ovarian stimulation for IVF are multiple times higher than those present during a natural cycle, their impact on the probability of pregnancy has been extensively evaluated.

A sustained interest in a potentially adverse effect of high E2 concentration on endometrial receptivity, as well as on oocyte/embryo quality is present in the published literature. A systematic review published in 2004, including 3352 patients treated by IVF using GnRH agonists and gonadotropins, evaluated the above association. Both a positive and a negative association between the serum E2 levels on the day of human chorionic gonadotrophin (hCG) administration and the probability of pregnancy were reported. On the other hand, the majority of studies failed to show an association. However, the low quality of the published studies limits the importance of the results reported.

Since the publication of that systematic review, several relevant retrospective studies have been published, as well as one prospective study. These studies, using different arbitrary E2 threshold levels in order to classify patients as those with low or high E2 on the day of hCG administration, supported a positive association, a negative association, or no association at all between E2 levels and pregnancy achievement (Table 13.1). The variability in the reported results might be due to the different E2 thresholds used during the evaluation of the above association. It should also be noted that the number and quality of the available studies still remain low, not allowing for solid conclusions to be drawn.

Overall, based on the currently available evidence, it appears that the majority of studies support the absence of an association between E2 levels on the day of triggering and the probability of pregnancy. Thus, its involvement in RIF appears unlikely, while its assessment does not offer useful information other than the evaluation of the intensity of ovarian response and the associated risk for ovarian hyperstimulation syndrome (OHSS).

CONCLUSIONS AND RESEARCH IMPLICATIONS FOR RIF

Based on the currently available evidence, it appears that no association can be supported between low LH levels or high E2 levels on the late follicular phase of ovarian stimulation for IVF and the probability of implantation in GnRH agonist or antagonist cycles. However, whether or not the absence of such an association is also true also for patients with RIF is not clear, since studies addressing the above question in these specific patients have not been performed.

Nevertheless, since abnormal development of endometrium in the late follicular phase is present in all cycles stimulated with GnRH analogues and gonadotropins, strategies that may assist in increasing endometrial receptivity need to be explored. In this respect, milder stimulation protocols might be an option. These protocols might be associated with an improved endometrial receptivity by decreasing the adverse effect of standard ovarian stimulation, albeit with the cost of producing fewer numbers of embryos for transfer and hence a decreased chance of pregnancy achievement.

Alternatively, the freeze-all strategy in which all created embryos are frozen for later transfer, needs to be explored in RIF patients. Apparently, transfer of embryos in the normal endometrium of a natural or artificially prepared cycle bypasses whatever effect of ovarian stimulation on endometrial receptivity, at the expense of introducing an additional intervention such as embryo
Table 13.1  Association between serum E2 levels (pg/mL) on the day of hCG administration and the probability of pregnancy per patient reaching hCG.

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>E2 groups/pregnancy rate per patient reaching hCG</th>
<th>Association (per patient reaching hCG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ng et al.37</td>
<td>&lt;2724 2724–5448  &gt;5448 19.5 (30/154) 13.95 (12/86) 7.9 (3/38)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Saucedo de la Llata et al.61</td>
<td>&lt;1000 1001–3000  &gt;3001 21.7 (33/152) 35.6 (52/146) 25.7 (9/35)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Thelmo et al.58</td>
<td>&lt;1000 1000–2000  2001–3000 3001–4000</td>
<td>Negative</td>
</tr>
<tr>
<td>OR 0.29, 95% CI 0.09–0.94</td>
<td>OR 0.43, 95% CI 0.18–0.98</td>
<td>Not reported OR 0.28, 95% CI 0.11–0.71</td>
</tr>
<tr>
<td>Ulug et al.57</td>
<td>113–1349 1350–3249  &gt;3250 32.4 (263/810) 49.9 (1662/3325) 54.4 (895/1643)</td>
<td>Positive</td>
</tr>
<tr>
<td>Wu et al.68</td>
<td>≤2000 2001–3000  3001–4000 4001–5000  &gt;5000 35.4 (46/130) 39.6 (21/53) 30.4 (14/46) 31 (9/29) 25 (4/16)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Kong et al.59</td>
<td>&lt;1634 1634–3268  3268–4903  &gt;4903 22.1 (53/240) 21.2 (83/392) 24.8 (57/230) 26.7 (68/255)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Kyrou et al.56</td>
<td>&lt;1142 1142–2446  &gt;2446 23.5 (12/51) 32.4 (34/105) 29.4 (15/51)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Farhi et al.59</td>
<td>&lt;1362 1362–2724  &gt;2724 38.3 (41/107) 38.5 (47/122) 47.1 (24/51)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Joo et al.60</td>
<td>&lt;1000 1000–2000  2000–3000 3000–4000  &gt;4000 13.9 (10/72) 13 (13/100) 19.7 13/66 32 (16/50) 19.8 (33/167)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Núñez et al.49</td>
<td>&lt;3000 &gt;3000 36.48 (27/74) 23.07 (9/39)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Imudia et al.51</td>
<td>≤2991 &gt;2991 49.4 (1331/2696) 45.5 (136/299)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Gursoy et al.52</td>
<td>&lt;2000 2000–4000  &gt;4000 24.6 (16/65) 14.5 (11/76) 28.2 (20/71)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Taskin et al.70</td>
<td>AUC 0.532, 95% CI 0.471–0.593</td>
<td>Not significant</td>
</tr>
<tr>
<td>Zavy et al.54</td>
<td>&lt;2000 2000–4000  &gt;4000 47.2 (58/123) 59 (170/288) 61.5 (48/78)</td>
<td>Positive</td>
</tr>
<tr>
<td>Prasad et al.53</td>
<td>&gt;1400, AUC 63.4 (p = 0.007)</td>
<td>Positive</td>
</tr>
<tr>
<td>Foroozanfard et al.55</td>
<td>&lt;1500 1500–3500  &gt;3500 8.9 (4/45) 11.4 (4/35) 31.2 (15/48)</td>
<td>Positive</td>
</tr>
</tbody>
</table>
freezing. This strategy has been shown to increase the probability of pregnancy in high responders; however, data for patients with RIF are still not available.

REFERENCES


Can recurrent implantation failure be managed by inducing endometrial inflammation or by the use of novel research therapies?

Recurrent implantation failure (RIF) remains a challenge for reproductive medicine. The patients characterized as RIFs consist of a heterogeneous group mainly due to the fact that RIF definitions vary. In general, though, RIF patients are the ones that fail to achieve a clinical pregnancy at least three times after embryonic transfers with good-quality fresh embryos.

Implantation success is a multifactorial process. However, two contributing components are considered critical for an embryo to implant and evolve. Embryo quality is an obvious factor for a successful pregnancy; endometrial receptivity is the other component. It is now clear that endometrial physiology may predict the potential for an ongoing clinical pregnancy. The endometrium, under the influence of progesterone, is normally transformed to decidua. Decidualization is a process featured by aseptic inflammation of the endometrial stroma. Several inflammatory agents, mainly cytokines, trigger chemotaxis and differentiation of several immune cells. Thus decidua is characterized by dendritic cells and natural killer cells, as well as other immune populations. This inflammation is under hormonal regulation, with progesterone playing the major role. The morphological outcome of endometrial decidualization is the formation of pinopodes on the luminal surface of the endometrium. This finding has been recognized as the key morphological alteration that happens during the window of implantation, the time point at which endometrial receptivity is maximized and synchronized with the maximum infiltrative potential of the blastocyst. The cytokine profile that prevails during the implantation has been characterized as the Th2 profile. Th2 has been correlated to local immune suppression and is considered favorable for implantation and early fetal development. A possible switch from the Th2 to the Th1 profile has been reported to be found in cases of implantation failures, including spontaneous or recurrent miscarriages.

Taking into account that implantation is a process featured by a specific aseptic inflammation, an inspired overview tried to link decidual inflammation to local stress. Indeed, stress mediators, with corticotropin releasing hormone (CRH) being the main representative of this neuropeptide family, have been reported to be expressed by the endometrium in a timely spatial pattern. CRH is overexpressed during the secretory phase and, most importantly, at the implantation site. Interestingly, CRH has been reported to be expressed by the trophoblast cells of the blastocyst. This increased CRH concentration, located at the fetal maternal interphase, seems to contribute to the establishment of local inflammation. CRH has, in turn, been reported to induce Fas ligand
Manipulating the endometrium

(FasL) expression to decidua cells and trophoblasts, mediating in that way the apoptosis of the local Fas-expressing T-cell population. Such an immune modification (the local downregulation of cellular immunity) has been reported as crucial to achieving maternal immune tolerance of the semiallogenic fetus.

Recently, the decidualized endometrium was proved to act as a biological selector of embryos of good quality. It was shown that apart from the window of implantation, a window of selection follows. During this time point, the endometrium selectively rejects embryos recognized as of bad quality. This mechanism is based on a further crosstalk between the recently implanted embryo and the decidua, aiming to scrutinize potential embryonic defects and thereafter proceed to embryonic rejection early enough. In that view, the window of selection may indeed explain the case of “superfertility” during which an increased number of embryos—that later reported to fail—may implant.

The essential role of inflammation on successful implantation has led to the formulation of several hypotheses supporting the exogenous trigger of inflammation as a key act in reversing RIF.

**A. Induction of local endometrial injury.** Performing local endometrial injury was reported early in the beginning of the 2000s. Barash et al. had conducted a randomized controlled trial (RCT) investigating whether local injury (represented by endometrial biopsy) could have an impact on the reproductive outcome of women with RIF when offered assisted reproductive technology (ART). The results were very promising since all basic parameters (implantation, clinical pregnancy, and live birth rates) were significantly improved after local endometrial injury. The impact of this study on ART practice was rather strong. Several clinical groups had started applying the technique prior to in vitro fertilization (IVF) treatment and further investigated all related clinical issues that arose during clinical practice. In that view, several reports using rather weak samples have addressed timing prior to IVF treatment as well as instrumentation to be used. Initially, the majority of the reports (reviewed by Almog et al.) converged to the theses that local injury could be beneficial if applied during the cycle prior to treatment and that either pipelle or hysteroscopy produce comparable results. The initial enthusiasm was further boosted by basic science reports aiming to delineate the mechanisms through which endometrial injury modulates endometrial receptivity. It was shown that endometrial injury could induce both inflammation and endometrial receptivity genes, thus favoring implantation. Clinical reports have been summarized in meta-analyses supporting that endometrial injury indeed can improve the reproductive outcome of women with RIF. However, new emerging results from RCTs, as well as recent meta-analyses, put endometrial injury in doubt. The recent concept, upon a possible wider acceptance of the method, is rather reluctant to be recommended in the frame of lack of data regarding complications. Future studies are expected to clarify whether endometrial injury is to be incorporated in the relevant guidelines.

**B. Intrauterine administration of immune cells.** An alternative to mechanical injury was proposed when the intrauterine administration of autologous peripheral blood mononuclear cells (PBMCs) was investigated as an inflammation inducer. Yoshioka et al. conducted a clinical trial to delineate a possible impact of autologous PBMC administration prior to embryo transfer in case of RIF. PBMCs were primed with human chorionic gonadotrophin (hCG) and administered two days prior to blastocyst transfer. The results were also promising since reproductive outcomes were significantly improved as well. To the same direction, others have modified the initial protocol in the aim of maximizing the immunomodulatory effect of the PBMCs on endometrial receptivity. Our group performed a pilot study administering CRH-primed PBMCs two days prior to blastocyst transfer to women diagnosed with RIF. Our results were in line with that of hCG-primed PBMCs since clinical pregnancy rates were significantly increased. However, to date, the idea of manipulating autologous immune cells to improve endometrial receptivity is not as appealing as was initially expected, perhaps due to the need of laboratory
involvement, not to mention the reluctance of the pharmaceutical industry to be involved in biological material manipulation.

C. Administration of hCG. The issue of administration of immune cells was bypassed by other researchers that have introduced the concept of cytokine/hormonal intruterine administration as endometrial priming agents prior to embryo transfer. The first approach reported was the administration of hCG. By the time this was suggested, the role of HCG on endometrial receptivity had already been investigated using *in vitro* and *in vivo* models. The results were initially very promising, since reproductive outcome parameters were significantly improved. The initial meta-analysis was supportive for hCG as an endometrial receptivity modulator. However new emerging data have also introduced an amount of skepticism. The most recent meta-analysis has revealed conditional success, showing that, only in certain cases, HCG may produce favorable results.

D. The role of granulocyte-colony stimulating factor (G-CSF). Alternatively to hCG, it was proposed to administer G-CSF as an endometrial receptivity primer. This approach was mainly based on the chemotactic effect that G-CSF exerts on macrophages, a cellular component essential in maintaining an inflammatory profile during implantation. Although initial reports were rather promising, data from randomized trials are rather short. Recent RCTs show controversial reproductive outcomes with no effect on reproductive outcome in older women. However, recent meta-analyses showed that there could be a role for G-CSF in cases of women diagnosed as RIF. The results presented in the meta-analyses revealed that G-CSF treatment is superior to controls in terms of reproductive outcomes. No significant difference was noted in terms of endometrial thickness changes. Furthermore, no significant difference was found when comparing the efficacy of G-CSF administration via different routes (vaginal vs. systematic administration).

E. Other promising agents.

a. Atosiban. Recent emerging data have revealed a role for atosiban in improving reproductive outcomes in women with RIF. The concept was based on the finding that atosiban is interfering with the oxytocin/prostaglandin F2a system, minimizing uterine contractility after embryo transfer. Few studies have been published so far and have already been analyzed via meta-analysis. The authors included 1756 cases from 6 studies. Meta-analysis revealed a significant improvement in implantation and clinical pregnancy rates, findings that remained significant even when subgroup analysis was focused on repeated implantation failures. Properly powered randomized trials are needed to strengthen such a finding at the level of acceptance in clinical practice.

b. Growth hormone (GH). To date, a single RCT exists in the literature comparing reproductive outcomes with or without GH treatment in a donor oocyte program. The study included 105 infertile patients, of which 70 were diagnosed with RIF. RIF patients were randomly assigned to receive GH or not (n = 35 for GH group and n = 35 for non-GH [negative control] group), while the remaining 35 non-RIF patients received their first oocyte, acting as a positive-control group. The results showed that GH treatment significantly improved implantation, clinical pregnancy, and live birth rates compared with controls. New emerging data are expected to clarify the role of GH in human reproduction.

**CONCLUSIONS**

The role of inflammation is pivotal in human reproduction. It has been involved in decidualization and implantation. This finding being established has triggered several hypotheses aiming in improving the reproductive outcomes of women with RIF. Although promising, most results need further verification to be considered established knowledge and thus eligible for application in clinical practice. Until such proof exists, RIF is to be treated with the to-date established protocols.
REFERENCES


The embryo in recurrent implantation failure

*Genetic selection and strategies for improving its implantation potential*

KATERINA CHATZIMELETIOU

The causes behind failure of an embryo to implant into the endometrial lining are multiple. Although in certain cases it may simply be a failure to properly synchronize the timing of transfer of a chromosomally normal embryo with the patient’s personalized window of implantation, embryonic abnormalities and uterine pathologies play undoubtedly important roles. Various uterine pathologies, including a thin endometrium or altered expression of adhesive molecules, immunological factors, endometriosis, and hydrosalpinges, have been shown to exert an adverse effect and reduce endometrial receptivity. Patient karyotype abnormalities (including balanced reciprocal translocations, Robertsonian translocations, qh-polymorphisms, inversions, or mosaicism) that can result in embryos with chromosomal imbalance and aneuploidies that are incompatible with implantation and further development, genetic and epigenetic defects of the male and female gametes, postzygotic chromosomal abnormalities during cleavage, and early blastocyst stages as well as zona hardening are among the most common embryonic reasons for failure of implantation.

**CHROMOSOMAL ABNORMALITIES IN EMBRYOS FROM PATIENTS WITH RECURRENT IMPLANTATION FAILURE (RIF)**

The origin of chromosomal abnormalities may vary in embryos from recurrent implantation failure (RIF) patients, and although meiotic errors account for a large proportion, postzygotic errors also play a significant role. Voulaire et al. detected chromosomal abnormalities in 60% of embryos from women with RIF. The abnormalities included aneuploidy for one or two chromosomes (25%) as well as more complex chromosomal abnormalities (29%). Small segmental translocations have also been identified in embryos from patients with RIF using next-generation sequencing. Voulaire et al. reported that the incidence of complex chromosome abnormalities involving three or more chromosomes was independent of maternal age but highly associated with RIF, suggesting that the pathology underlying complex abnormality is different from that resulting in aneuploidy of one to two chromosomes. Chatzimeletiou et al. provided the first cytoskeletal analysis of human embryos at all stages of preimplantation development and documented the mechanisms leading to postzygotic chromosomal abnormalities, embryonic arrest, and implantation failure via multipolar spindle formation, binucleation, multinucleation, and chromosome lagging (Figure 15.1). The combination of meiotic and postzygotic errors can lead to embryonic arrest and may arise from disruption of the normal sequence of chromosome replication and segregation, caused by maternal cytoplasmic factors, mutations in cell-cycle control genes, or sperm-related factors.
The embryo in recurrent implantation failure

SPERM FACTORS AFFECTING EMBRYO QUALITY

Normal embryonic development in vitro has been shown to be subject to strong paternal (sperm-derived) effects. Centriolar defects can lead to failure in fertilization through impairment or absence of aster formation while centrosomal abnormalities can cause embryonic arrest through the formation of abnormal spindles and the accumulation of chromosomally abnormal cells that derive from them. Tesarik et al. compared the quality of embryos resulting from sibling donor oocytes fertilized by spermatozoa from different patients. They reported that fertilization with spermatozoa from certain individuals repeatedly resulted in the formation of zygotes with abnormal pronuclear morphology that tended to cleave slowly and had high levels of fragmentation and uneven blastomeres. These paternal effects are most likely due to a combination of genetic origin, related to the minor gene activity of the male pronucleus; epigenetic origin, related to the sperm-derived oocyte-activating factor; and extragenetic origin, involving defective sperm centriolar function.

Centriolar abnormalities are more frequently observed in immotile or nonprogressively motile spermatozoa than in normal motile spermatozoa. Since the distal centriole gives rise to the axoneme during spermiogenesis, it is possible that distal centriolar defects are responsible for problems associated with sperm motility. Abnormalities in pronuclear apposition and aster formation have also been observed in vasectomized patients who produce antisperm antibodies against centriolar structures, and after intracytoplasmic sperm injection (ICSI) with severely abnormal spermatozoa.

Molecular cytogenetic analysis in embryos from patients with oligoasthenoteratozoospermia (OAT) and RIF that show slow or arrested development in vitro often reveal patterns of polyploidy and postzygotic malsegregation of chromosomes that could be explained by abnormal centrosomal distribution, following cytokinetic failure and defective spindles. Delayed division of blastomeres can theoretically result in tetraploidy, either through formation of an abnormal monopolar spindle or failure of both karyokinesis and cytokinesis. The tetraploid blastomere produced by failure of karyokinesis and cytokinesis has two centrosomes that, when duplicated, would produce four. If there is unequal allocation of these centrosomes on the spindle poles, for example, 3:1, then it is possible that unequal numbers of spindle fibers will be present and one-sided divisions will ensue, as most of the chromosomes will be attached and move toward the spindle pole with the greatest number of centrosomes. The presence of four centrosomes in an unbalanced arrangement (3:1), three distinct foci at one pole of a spindle and one at the other pole, has been previously reported.
by Chatzimeletiou et al.\textsuperscript{9} following cytoskeletal analysis of human preimplantation embryos, but can also occur via mechanisms of centrosome splitting in the presence of impaired DNA integrity and replication.\textsuperscript{26} Confocal laser scanning microscopy has also highlighted the presence of tripolar and tetrapolar spindles with the characteristic Y- and cruciform X-shaped organization, at cleavage and early blastocyst stages, respectively, in human embryos, following labeling with antitubulin antibodies and a DNA-specific fluorochrome.\textsuperscript{9} As there are no checkpoints to monitor excess spindle poles, multipolar spindles progress in the cell cycle and lead to chaotic chromosomal constitutions. This study provided the first direct evidence of spindle abnormalities throughout human preimplantation development, and led to the realization that nuclear and chromosomal aberrations are interrelated through abnormalities in cytokinesis and spindle formation.\textsuperscript{9}

**TREATMENT OPTIONS IN COUPLES WITH SEVERE MALE FACTOR AND RIF**

Intracytoplasmic morphologically selected sperm injection (IMSI) could be of value to patients with RIF that have abnormal sperm parameters. Balaban et al.\textsuperscript{27} reported that patients with RIF who had IMSI experienced higher implantation and clinical pregnancy rates compared with those who had ICSI, (28.9% vs. 19.5% and 54.0% vs. 44.4%, respectively; $p \geq 0.05$). El Khattabi et al.\textsuperscript{28} suggested that IMSI is beneficial for patients with severe teratozoospermia at their first or second attempts, but it does not improve the pregnancy rate in patients with repeated ICSI failures in the absence of severe male factor. In particular, these authors showed that live birth rate was significantly higher when IMSI was used in men with teratozoospermia and RIF compared with ICSI (38% [50/132] vs. 20% [25/126]) but was similar between IMSI and ICSI procedures (21% [19/90] vs. 22% [28/130]) in men with RIF and no severe male factor. Delaroche et al.\textsuperscript{29} reported that the percentage of top quality embryos at cleavage stages was higher in IMSI compared with IVF/ICSI cycles (89.8% vs. 79.8%; $p = 0.009$), the mean number of blastocysts was higher in IMSI cycles (1.5 ± 1.9) than in in vitro fertilization (IVF)/ICSI cycles (1.0 ± 1.2; $p = 0.03$), and the clinical pregnancy and live birth rates were higher in IMSI versus IVF/ICSI cycles.

It has been postulated that spermatozoa are subjected to post-testicular damage during sperm transport between the seminiferous tubules and epididymis. It is therefore possible that the injection of damaged spermatozoa may be a cause behind cases of RIF in which no female factor can be identified. Increased sperm DNA fragmentation and aneuploidy may also lead to embryos with increased levels of fragmentation and chromosomal abnormalities that are not compatible with implantation (Figure 15.2).\textsuperscript{30} Testicular spermatozoa have been isolated and used to fertilize oocytes with promising results regarding embryo quality, implantation, and pregnancy rates.\textsuperscript{31} However, currently there is limited, low-quality evidence suggesting that a higher probability of pregnancy might be expected using testicular rather than ejaculated spermatozoa, only in men with high DNA fragmentation index (DFI) and oligozoospermia.\textsuperscript{32}

Finally, Blazquez et al.\textsuperscript{33} investigated the efficacy of using donor sperm in addition to oocyte donation after RIF in improving pregnancy and live birth rates. There was no difference in fertilization rates (75.3% vs. 75.2%, $p = 0.97$), biochemical pregnancy rates (52.2% vs. 54.1%, $p = 0.79$), clinical pregnancy rates (41.2% vs. 45.9%, $p = 0.51$), ongoing pregnancy rates (38.2% vs. 37.1%, $p = 0.87$), and live birth rate (38.2% vs. 35.8%, $p = 0.73$) between the double donation (DD) and oocyte donation (OD) groups.

**THE ROLE OF EMBRYO BIOPSY AND PREIMPLANTATION GENETIC SCREENING IN RIF**

Preimplantation embryo biopsy by removing polar bodies at the zygote stage (day one), a single blastomere at the cleavage stage (day three), or trophectoderm cells at the blastocyst stage (day five), in combination with preimplantation genetic diagnosis for aneuploidy screening (PGD-AS), has paved the way to detect and eliminate chromosomally abnormal embryos, thereby increasing the chance of implantation and a healthy pregnancy (Figure 15.3). Initial reports using fluorescence
The embryo in recurrent implantation failure

in situ hybridization (FISH) to screen for a limited number of chromosomes showed no significant increase in pregnancy rates in RIF patients.\textsuperscript{34–37} In a pilot study, Greco et al.\textsuperscript{38} explored whether preimplantation genetic screening (PGS) by array comparative genomic hybridization (array CGH) and transfer of a single euploid blastocyst in patients with RIF can improve clinical results. Three patient groups were compared: (group RIF PGS) versus (group RIF NO PGS) versus (group NO RIF PGS), and higher implantation and clinical pregnancy rates were observed in the group RIF PGS compared with the group RIF NO PGS.\textsuperscript{38} Fragouli et al.\textsuperscript{39} compared the incidence of aneuploidy and the implantation and pregnancy rates following blastocyst biopsy and polar body (PB) biopsy in patients with RIF. The oocyte and blastocyst aneuploidy rates were 65.5% and 45.2%, respectively, and the implantation and pregnancy rates for the patients with blastocyst biopsy were higher compared with those who underwent PB biopsy (58.3% and 69.2% vs. 11.5% and 21.4%, respectively).

Blastocyst biopsy is followed by immediate vitrification as the time needed for the PGS result to be given exceeds the window of implantation. Vitrification has high survival rates following warming, and cytoskeletal analysis by confocal laser scanning microscopy has demonstrated that it does not adversely affect embryonic development and the ability of cells to carry out normal cell divisions, as the vast proportion of spindle/chromosome configurations are normal.\textsuperscript{40} However, due to mechanical stress during exposure to cryoprotectants, a significantly higher incidence of spindle abnormalities is evident in vitrified embryos compared with fresh embryos. The abnormalities

![Figure 15.2](image-url)
TREATMENT OPTIONS IN PATIENTS WITH RIF THAT HAVE EMBRYOS WITH NO CHROMOSOMAL ABNORMALITIES

Stamenov et al. evaluated the efficacy of frozen mixed double-embryo transfer (MDET; the simultaneous transfer of day-three and day-five embryos) in comparison with frozen blastocyst double-embryo transfer (BDET; transfer of two day-five blastocysts) in patients with RIF. The results revealed significantly higher implantation and clinical pregnancy rates in patients who underwent MDET than in those who underwent BDET (60.4% vs. 39.3%, p = 0.03 and 52.1% vs. 30.4%, p = 0.05, respectively). A significantly lower miscarriage rate was observed in the MDET group (6.9% vs. 10.7%, p = 0.05). In addition, the multiple pregnancy rate was slightly, but not significantly, higher in the MDET group (27.1% vs. 25.0%), suggesting that MDET could be regarded as an alternative, useful approach to improve implantation/pregnancy rates in RIF patients. Esfandiari et al. also reported a successful pregnancy following a novel technique of sequential double

Figure 15.3 Chromosomal constitution and development on day five of embryos from a patient with RIF following embryo biopsy on day three and PGS with array CGH. (a) Hatching blastocyst with normal chromosomal constitution, (b) hatching blastocyst with Monosomy 21 and Trisomy 22, (c) hatching blastocyst with monosomy 6, and (d) arrested embryo with chaotic chromosomal constitution.

include spindles with a focused and an unfocused pole, chromosome bridging, chromosome lagging, and congression failure, reflecting mechanisms that can lead to chromosomal mosaicism in early human development. The latter can potentially result in implantation failure or miscarriage depending on the extent and type of the abnormality. In cases of biopsied vitrified embryos, a hole in the zona has already been introduced that can help the embryo to hatch out and implant following warming and transfer. During standard cryopreservation of embryos, including cases of “freeze-all” protocols, which are used in order to optimize better the endometrium or to overcome problems associated with severe ovarian hyperstimulation syndrome, the zona is intact. Assisted hatching may be beneficial in those cases to minimize embryo entrapment in the zona following zona hardening imposed after cryopreservation. Assisted hatching has been previously proposed as a method to overcome zona hardening in fresh embryos of RIF patients.
transfer of two frozen embryos at the same developmental stage (eight-cell) at five and seven days after the luteinizing hormone (LH) surge to a patient with seven previous failed assisted reproductive technology (ART) cycles.

Loutradis et al. 49 also compared pregnancy rates in patients with RIF who underwent single or double-embryo transfer (group A1 underwent embryo transfer on day two and day four after pick-up, group A2 underwent embryo transfer on day two and day five after pick, group B-control had a day four or five only transfer without having an additional day two transfer). These authors concluded that double-embryo transfer had beneficial effects on patients with good-quality embryos and RIF. In particular, if the additional embryo transfer was done on day four, a 38.2% clinical pregnancy rate and a 50% total pregnancy rate were reported, while if the additional embryo transfer was done on day five, a 60% clinical rate and 60% total pregnancy rate were achieved.

Rahman et al. 50 evaluated the efficacy of recombinant follicle stimulating hormone (r-FSH) supplemented by recombinant LH (r-LH) in the late luteal phase of ovarian stimulation in patients with RIF. The authors concluded that the number of metaphase II oocytes with cytoplasmic maturation, the number of gestational sacs, as well as implantation and pregnancy rates, were significantly higher in the r-LH group compared with the r-FSH only group.

Patients who fail to conceive following multiple assisted reproduction cycles often seek treatment options that include immunological testing and treatment, allogenic lymphocyte therapy, blastocyst transfer sequential embryo transfer in fresh or frozen cycles, assisted hatching, coculture of embryos on homologous endometrial cells, PGD-AS, and mitochondrial DNA assessment.3,38,39,51–55 PGD-AS by array CGH or next generation sequencing (NGS) may be beneficial for RIF patients with no uterine pathologies who have a high incidence of chromosomally abnormal embryos. In those cases, correct synchronization of the window of implantation with the transfer of the chromosomally normal embryo in a well-prepared, receptive endometrium may lead to successful implantation.

REFERENCES

References


Is oocyte donation efficient in patients with recurrent implantation failure?

GUSTAVO NARDINI CECCHINO and JUAN ANTONIO GARCÍA-VELASCO

INTRODUCTION
Implantation is a complex process whereby the embryo adheres to the maternal uterine endometrium. In the clinical setting, it is assured whenever an intrauterine gestational sac can be sonographically detected, following either a natural conception or an assisted reproductive treatment. The impossibility of identifying the gestational sac is referred to as implantation failure.

Regarding in vitro fertilization (IVF) treatments, implantation depends on the balance of several aspects, ultimately related to: gamete and embryo quality, maternal factors, and technical issues. Curiously, a considerable proportion of infertile couples undergoing multiple IVF attempts are unable to achieve a successful pregnancy due to recurrent implantation failure (RIF).

The definition of RIF varies widely. Andrological causes for RIF are still controversial and basically linked to sperm quality. In contrast, maternal factors are well known and include not only age, but also lifestyle, congenital and acquired uterine conditions, altered endometrial receptivity, and endocrine, immunological, and hematological disorders. Technical issues involve detrimental effects of ovarian stimulation protocols, inadequate endometrial priming, gamete and embryo manipulation, nonoptimized culture conditions and poor transfer efficiency. These topics were extensively discussed in the previous chapters.

Somehow, the figurative question remains unclear: is the seed or the soil responsible for RIF?

IMPORTANCE
In Western countries, there is an increasing trend to postpone parenthood. A comprehensive systematic review on behalf of the European Society of Human Reproduction and Embryology (ESHRE) has shown that the main critical reasons for this phenomenon are the ever-increasing educational level and labor-force participation among women to compensate for a society in which gender inequity still predominates. Also, immature and ineffective social policy incentives lead to delayed motherhood.

In spite of remarkable advances in the field of assisted reproductive technologies (ART), not all embryos transferred implant, and these rates significantly decline as women age. In relation to the fraction of transferred embryos progressing to delivery, available data are even more frustrating, as reported by the Centers for Disease Control and Prevention (CDC) in their latest National Summary Report.3

Taken together, this may contribute for the recurrence of implantation failure. Additionally, the number of couples requiring oocyte donation (OD) to conceive is continuously increasing. As the percentage of cycles with women’s own eggs declines with age, the use of donated eggs becomes more prevalent, reaching over 70% of all ART cycles after the age of 44. Among all ART treatments, OD shows the best results. Nevertheless, multiple implantation failures may also affect recipients of OD.
Importantly, women with RIF experience significantly higher psychological stress levels than healthy, fertile women. Feelings of social isolation, diminished sexual enjoyment, and need for parenthood are more frequent among this group of patients. These feelings may vary according to the supportive care received and the outcomes of consecutive attempts.\(^5\)

Furthermore, limited knowledge to fully comprehend the components implicated in RIF has motivated the employment of countless alternative therapies. In general, these interventions lack rigorous scientific evidence. Besides not being cost effective, they create unrealistic expectations among patients who are already emotionally vulnerable.

**ADVANCES IN OVUM DONATION PROGRAM**

In 1983, a successful pregnancy was established for the first time following a rudimentary OD procedure. Unfortunately, it ended up in a spontaneous abortion after 10 weeks.\(^6\) Later in 1984, the first newborn was achieved using such a technique.\(^7\) Ever since, decisive initiatives have supported the creation of structured OD programs worldwide.

OD was originally applied to overcome cases of primary or secondary ovarian failure. Since 1989, the use has been extended to women with poor response to ART, repeated IVF failure, and age-related infertility.\(^8\) The first clinical trial comparing the results of OD between women over and under 40 years of age showed similar reproductive outcomes. Moreover, the IVF results of women $\geq$ 40 years undergoing OD were notably superior than those presented by women of the same age that used their own eggs.\(^9\)

Currently, women from 35 to 44 years old represent the main group of patients treated with OD, and the mean age is around 40 years.\(^3\) Implantation rates and reproductive outcomes are stable and comparable among OD recipients from different age groups. This suggests that, rather than the uterus, a poor oocyte competence is strongly linked to aging. Data regarding age and uterine receptiveness are still controversial. However, the best evidence available exhibited declining rates of implantation, clinical pregnancy, and live birth, along with increased miscarriage rates only in OD recipients beyond 45 years.\(^10\)–\(^12\) Figure 16.1 illustrates these facts.

OD accounts for approximately 9.2% of all ART cycles performed in the US.\(^3\) The following graphs show a significant rise concerning egg donation cycles in both the US and Spain over a 10-year period. Noteworthy, the use of frozen gametes is becoming more common due to the refinements in cryopreservation techniques (Figure 16.2).

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**Figure 16.1** Clinical outcomes in (a) fresh and (b) frozen donor egg cycles according to recipient age. It is important to note the stability until the late 40s and the changes after the age of 45. $\Diamond =$ clinical pregnancy rate; $X =$ delivery rate; $O =$ miscarriage rate. (Adapted from Toner JP et al. Fertil Steril. 2002;78(5): 1038–45, with permission.)
Is oocyte donation efficient in patients with recurrent implantation failure?

A recent analysis from the CDC’s National ART Surveillance System revealed not only a boost in the number of donor oocyte cycles carried out in the US last decade, but also a remarkable reduction in preterm delivery and low birth weight. Similarly, it was well demonstrated that the risks involved in OD are relatively low, with an incidence of serious complications below 1%, such as moderate or severe ovarian hyperstimulation syndrome, severe infection, ovarian torsion, or any other complication requiring hospitalization.

Budak et al. noticed several improvements while evaluating 10,537 OD cycles performed from 1995 to 2005 in the Valencian Institute of Infertility (IVI) clinics in Spain, such as a sequential increase in both implantation and pregnancy rates (PR), as well as a reduction in rate of multiple pregnancies due to a progressive decrease in the number of embryos transferred in each cycle. Some of these trends are synthesized in Figure 16.3.

In OD programs, a rigorous screening of donors and recipients is highly recommended. This is especially important considering the massive dissemination of oocyte cryobanks. Large studies validated the use of cryo-stored oocytes in ovum donation programs by confirming its noninferiority in relation to success rates, including obstetric and perinatal outcomes.

Recently, a new approach to assess IVF success has been described. The cumulative live birth rate (CLBR) per total number of embryos transferred gives a more realistic estimation of a couple’s probability to reach a newborn after an IVF treatment. Despite the increasing number of embryos transferred, the CLBR dramatically declines with age and reaches a plateau on women older than 40 years using their own eggs. Conversely, it has been proved that in an OD program, the number of...

![Figure 16.2](image-url) Distribution of egg donation cycles over a 10-year period: (a) US; (b) Spain. (Adapted from [a] National Center for Chronic Disease Prevention and Health Promotion - Division of Reproductive Health. Assisted Reproductive Technology National Summary Report; [b] Spanish Fertility Society, National Summary Report, with permission.)
of embryos needed to achieve a newborn is lower and the CLBR rates are very similar between women of distinct ages, as shown in Figure 16.4.21

**OOCYTE DONATION X RIF**

Apparently, poor oocyte quality is the central cause of age-related deterioration of reproductive capacity. By the age of 40, almost 80% of women’s oocytes are aneuploid.22 Equally, the rate of aneuploid embryos starts to increase rapidly after 37 years and reaches its peak at the age of 44, when almost 90% of the embryos are aneuploid.23

![Figure 16.3](image.png)

**Figure 16.3** Trends in different reproductive outcomes over time in an OD program. (Adapted from Budak E et al. *Fertil Steril.* 2007;88(2):342–9, with permission.)

![Figure 16.4](image.png)

**Figure 16.4** CLBR according to the total number of embryos transferred using (a) own eggs and (b) OD. (Adapted from [a] Garrido N et al. *Fertil Steril.* 2012;98(2); [b] Geraedts J et al. *Hum Reprod.* 2011;26(11): 3173–80, with permission.)
These facts certainly contribute for multiple implantation failure and inferior ART outcomes. Fortunately, the use of preimplantation genetic testing for aneuploidy (PGT-A) improves the implantation and live birth rates in both women with advanced maternal age and selected RIF patients whenever transferring chromosomally normal embryos.

In addition, when analyzing the oocyte-to-infant ratio, it is possible to observe tremendous biological wastage. Such a biological loss is even greater in patients undergoing PGT-A. A prospective study including couples with recurrent pregnancy loss, multiple IVF failures, and advanced maternal age showed that up to 99% of the retrieved oocytes did not produce live births. In the RIF group, less than 5% of the inseminated oocytes were capable of producing euploid embryos.

Human ooplasmic transplantation emerged in the 1990s as an innovative approach to overcome the detrimental effects of compromised oocytes on embryonic development. Experiments conducted by Cohen et al. in women experiencing RIF confirmed that the injection of donor ooplasm into patient eggs was able to enhance embryo developmental potential. Indeed, as a result of these experiments, women with multiple IVF failures got pregnant and gave birth. Nonetheless, the US Food and Drug Administration banned the use of this technique in 2001 due to ethical and biological concerns, essentially related to the pattern of mitochondrial DNA transmission and the risk of mitochondrial disease inheritance. Lately, research involving meiotic spindle transfer and other nuclear genome transfer techniques are being investigated in animal-based models, but it is still too early to claim any clinical utility.

It is reasonable to assume that a great proportion of women suffering from RIF systematically produce impaired oocytes, which often generate poor-quality embryos. So, it is expected that changing the gamete can lead to better results. Outcomes from OD models support this concept. Figure 16.5 highlights that regardless of the indication for OD, reproductive results are excellent even for cases of RIF.

Once more, the oocyte is probably the main factor responsible for the occurrence of RIF, whereas the endometrial receptivity seems to play a minor role. Almost 75% of RIF patients show a normal window of implantation (WOI). Though, in some cases, an endometrial receptivity array (ERA) permits the diagnosis of a displaced WOI and a personalized embryo transfer, which can normalize pregnancy and implantation rates.

Cobo et al. recently demonstrated that in a well-developed OD program, the overall probability of achieving a child can be as high as 97.3% until the late forties. It is worth noting that such an elevated CLBR is usually achieved when 25–40 oocytes are consumed, which represents nearly three or four donation cycles. By all means, this certainly endorses the efficiency of the OD irrespective of the recipient’s age and infertility cause.

Over the last few years, new observations with respect to maternal killer-cell immunoglobulin-like receptor (KIR) and fetal HLA-C compatibility have shown that it could also influence implantation and live birth rates among patients with RIF, including in OD cycles. The mechanisms through which the KIR-HLA matching could influence implantation and live birth rates are discussed elsewhere. It must be recognized that these are preliminary observational data at best, and randomized clinical trials are still needed. Future studies are urged to prove the clinical utility of the KIR-HLA matching, especially in RIF.

CONCLUSIONS

Finally, we go back to the initial question: is the seed or the soil responsible for RIF? The best guess would be both. The key for couples with an adequate control of maternal and technical elements probably relies on improving embryo potential, which, in some cases, can only be achieved using OD.

Thus, as most couples struggling with RIF are prone to accept alternative therapies, OD might be an attractive treatment option to achieve their reproductive goals.
Figure 16.5 OD model: (a) CLBR according to the total number of embryos transferred and (b) PR according to donor cycle number. (Adapted from [a] Geraedts J et al. Hum Reprod. 2011;26(11):3173–80; [b] Budak E et al. Fertil Steril. 2007;88(2):342–9, with permission.)

REFERENCES

Is oocyte donation efficient in patients with recurrent implantation failure?


Sperm donation and recurrent implantation failure

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INTRODUCTION

Sperm donation refers to the provision by a male (donor) of his sperm (donor sperm), which has been manipulated outside the human body with the purpose of achieving a pregnancy in a female who is not his sexual partner. The donor, typically, intends to have no legal relationship to any resulting offspring. The sperm may be donated privately and directly to the recipient or through a sperm bank or fertility clinic. Donor sperm is commonly used in artificial insemination, which refers to the introduction of sperm into the vagina (intravaginal insemination), uterus (intracervical or intruterine insemination), or oviduct by a means other than sexual intercourse. Artificial insemination using sperm from a male other than the patient’s partner is called therapeutic donor insemination (TDI). Less commonly, donor sperm may be used in in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedures, especially in women who are not good candidates for TDI, such as those with subfertility due to tubal disorder, uterine malformation, active pelvic infection, or uncorrected anovulation.

TDI is the oldest artificial reproductive technique in use for the treatment of male infertility. In the US, over 170,000 women were treated with TDI in 1987, while in 1990, it has been estimated that 11,400–23,400 pregnancies were achieved annually by TDI. The advent of ICSI has decreased the indication for sperm donation; nevertheless, recently emerged reproductive choices have expanded the therapeutic role of TDI. Currently, the main reasons for TDI cycles, according to ESHRE Capri Workshop Group, are

- At least three failed IVF or ICSI cycles with partner’s sperm
- Nonobstructive azoospermia (NOA), confirmed by testicular sperm extraction (TESE)
- Women without a male partner, such as single women seeking conception or women in a female–female relationship (lesbians)

Moreover, TDI should be considered in

- Couples in whom the male partner has severe sperm or seminal fluid abnormalities or ejaculatory dysfunction
- Couples in whom the male or both partners are carriers of a heritable disease
- Couples who are serodiscordant for sexually transmissible viral infections
- Couples who are incompatible for red-cell antigens, associated with hemolytic disease of the newborn, and with a history of a severely affected infant

Unsuccessful IVF or ICSI treatment could be due to implantation failure. Repeated implantation failure (RIF) refers to a situation in which implantation has repeatedly failed to reach a stage recognizable by pelvic ultrasonography; nevertheless, as yet, no universally accepted definition exists. According to a proposed definition, RIF is the failure to achieve clinical pregnancy after the 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. RIF could be due to embryo quality or endometrial factors or both. Regarding the relationship between sperm donation and RIF, two clinically important
questions arise: (a) What is the incidence of RIF in TDI, used as primary treatment for infertility? and (b) What is the therapeutic role of TDI in the management of couples with RIF? Unfortunately, there are no or few data published as yet that can provide a clear answer to either questions.

FAILURE OF TID CYCLES AS A PRIMARY INFERTILITY TREATMENT

There are no studies so far reporting on the incidence of RIF in sperm-donation cycles. This is probably due to the fact that no universally accepted definition of RIF is established and the fact that the number of recipients not achieving a pregnancy is usually not reported. Instead, the clinical outcome is expressed as the pregnancy and delivery rates, which depend upon the method of insemination used, on the semen parameters, and on the age and body weight of the female recipient.

One of the main indications for using TDI is NOA of the male partner. The largest amount of data comes from the French Federation of Centre d’Etudes et de Conservation des Oeufs et du Sperme humain (CECOS), which has organized one of the largest sperm-donation programs in Europe. Between 1998 and 2007, 2757 couples have requested treatment with TDI and have been included in the CECOS Paris-Cochin study. The indication for sperm donation was azoospermia in 69% and other sperm defects in 27% of the couples. The first treatment was intracervical insemination (ICI) in 26% of couples, intrauterine insemination (IUI) in 68%, IVF in 4%, and ICSI in 2% of the couples, while the pregnancy and delivery rates were 6% and 4.8%; 14.9% and 12.1%; 27.2% and 16.3%; and 26.1% and 16.8%, respectively. Unfortunately, no data regarding the rate of RIF in IVF or ICSI cycles were published.

Another large retrospective study examined the crude and expected cumulative delivery rates in 364 women aged <40 years undergoing a total of 660 donor ICSI cycles. The main indications were severe male infertility, including azoospermia (n = 190), requests from lesbian couples (n = 102), and requests from single women (n = 72). The crude cumulative delivery rate after three ICSI cycles in the whole population and in women aged 20–29, 30–37, and 38–39 years were 62%, 79%, 61%, and 50%, respectively. It can be assumed that the failure rates after three donor ICSI cycles, consistent with the definition of RIF, were 38%, 21%, 39%, and 50%, respectively. However, these numbers do not reflect the actual incidence of RIF, which is probably significantly less, because failure to deliver a baby is not only due to implantation failure. After the third cycle, the increase in delivery rates became less pronounced and reached a plateau in the fourth cycle in all age groups. As expected, this study has shown that failure rates in donor ICSI cycles increased significantly with increasing female age. The same group reported on the cumulative delivery rates in women aged >40 years undergoing either TDI or IVF/ICSI with donor sperm or switched from TDI to donor IVF/ICSI. Crude cumulative delivery rates after six IUI cycles and three primary ICSI cycles (no previous IUI) were similar in both groups (24% vs. 26%). An older and smaller (39 cycles included) study published data regarding the cumulative pregnancy rate (PR) after IVF with frozen donor sperm. The cumulative PR for one year (three donor IVF cycles) was 44.7%. Finally, a recent study examined the pregnancy rates after TDI or IVF with donor sperm, according to treatment indications. They found that pregnancy rates associated with the first treatment cycle were higher in women undergoing TDI due to NOA than those after ICSI failure or without a male partner (29.1%, 27.6%, and 22.6%, respectively), while in women treated with donor IVF cycles, the respective figures were 42.1%, 48.7%, and 38.2%. It seems that donor IVF cycles have higher success rates than TDI, irrespective of treatment indications, and single women have the highest failure rates, probably due to their advanced age.

SPERM DONATION CYCLES AS A TREATMENT OPTION FOR RIF

Rationale for the use of donor sperm

According to a recently proposed definition, RIF is expected to result primarily from endometrial/uterine factors. However, given the fact that current methods for the assessment of embryo quality
are still quite subjective and not always accurate, inevitably there will be a proportion of RIF cases due to gamete or embryo factors. The embryo quality depends both on the quality of oocytes and spermatozoa. Poor-quality sperm may produce poor-quality embryos. It is known that the genetic and epigenetic integrity of sperm is essential for fertilization, normal embryo development, and successful implantation.

Animal and human studies have linked sperm DNA damage to poor embryo development and failure to achieve either spontaneous or assisted conception. A recent meta-analysis has found a modest but statistically significant negative effect of sperm DNA damage on pregnancy rates after IVF and/or ICSI treatment. Increased DNA fragmentation has also been associated with increased miscarriage rates and recurrent pregnancy loss after IVF or ICSI; nevertheless, its association with RIF has not been elucidated yet, despite a recent study that did not find any effect. Moreover, sperm aneuploidy and chromosomal structural aberrations have been implicated in the pathogenesis of recurrent pregnancy loss and RIF. A more detailed presentation of the andrological causes of RIF can be found in Chapter 9.

All couples experiencing RIF should undergo a thorough investigation in order to elucidate the cause. The investigation plan should include hysteroscopy, hysterosalpingography, pelvic ultrasonography, assessment of ovarian reserve and function (follicle-stimulating hormone [FSH], anti-Müllerian hormone [AMH], antral follicular count), and parental karyotyping. Investigations of research value are thrombophilia screen, sperm DNA integrity tests, and sperm fluorescence in situ hybridization (FISH). If the diagnostic work-up reveals no female factor and the problem lies with the embryo due to impaired semen quality, a number of treatment modalities have been proposed. In the case of increased DNA fragmentation (DF), treatment options include: lifestyle modifications (healthy diet, weight loss, cessation of smoking, reduced alcohol consumption); antioxidant therapy; selection of sperm with low levels of DNA damage using a number of techniques, such as annexin-V columns or sperm hyaluronic acid binding; the implementation of intracytoplasmic morphologically selected sperm injection (IMSI), which utilizes spermatozoa selected under high-power magnification according to a predefined set of morphological criteria; and ICSI using surgically retrieved testicular sperm instead of ejaculated sperm. However, data regarding the effectiveness of the above-mentioned approaches in increasing the implantation and pregnancy rates in couples with RIF are limited and inconsistent.

In the case of chromosomal numerical or structural abnormalities in the male partner, genetic counseling is essential to inform the couple about the associated risks in the offspring and their reproductive options. Preimplantation genetic diagnosis (PGD) is recommended, especially in cases of reciprocal translocations, as it has been shown in some studies to produce a more favorable reproductive outcome. However, pregnancies conceived with a male partner with sperm aneuploidy, even if PGD has been employed, still carry an increased risk of aneuploidy; thus, appropriate prenatal screening and testing should be offered.

It is logical to assume that the only way to eliminate the impaired semen as a contributing factor to RIF and to alleviate the genetic risk to the offspring associated with chromosomal abnormalities of the sperm would be to proceed with donor sperm cycles. However, studies comparing the efficacy of sperm donor cycles to any of the above-mentioned treatment strategies, as the preferred first-step management option of couples with RIF due to impaired semen quality, are lacking. All couples with RIF, after undertaking an appropriate diagnostic evaluation, should be informed about their reproductive options. Sperm donation may be advised when there is

- Absence of a female factor causing RIF
- Clinical or laboratory evidence of impaired sperm or embryo quality (especially chromosomal aberrations)
- Implantation failure, despite other treatment attempts or if the prognosis of further IVF cycles is considered poor
Efficacy of sperm donation cycles after IVF/ICSI failure

There are very few studies, retrospective only, examining the outcomes of sperm donation cycles after IVF/ICSI failed attempts. All these studies, however, included couples with at least one unsuccessful IVF/ICSI procedure and did not evaluate the reproductive efficacy of sperm donation specifically in couples with RIF. The clinical PR per cycle and the cumulative PR per couple after six or seven donor sperm cycles vary significantly across studies. A multicenter study conducted in France within CECOS included 162 couples treated with TDI after intraconjugal ICSI failure and reported a pregnancy rate per cycle of 5.8% in women who had obtained poor-quality embryos in previous ICSI cycles and above 19% in those who had obtained good-quality embryos.35 Gorrill et al. analyzed a series of 61 TDI cycles in 19 couples who had previous failed ICSI attempts performed for severe male factor infertility and reported a PR per cycle of 27.9% and a cumulative PR of 84.2%. Pregnancies occurred within a mean of $3.2 \pm 1.8$ cycles.36 The largest study so far included 319 couples, who underwent a total of 1159 TDI cycles after a total of 1011 unsuccessful intracouple ICSI cycles. The mean PR per cycle and the cumulative PR after seven TDI cycles were 12% and 43.6%, respectively. Three-quarters (78.4%) of pregnancies were achieved during the first four cycles of insemination.37 The variation in the results could be due to differences in the number of couples included in each study and in population characteristics.

One study13 reported on the pregnancy rate associated with the first treatment cycle of TDI or IVF using donor sperm (IVF-D) in couples with at least three previous failed ICSI cycles. The indications for IVF-D were tubal obstruction, moderate/severe endometriosis, and advanced maternal age ($\geq 39$ years old). The success rates for IVF-D and TDI were 48.7% and 27.6%, respectively. The same study reported that previous ICSI failure was the most prevalent indication for sperm donation cycles, the other ones being NOA and single women seeking fertility. In TDI procedures, higher pregnancy rates were achieved in patients with azoospermia (29.1%) than in cases of ICSI failures (27.6%) and single women (22.6%) ($p = 0.020$), whereas in IVF-D cycles, better results were obtained in cases of ICSI failure (48.7%) than in NOA (42.1%) and single women (38.2%) ($p < 0.001$), whose advanced age tended to be a factor.

Apart from pregnancy rates, live birth rate is considered to be clinically a more useful parameter in expressing the success rate of an assisted reproductive technology (ART) program, especially the cumulative live birth rate after a defined number of treatment cycles.36 According to the study by Gorrill et al., the application of TDI after ICSI failure produced a live birth rate per cycle of 24.6% and a cumulative live birth rate of 88% after seven cycles of insemination.36 However, these high rates were not confirmed in more recent studies. Leguy et al. evaluated retrospectively the reproductive outcome of sperm donation cycles in 71 couples who failed to conceive after intracouple ICSI treatment for male infertility and reported that, after donation, 30 couples (42.2%) succeeded in being parents.39 Frapsauce et al. reported on the outcome of 175 TDI cycles in 47 couples after 120 failed ICSI cycles for various reasons. The mean live birth rate per cycle and the cumulative live birth rate after six cycles were 16.6% and 62%, respectively. Overall, a live birth occurred after $3.7 \pm 1.8$ cycles.

It can be concluded that TDI is an effective reproductive option for couples with previous failed IVF/ICSI treatments, as at least half of them can achieve parenthood. The success rates are comparable to those obtained in couples to whom TDI is proposed as an initial treatment.37 However, in cases of couples experiencing RIF during oocyte donation (OD) cycles with the partner’s normal semen, switching to donor sperm in subsequent cycles does not seem to improve the reproductive outcome in terms of either birth rate or live birth rate.

Prognostic factors affecting the outcome of TDI

It is of great importance for the clinician involved in the management of couples with RIF to be able to differentiate those who would have a favorable reproductive outcome with TDI. However,
Sperm donation and recurrent implantation failure

Evidence is limited to only a few studies, which suggest that the main factors implicated in the prognosis of TDI cycles are female partner’s age, ovarian response, number of good-quality embryos obtained in previous IVF/ICSI cycles, semen characteristics, and number of TDI cycles.

**Female age:** Increasing female partner’s age decreases the success rate of TDI cycles. Hennebicq et al. have shown that cumulative PR was 51.7% for women under 34 years of age, 38.1% for women between 34 and 40 years, and 23.8% for women aged over 40 years ($p = 0.010$). The negative impact of woman’s age on the reproductive outcome of TDI has also been reported in other studies.$^8,42,43$

**Ovarian response:** Poor ovarian response during antecedent intracouple ICSI cycles has been proposed as a negative prognostic factor for the success of subsequent TDI treatment.$^8,35$ In a larger recent trial,$^37$ although cumulative PR tended to be higher (44.5%) in cases when $>3$ oocytes were retrieved in previous intracouple ICSI cycles compared with cases when $\leq 3$ oocytes were obtained (33.5%), no statistical significance was reached ($p = 0.353$). Moreover, using a saturated logistic regression model, no significant relationship was found between ovarian response during intracouple ICSI and clinical PR during TDI (odds ratio [OR] 1.44, 95% CI 0.46–4.51; $p = 0.115$).$^{37}$

**Embryo quality:** The results of studies evaluating the impact of the availability and the number of good-quality embryos obtained during intracouple ICSI cycles on the success of TDI are inconsistent. Saias-Magnan and Mandelbaum have demonstrated that the PR during TDI was significantly higher (>19% vs. 5.8%), when couples had morphologically good embryos during previous ICSI cycles compared with those who had only poor-quality embryos.$^{35}$ Frapsauce et al. have found higher, although not statistically significant, live birth rate per cycle (20% vs. 13.3%) and cumulative live birth rate after six cycles of TDI (68% vs. 54.5%) in couples in whom at least one top-quality embryo (TQE) was obtained in previous intracouple ICSI cycles compared with those without TQE.$^{40}$ The same study also reported that couples with $\geq 1$ TQE achieved live births faster than couples without TQE.$^{40}$ By contrast, Gorrill et al. demonstrated a high rate of TDI pregnancies irrespective of the embryo quality during previous failed ICSI procedures.$^{36}$ A French CECOS study, which analyzed the results of TDI according to embryo quality on day two after ICSI, has shown that the rate of TQE was similar in couples who achieved live births after TDI cycles and in those who failed.$^{39}$ In a recent larger multicenter study, also from CECOS, no significant difference was detected in pregnancy rate per TDI cycle (10.5% vs. 12.9%; $p = 0.278$) or cumulative PR (40.7% vs. 43.6%, $p = 0.651$) between couples in whom no TQE and couples in whom at least one TQE was transferred during intraconjugal ICSI procedure.$^{37}$ However, the authors of the latter study have underlined that embryo quality was not addressed properly, and the absence of an effect on subsequent TDI success could be also due to the multicenter design of the study, leading to a bias in embryo classification.$^{37}$

**Semen characteristics:** Couples with male partners diagnosed with azoospermia or cryptozoospermia seem to have better outcomes in TDI performed after repeated ICSI failures compared with couples with male partners diagnosed with oligoasthenoteratozoospermia or normal semen parameters. The recent large multicenter study from CECOS demonstrated that clinical pregnancy rates during TDI were higher when male partners presented with azoospermia or cryptozoospermia compared with those with oligoasthenoteratozoospermia or normal sperm: 14.8% versus 10.1% ($p = 0.015$) per cycle and 53.1% versus 37.2% ($p = 0.005$) per couple, respectively. According to the origin of sperm used in previous failed ICSI cycles, the TDI cumulative PRs were higher when the sperm was surgically obtained in comparison with ejaculated sperm: 55.6% versus 36.2%, respectively ($p = 0.001$).$^{35}$ It can be assumed that in cases of azoospermia or cryptozoospermia, the intraconjugal ICSI failure is mainly due to male factors; therefore, the use of donor sperm would alleviate the problem and allow an embryo with implantation capacity to be obtained.$^{37}$ On the contrary, in an older study with a smaller sample size, azoospermia was found to be a poor prognostic factor for the success of TDI.$^{39}$

**Number of TDI cycles:** The probability of achieving a clinical pregnancy with TDI decreases as a function of increasing number of TDI cycles (OR 0.87, 95% CI 0.78–0.97; $p = 0.010$). The reported
mean PR per cycle during the first four TDI cycles was 21.7% and dropped after the fourth cycle to 4.5%.37 This finding has also been reported in other studies.44 It seems that as the number of TDI cycles increases, the woman's age also increases, and the negative impact of advanced female age on reproductive outcomes is well documented.45 Moreover, it could be due to the fact that the good-prognosis women fall pregnant quicker and this leaves women of poorer prognosis in the higher order TDI cycles.

Taken together, the above-mentioned evidence indicates that the transition to sperm donation cycles should be proposed at key moments, taking into account especially the woman's age and the sperm characteristics of the male partner in order to achieve the highest probability of pregnancy. The best results should be expected when TDI cycles are offered, as a treatment option after repeated IVF/ICSI failures, to women aged under 34 years whose partners have been diagnosed with azoospermia or cryptozoospermia.

CONCLUSIONS

RIF is a distressing condition both to the couples and the treating physician. Data regarding the incidence of RIF among sperm donation cycles performed as initial treatment for infertility are lacking. On the other hand, sperm donation could be an effective treatment option for couples struggling with repeated IVF/ICSI failures only after an extensive diagnostic evaluation and detailed consultation with the couple has been performed. Before offering TDI, the clinician should take into account specific characteristics of the couple, such as the female age and the semen status in order to maximize the clinical benefit. However, large-scale prospective studies evaluating the reproductive efficacy of TDI and the factors affecting the outcome specifically in couples with RIF are warranted.

REFERENCES

2. Ginsburg ES, Srouji SS. Donor insemination. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA (accessed on December 02, 2017).


14. Racowsky C, Jackson KV, Cekleniak NA, Fox JH, Hornstein MD, Ginsburg ES. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. *Fertil Steril*. 2000;73(3):558–64.


INTRODUCTION

The enigmatic condition of recurrent implantation failure (RIF) may be much related to conditions that characterize the reproductive process in the human species. With optimal fertility in the second and third decade of life, a rapid decline is observed in the fourth, resulting in a state of natural sterility on average in the early forties. The majority of explanatory factors seem to be present in the female, as males tend to have uncompromised fertility up to the age of 50, with a slow decline thereafter. Factors that are believed to highly contribute to the limited fertile lifespan in the human female are oocyte and subsequent embryo quality, for which the mechanisms are largely unknown.1–3 Next to embryonic aneuploidy, mostly related to meiotic errors, many other failures in the oocyte-follicle complex may contribute to the overall low, and with age rapidly declining, quality of the human embryo.4,5 Still, aging or dysfunction of the endometrium may have a certain degree of contribution, although oocyte donation studies have excluded a major role at this level.

It is therefore no surprise that failures occurring in the process of assisted reproductive technology (ART), after the moment the embryo has been placed in the uterus, are so frequent. From this knowledge and awareness, it is within expectation that many couples will not achieve a pregnancy in spite of repeatedly receiving morphologically well-behaving embryos. This is much to the frustration of both couple and physician and elicits a heart-breaking quest into the “why.” Although part of the answer can be drawn from the aforementioned, the obligation for the doctor is obvious to rule out any adjustable factor of “substandard” care.

The process of ART called in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) comprises three distinct phases: the retrieval of gametes, simple for the human male, complex for the female, where getting eggs may be more important than a strenuous strive for all that can be squeezed out; the laboratory phase, where some quality characteristics can be observed such as fertilization rate, embryo development rate, and embryo implantation rate; and finally, the luteal phase with the embryo transfer, a handling with only indirect and incomplete information on the correct “arrival” of the embryo, but also the endocrine management of endometrium development and timing. The subsequent event, implantation, will then unveil itself at the moment of human chorionic gonadotrophin (hCG) testing, or not.6

It is important to realize that, although each of the aforementioned three phases are crucial for the ART process, the relative contribution to the final aim, a live birth, is not so clear. Will the approach in the stimulation and egg retrieval phase make a difference? Recent studies seem to indicate that, apart from a few basic concepts, any approach will be good enough.7–9 The IVF laboratory phase must demonstrate that gametes handling leads to fertilization and embryo development, where many tools and gadgets on embryo quality and selection may or may not give the big advances that are frequently hoped for.10–13 Finally, the management of luteal function toward
the endometrium may have been a slightly neglected area, as it has surfaced through the increasing application of the agonist trigger, where luteal phase insufficiency developed by the elimination of the powerful hCG stimulus.

For the latter phase, the execution of the embryo transfer must be considered as a distinct factor of success of the ART process, where knowledge is both present and absent. We know the role of a smooth procedure, using specific materials and tools such as ultrasound guidance, but the suggestion that the physician performing the transfer could make an important difference has never led to robust research to sort out this feature. In this chapter, we will review the existing knowledge on how the embryo transfer procedure can be optimized, and where research could fill up open holes in our knowledge. It goes without saying, that, if the transfer procedure has been carried out according to current best standards, recurrent non-implanting of the embryo(s) cannot easily be explained from the transfer process. Still, rigorous attention for this step may help in minimizing the rates of non-implantations.

According to a survey performed in 2014\textsuperscript{14} and 2017,\textsuperscript{15} there may be quite some practice variation going on in the steps applied in the embryo transfer procedure. When we would define the common denominator of what we do daily, then we will allow any trained member of the team to participate in this activity; we tend to clean the upper vagina and the outer surface of the cervix with sterile water, we either perform a direct transfer with preload or routinely apply a mock transfer to probe the accessibility of the cavity or use an afterload approach after the catheter has been introduced. Most of us, if not all, will have abdominal ultrasound guidance to ensure the optimal position of the catheter tip at the moment of push-out, which will be generally in the middle-upper part of the endometrium echo, at least halfway between fundus and internal os, but not closer than 1.0 cm from the fundus. In cases in which we have trouble accessing the cavity, most physicians will revert to the use of a malleable stylette catheter and/or a tenaculum to reposition the cervical-uterine alignment. In such conditions, the embryo is frequently returned to the incubator until the passage into the cavity has been accomplished. The injection or push-out procedure is typically executed by mechanical force on the plunger of the attached syringe, mostly by physician, after which most of us will wait a few seconds, rotate the catheter, and gently pull it back. Then, over 50\% of clinics will have the patient rest supine for 15–30 minutes. With a difficult transfer completed, many of us will consider dilating the cervix one month prior to a subsequent transfer.

It is beyond doubt that we all have an idea of what the optimal embryo transfer (ET) procedure is. In fact, it is the one that leads to a healthy live birth born months later. Still, “suboptimal” ET procedures do lead to live births, and the characteristics of the ideal ET procedure may therefore be difficult to identify. From studies that have inventoried the optimal transfer execution, it has appeared that up to 50\% of real day life transfer procedures may not be classified as optimal. Reasons for this are various, such as difficult passaging of the internal os, the need to bend and steer the catheter around a “corner,” the presence of blood at the tip of the catheter after procedure completion, the need to use a stiff catheter or a tenaculum to steer, and the need to use ultrasound guidance if not applied routinely.\textsuperscript{16–18} One could pose the question: what could the effect be if all these supposedly nonoptimal transfers could be transformed into optimal ones? From studies comparing ongoing pregnancy rates among these two groups, it has been demonstrated that, while background factors, such as female age, duration of infertility, and primary versus secondary, and treatment factors, such as oocyte and embryo number and endometrial thickness, do not differ, the effects on success are pronounced. From the review by Phillips\textsuperscript{18} it becomes clear that the reductive effect is \(\sim 25\%\) (CI \([-34\%] - [-14\%]\)) for difficult transfers, while the effect of only blood at the catheter after withdrawal may be as low as 4\% (CI \([-18\%] - [+14\%]\)).

The question then will be how to identify the factors that really help in increasing the rate of optimal transfers. In the following is presented the scientific evidence on factors or procedures or approaches that will or may make a difference. On the basis of that, a best (possible) practice can be identified that helps in getting the best out of the results of the two preceding phases of the ART process: getting oocytes (and sperm) and creating embryos.
THE PHYSICIAN

Most IVF centers will apply evaluation of certain key performance indicators (KPIs), among which the success rates of the physician performing the transfer will be present. In many cases there will be remarkable differences between operators, but a large part of these differences can be explained by variation in patient (type, duration and cause of infertility, female age) and treatment (number of oocytes, embryo usage rate) factors. As manual dexterity may vary among physicians and the smoothness level of an embryo transfer is strongly associated with the proportion of ongoing pregnancies, the question can be how much it matters whether doctor A or B will do your transfer. Several articles have suggested that the physician indeed is an independent success factor, provided that the procedure of catheter loading, the type of catheter, and the use of ultrasound guidance all have been standardized. However, when correction for patient and treatment factors was applied, the differences between operators seemed to become reduced, or even absent. Especially in the studies by van Weering and Lalwani, patient and treatment factors seemed to be evenly distributed across physicians, and differences in ongoing pregnancy rates were not statistically significant. Still, differences in skills and troubleshooting between operators do exist, and the question is open whether or not these differences are large enough to affect the overall performance of an ART program. In a high-quality study, comparing the effect of the type of ET catheter on live birth rates, no overall difference was observed between the two treatment arms. However, the three experienced physicians that were assigned to perform transfers in this study did perform differently when applying one or the other ET catheter, thereby indicating that the “physician” factor does exist, albeit coupled to a specific embryo transfer catheter. Final proof for this may come from a study where, in a blinded fashion, patients are randomized to either undergo the transfer by one of the team members under routine care planning, or, by a selection of the “best” performing physicians of this team. Such trial design may imply quite some challenges.

If we cannot exclude the role for the physician next to patient and treatment factors, the realistic challenge may be training and frequent supervision. Typically, starting physicians will need between 25 and 50 supervised transfer procedures to reach levels of performance comparable to experienced colleagues. Several studies have indicated that structured training of this procedure will help starting physicians to perform at the same level as experienced physicians within a short time period. The implementation of intrauterine inseminations as a training tool has been shown to reduce the time period necessary to reach an adequate level of performance, while only minimally jeopardizing the interest of the IVF/ICSI patient. Today, simulators for embryo transfer procedures have entered the market and seem to improve the learning curve toward optimal proficiency in starting physicians in reproductive medicine, although direct comparison to more “classic programs” are currently lacking. As mentioned, even among well-trained and experienced physicians, real differences in performance may remain present and may urge to install, or at least research, regular refreshing courses, in the form of peer-to-peer learning.

THE CATHETER

As the smoothness of the transfer procedure is consistently related to the outcome live birth, the choice of transfer catheter may be an important step in optimization. While soft catheters have the property of doing the least physical harm, the stiffer catheters have the benefit of steering “potential” when access to the uterine cavity is difficult. For many years, soft catheters now have the preference, based on several comparative trials. Still, testing catheters in trials has in fact been a work in which one catheter is combined with several physicians, under the assumption that the combination catheter-with-doctor would not affect the results of the comparison. The contribution of several doctors to the execution of the transfer therefore was not seen as a potential confounder of the interpretation of results.

Therefore, we tend to embrace the results of large meta-analyses that have appeared over the last two decades and that have shown an obvious benefit for the soft catheters. The most recent review
The ultrasound

has been done quite some years ago by pooling available comparative studies and demonstrating that the soft catheter types offered a 30%–40% increase in clinical pregnancy rates compared with hard catheters.\(^{30,31}\) Therefore, the current image is that soft catheters are the standard of practice (Figure 18.1). Still, the current preference for the use of abdominal ultrasound guidance of the transfer procedure may have slightly changed our views. In two studies, the claim was such that comparing a soft versus firm catheter with accompanied ultrasound (US) guidance did not produce differences in pregnancy rates.\(^{32}\) Although the reasoning was that navigation could become more smooth with the stiff catheters using the ultrasound information, the power of the studies was insufficient to ensure an equipoise between the two approaches.

Comparisons of various soft catheters has never resulted in a systematic review, due to the scarcity of high-quality comparisons.\(^{33}\) Yet, the large study by Yao revealed that the overall comparison of two soft catheters, applying the fixed distance technique without US guidance, demonstrated an equal rate of clinical pregnancy. Interestingly, for two of the three physicians executing all of the transfers, a significantly higher clinical pregnancy rate was observed using either the one or the other catheter. This indicates that the combination of physician and transfer catheter can make a difference in the prospect for the patient. Even if the soft catheters remain the preferred tool, physicians may have a more successful job when more than one type of catheter is available in a center to choose from and get experienced with.

**THE ULTRASOUND**

Many of the physicians in reproductive medicine have been trained in performing “blind” embryo transfer procedures. Roughly two distinct methods existed, the “clinical-touch” and the
“fixed-distance” approaches, in order to deal with positioning of the catheter at the moment of push-out of the embryo(s). The clinical-touch method implied a sounding procedure until touching the uterine fundus, with subsequent withdrawal of the catheter by 1 cm and push-out. This approach was considered as potentially disadvantageous in the hands of some physicians, especially when using a firm catheter, as the touching could imply harm done to the endometrium lining, with bleeding and uterine contractions as the result. In contrast, the fixed-distance technique, with or without prior assessment of uterine sounding depth, and limiting the introduction of the catheter to \( \sim 6 \) cm, with subsequent push-out, was proposed as the more atraumatic, and thereby more successful, way of working, in the hands of any physician. 34

The visualization of the process of catheter introduction, as well as the deposition position of the embryo(s) by using abdominal ultrasound has professionalized the fixed-distance technique. Ultrasound guiding has thereby become the standard of care, and systematic reviews have provided a solid basis for this (Figure 18.2). Interestingly, using US guidance in fact implies the use of two distinct processes: looking at (and possibly guidance of) the entering catheter and defining the position for push-out but also altering the uterine position by instructing the patient to have a

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>UGET n/N</th>
<th>CTET n/N</th>
<th>Odds ratio M-H, Fixed, 95% CI</th>
<th>Weight</th>
<th>Odds ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Live birth</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Azmy 2009</td>
<td>181/435</td>
<td>80/418</td>
<td>*</td>
<td>10.4%</td>
<td>3.01 (2.21, 4.10)</td>
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<tr>
<td>Drakeley 2008</td>
<td>190/834</td>
<td>187/815</td>
<td>31.8%</td>
<td>0.99 (0.79, 1.25)</td>
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</tr>
<tr>
<td>Martin 2004</td>
<td>23/50</td>
<td>17/50</td>
<td>2.0%</td>
<td>1.65 (0.74, 3.71)</td>
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<tr>
<td>Matorras 2002</td>
<td>56/255</td>
<td>36/260</td>
<td>6.1%</td>
<td>1.75 (1.11, 2.77)</td>
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<tr>
<td>Subtotal (95% CI)</td>
<td>1574</td>
<td>1543</td>
<td>50.2%</td>
<td>1.53 (1.29, 1.80)</td>
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<td>Total events:</td>
<td>450 (UGET), 320 (CTET)</td>
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<td>Heterogeneity: ( \text{Chi}^2 = 32.47, \text{df} = 3 ) (p &lt; 0.00001); ( I^2 = 91% )</td>
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<td>Test for overall effect: ( Z = 5.03 ) (p &lt; 0.00001)</td>
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</table>

2. Ongoing pregnancy

<table>
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<tr>
<th>Study or subgroup</th>
<th>UGET n/N</th>
<th>CTET n/N</th>
<th>Odds ratio M-H, Fixed, 95% CI</th>
<th>Weight</th>
<th>Odds ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammar 2013</td>
<td>9/45</td>
<td>5/45</td>
<td>0.9%</td>
<td>200 (0.61, 6.52)</td>
<td></td>
</tr>
<tr>
<td>Coroleu 2000</td>
<td>85/182</td>
<td>52/180</td>
<td>6.1%</td>
<td>2.16 (1.40, 3.33)</td>
<td></td>
</tr>
<tr>
<td>Dvar 2007</td>
<td>17/90</td>
<td>11/90</td>
<td>1.9%</td>
<td>1.67 (0.73, 3.81)</td>
<td></td>
</tr>
<tr>
<td>Eskander 2008</td>
<td>68/183</td>
<td>50/190</td>
<td>6.7%</td>
<td>1.66 (1.07, 2.57)</td>
<td></td>
</tr>
<tr>
<td>Garcia-Velasco 2002</td>
<td>100/187</td>
<td>94/187</td>
<td>9.5%</td>
<td>1.14 (0.76, 1.71)</td>
<td></td>
</tr>
<tr>
<td>Kosmas 2007</td>
<td>36/101</td>
<td>38/95</td>
<td>5.5%</td>
<td>0.83 (0.47, 1.48)</td>
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</tr>
<tr>
<td>Marconi 2003</td>
<td>22/41</td>
<td>12/42</td>
<td>1.2%</td>
<td>2.89 (1.17, 7.18)</td>
<td></td>
</tr>
<tr>
<td>Tang 2001</td>
<td>94/400</td>
<td>76/400</td>
<td>12.7%</td>
<td>1.31 (0.93, 1.84)</td>
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<tr>
<td>Weissman 2003</td>
<td>36/160</td>
<td>28/124</td>
<td>5.3%</td>
<td>1.00 (0.57, 1.74)</td>
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</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>1389</td>
<td>1353</td>
<td>49.8%</td>
<td>1.40 (1.19, 1.66)</td>
<td></td>
</tr>
<tr>
<td>Total events:</td>
<td>467 (UGET), 366 (CTET)</td>
<td></td>
<td>Heterogeneity: ( \text{Chi}^2 = 13.05, \text{df} = 8 ) (p = 0.11); ( I^2 = 39% )</td>
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<td></td>
</tr>
<tr>
<td>Test for overall effect: ( Z = 3.95 ) (p = 0.000079)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| Total (95% CI)    | 2963     | 2896     | 100.0%                      | 1.47 (1.30, 1.65) |
| Total events:     | 917 (UGET), 866 (CTET) | | Heterogeneity: \( \text{Chi}^2 = 45.91, \text{df} = 12 \) (p < 0.00001); \( I^2 = 74\% \) |
| Test for overall effect: \( Z = 6.36 \) (p < 0.00001) |
| Test for subgroup differences: \( \text{Chi}^2 = 0.48, \text{df} = 1 \) (p = 0.49); \( I^2 = 0.0\% \) |

**Figure 18.2** Meta-analysis of trials showing live birth and ongoing pregnancy rates per woman randomized, comparing ultrasound-guided (UGET) versus clinical-touch (CTET) embryo transfer. (From Brown J et al. Cochrane Database Syst Rev. 2016;2016(3), with permission.)
full urine bladder at the time of embryo transfer. The latter may affect the ease of introduction by straightening of the uterine cavity. The relative contribution of the two processes has become clear from a systematic review in which US-guided transfer was compared in cases with bladder-full against bladder-empty instructions. With a relatively small sample size, the ongoing pregnancy rate was significantly better in the group with filled bladder, but the quality of the two studies included was considered quite low. The reviews on US-guided embryo transfer (UGET) compared with any form of blind (clinical) touch have demonstrated a considerable and consistent positive effect on ongoing pregnancy rate from using the abdominal ultrasound. The quality of this evidence was considered moderate to low by the authors, but when looking at only those trials that had low risk of bias, the evidence of an overall benefit for UGET remained. The size of the most likely effect was a ~20% increase in live birth rate (OR 1.17, 95% CI 1.01–1.36), while in the ultrasound guided transfers, only 8% of transfers were reported to have been difficult, compared with 12% in the clinical-touch group (risk ratio of non-easy transfer 0.69 [0.57, 0.82]).

As the use of abdominal ultrasound guiding necessitates the availability of a well-trained sonographer, efforts have been put on applying the transvaginal approach in steering both the catheter as well as the guiding ultrasound by the physician (VGET). The work-up for this varies across studies, but mostly, after positioning the outer sheath of an empty transfer catheter just passed the internal os, the speculum is removed, transvaginal probe with sterile cover put in position, and the loaded inner catheter sheath inserted by the embryologist. The catheter tip is then positioned by the physician for placement of the embryo at the center of the endometrial cavity. Studies that compared the abdominal to the transvaginal ultrasound-guiding approaches have not demonstrated any benefit for either of the approaches, indicating that the choice will depend on local availability of sonographer staff and the preference of the physician. Moreover, patients may appreciate the absence of the need to have a full bladder.

THE LOADING

The transfer of the embryo(s) into the uterine cavity basically takes place by flushing and filling off the transfer catheter with culture medium, by using a prefilled syringe, and subsequently positioning of the embryo(s) in the tip of the catheter in a drop of culture medium, by means of negative pressure created by gentle withdrawal of the syringe plunger. Push-out of the fluid column containing the embryo(s) will then be established by forward pressure on the plunger. The culture medium volume containing the embryo(s) will often be separated from the initial culture medium filling of the catheter and from the very tip by a small column of air, in order to prevent the fluid column from being lost during the introduction process (air trap). Also, a large volume may result in embryo expulsion out of the uterine cavity per se, while low volumes may result in implantation failure due to suboptimal ambient conditions. In most practices, the medium volume is typically within the range of 15–30 µL. Variation in this “replacing volume” may be quite considerable, probably depending on the volume and diameter specifications of the various transfer catheters used.

Studies that compare different loading techniques are limited in number and mostly focus on the loading volume and the air-trap system. In one study, a low (20–25 µL) was compared to a high replacement volume (40–45 µL), using the three-drop filling method. The randomized control trial (RCT), comprising 216 couples, demonstrated a nonsignificant difference in ongoing pregnancy rates in favor of larger volumes (40.0% vs. 33.3%; p = 0.31). This means that larger studies need to be executed in order to really exclude an important role for this aspect of catheter loading. The use of the air-trap method versus using fluid only has only been researched in two small studies with the overall outcome in a formal meta-analysis that one method is not superior over the other.

In a large survey (265 centers across the globe) on techniques of catheter loading, it was noted that the majority of units wash the catheter prior to loading the embryos. Moreover, a slight preference was reported for the loading by the medium-air-(medium + embryo)-air-medium approach (42%), although the methods of medium-(medium + embryo) in catheter tip (20%) or
medium-air-(medium + embryo) (15%) are also frequently used. As such, the role of the catheter loading may seem to be a relatively unknown factor in the chances of obtaining a live birth from the ART cycle. Comparative studies for different techniques are easy to execute and may unveil possible gaps in our knowledge on these matters.

THE DEPOSITION

Once positioned in the cavity, the forward pressure applied to the syringe plunger will push out the fluid column with associated air column(s) from the ET catheter, leaving air with fluid combined, positioned at some distance ahead of the tip of the inner catheter. This air-fluid combination (bubble) is well visible at ultrasonography and remains so for quite some time. It is believed to represent the position of the embryo(s) in the cavity. Over the past 15 years, studies have addressed the question in which part of the cavity the embryo’s may be best left “behind.”

In an initial systematic review covering over 2000 ET procedures, it was shown that pregnancy rates may be similar when the upper and lower halves of the endometrial cavity are compared, and that push-out with the catheter tip at ~20 mm from the fundal end of the endometrium may be superior to the traditional transfer at ~10 mm. In the period thereafter, however, much ground was made for the belief that the bubble position after the deposition could best be within a distance of 10 mm from the internal fundus in order to obtain optimal prospects for implantation. This would imply that the tip of the catheter at push-out may be best placed at a distance of 15–20 mm from the fundus, as in most cases the embryo bubble will attain a position ahead of the catheter tip (Figure 18.3). However, the control on the direct position of the embryo bubble after deposition may vary depending on push-out speed. In an attempt to control this process, the use of an automated push-out device was tested as to its effects on pregnancy rates. Although the position of the embryo bubble directly after the push-out could be better controlled, the effects on ongoing pregnancy rates were unfortunately not convincing.

More recent studies have noted that the position of the tip of the catheter may not automatically determine the position of the bubble. The action of introducing the catheter will first elicit an increase in uterine peristaltic wave frequency, with subsequent effects on fluid movement. Moreover, the intensity of uterine peristaltic was quantitatively correlated with the distance of fluid movement in the time period of 30 minutes after the embryo transfer. The belief that the position

Figure 18.3 (a) The length of uterine cavity, (b) the distance between the end of the fundal endometrial surface and the tip of inner catheter, and (c) the distance between fundal endometrial surface and air bubbles. (From Cenksoy PO et al. Eur J Obstet Gynecol Reprod Biol. 2014;172(1):46–50, with permission.)
of the bubble at the moment of push-out is related to the prospects for pregnancy may therefore be a misconception, as a recent study has revealed that the position of the bubble some 60 minutes after the transfer may be best related to the outcome pregnancy. So, pregnancy rates can be influenced by the change of the bubble position after the ET completion, although the embryo had been positioned in the “optimal” part of the uterine cavity. Currently, the data on the effect of changes in the embryo bubble position after ET on pregnancy rates are too scarce to draw firm conclusions.

Together, the evidence seems to support depositing the embryo(s) as gentle as possible with soft pressure on the plunger with the tip at a distance of 15–20 mm from the fundus, in order to bring the embryo(s) close to the fundus. However, the final position of the air bubble may be outside the control of the physician performing the transfer, as subsequent uterine peristalsis may further affect this position.

THE MOCK TRANSFER

As the use of ultrasound guidance has gained enormous ground in reproductive medicine today, the use of a trial or mock transfer may seem to have become an echo of the past. The philosophy behind the trial transfer is that by scouting the ease of entrance into the uterus will improve both the easiness of the real embryo transfer, as well as the prognosis for a pregnancy occurring. However, studies have noted that information obtained from the execution of the mock transfer may not reliably foresee conditions at the real transfer execution. This may be true for the position of the uterus, the uterine cavity depth, but the predictability of a difficult real transfer from the information of the trial transfer seems to be quite high (Figure 18.4). Difficulties with execution of the transfer may stem from various sources. In the comprehensive study by Larue, it was demonstrated that the five most common anatomical causes were endocervical crypts, tortuosity of the cervical canal, internal os contractions, pronounced ante- or retro-version of the uterus, and a heterogeneous group of anatomical factors (such as a cesarean section-induced niche, cervical synechiae, and endocervical polyps).

As such potential difficulties can be predicted prior to real transfer, adjustments of the ET procedure can be applied by, for instance, the choice of the catheter, endovaginal ultrasound guiding, or straightening of the uterus. Scientific evidence that prior knowledge of a difficult transfer will improve pregnancy rates has not been delivered, however. In a study by Mansour, 335 couples with an indication for IVF were randomized into either group A (n = 167), in which a trial transfer was...
carried out to base the choice of the catheter upon, or into group B (n = 168), in which patients started IVF treatment without a prior trial ET. The embryo transfer procedure was only difficult in 50 cases (29.8%) in group B, while pregnancy rates (22.8%) in the trial transfer group A were significantly higher compared with group B (13.1%).58 It may be questioned whether these findings are valid, since this study is the only fully published report on this matter, and the extended use of ultrasound guidance may have made prior information on the ease of transfer redundant.

THE BEDREST
What is next if the embryo(s) have been deposited gently in the uterine cavity? Sit up, get dressed, and go home? Or remain as horizontal as possible, do not move, get transported by stretcher to the relax room, and return home not before at least one hour later? It is important to wonder why bedrest after the ET would affect the chances of implanting. The assumption here is that reduced physical activity and the supine position will decrease the risk of expulsion of the embryo(s). Expulsion could be caused by uterine contractions, or by the mere effects of gravity. The latter seems not realistic. The weight of the bubble containing the embryo may be far too low to overcome the resistance that will be caused by the abutting endometrial layers. Moreover, an anteflexed uterus will be positioned almost in a horizontal position when standing, making the effects of gravity quite irrelevant. The former, uterine contractions that may push the fluid-air bubble with embryo around, may be a hazard, but the question will be whether bedrest will reduce such contractility. Indeed, the fluid-air bubbles frequently move after the embryo transfer, even when remaining horizontal, and this may be caused by contractions. When fluid-air bubble position was recorded immediately, 30 minutes later and when the patient stood up, after a transfer with the tip at a distance of 10–20 mm from the fundus, a rather random movement of the bubble within the endometrial area was observed.59 In fact, no signs have been found that standing upright after ET would remove the bubble from this area.60–62

The final decision on the role for bedrest comes from only a few studies that together reveal that there is insufficient evidence to support any specific length of time for women to remain recumbent, if at all, following embryo transfer.63,64 Prolonged bedrest may even reduce the implantation rate.65

ANYTHING ELSE?
Among interventions at or around the time of embryo transfer that have aimed at improving the chance of a live birth, there are many, in addition to those discussed in the previous sections. Below, a brief description will be given on a series of possible adjuvants that have been known in the literature, but that may have not gained much ground or may need more thorough research.

As the mock transfer has been considered a scouting maneuver too remote from the actual ET, the idea of afterloading was proposed, in which an empty catheter is placed just past the internal os. After this positioning, the inner catheter is replaced by a second inner sheath containing the embryo. This allows the benefit of a “mock” transfer, with only minimal manipulation that could lead to traumatizing the embryo, in case of a difficult introduction.66 With such an approach, the implantation rate per embryo was not significantly different (24.7% vs. 20.5%), but in the afterload group, the clinical pregnancy rate was higher (52.4% vs. 34.9%; p = 0.06). Interestingly, after this primary publication, no additional trials were published.

Contamination of the cavity with endocervical microbial flora is believed to affect the pregnancy rates significantly. Prophylactic antibiotics administered at the time of oocyte retrieval resulted in a reduction of positive microbiology cultures of embryo catheter tips, with a suggested improvement in pregnancy rates.67 However, from a review on studies using amoxicillin/clavulanic acid prior to embryo transfer, no alterations of clinical pregnancy rates were noted.68

Cervical mucus removal before transferring has been recommended to improve rates of live birth, by preventing a possible blocked passage of the embryo from the catheter tip and by eliminating the transfer of microorganisms into the cavity. However, the actual introduction of the catheter may become more difficult.69,70 In a review, comprising over 1700 cases in eight RCTs,
no statistically significant differences in terms of pregnancy, implantation, or live birth rates were noted.\textsuperscript{70}

Uterine contractility at the time of embryo transfer can be an important success factor, as it may affect the position of the embryo in the hours after deposition. It is therefore that uterine relaxants have been tested as possible success modifiers. Nonsteroidal antiinflammatory drugs (NSAIDs) will reduce the prostaglandin activity and thereby uterine contractility, with possible beneficial effects on pregnancy rates.\textsuperscript{71} Atosiban, as a receptor antagonist for vasopressin V1a and oxytocin, acts on the uterus to suppress uterine contractions. It may be an appropriate tool for women with RIF, as from two small studies on a total of 290 cases, an improving effect was noted on both implantation and live birth rates.\textsuperscript{72}

THE BEST PRACTICE

Today, the execution of the embryo transfer is very likely to obtain highly focused attention of the embryologist and the physician. Among the procedures to be considered as part of the current golden standard are the use of a soft embryo transfer catheter, abdominal ultrasound-based guiding, and deposition with the purpose to put the embryo bubble within a distance of 10–15 mm from the fundal ending of the endometrium. Much more to the individual preference of the transferor, one could apply a mock transfer procedure, afterloading (being a mock and real transfer in one) or perform a transvaginal “scout” ultrasound.

Applying such approaches and performing the smoothest transfer ever may still not lead to the desired outcome of a live birth. There, factors of other origin than the transfer execution will play the major role, albeit that somewhere in the knowledge of the embryo transfer process we may still be “missing a point.” The only best step in such cases of implantation failure is repeating the ART procedures with the “knowledge” that the embryo transfer is not likely to be the limiting factor. Obviously, we could think of many of the approaches that have been discussed in the aforementioned, for which solid evidence is unfortunately lacking. Specifically, measures like the use of a short period of bedrest, or the application of uterine relaxants or antibiotics may come into the doctor’s mind. It seems, however, best to adhere to applying measures that have been well researched and have been confidently shown efficacious.

In contrast, in RIF couples in which a transfer procedure has been shown difficult, it is without doubt a factor that needs correction; choosing the best replacer in the department, applying a prior trial or mock transfer in order to unravel the smooth way into the uterus, or simply using the afterload approach are all options to be applied. In fact, creating the transition from a previous difficult into a current smooth transfer is very likely to positively affect the remaining prospects for the couple.

REFERENCES


48. Rovei V, Dalmasso P, Gennarelli G et al. IVF outcome is optimized when embryos are replaced between 5 and 15 mm from the fundal endometrial surface: A prospective analysis on 1184 IVF cycles. *Reprod Biol Endocrinol*. 2011;9(1). [http://www.rbje.org/content/9/1/114](http://www.rbje.org/content/9/1/114)


INTRODUCTION

Recurrent implantation failure (RIF), although not universally accepted, is usually defined as the failure to achieve a clinical pregnancy after three consecutive in vitro fertilization (IVF) attempts and transferring one to two high-quality embryos in each cycle. RIF is a distressing situation, which is rather difficult for women to manage, since they have higher levels of stress than fertile women and are constantly uncertain whether or not they will remain childless despite further IVF cycles. Furthermore, in the absence of public funding of IVF, the couples have to make significant financial sacrifices to continue their treatment. It has been shown that IVF failure is one of the major reasons for discontinuation of treatment, and even when its cost is covered by insurance, dropout rates are as high as 17%–65%

The optimal tool to counsel such patients would be the epidemiological analysis based on large databases. As yet, data on cumulative clinical pregnancy and live-birth rates following various treatments in RIF patients are absent.

FEMALE AGE

Although it has been proposed that the diagnosis of RIF is not appropriate for women with poor prognosis based on age above 40 years, a large proportion of patients seeking fertility treatment are of advanced age. In 2013 in Europe, 17.6% of all oocyte aspirations for IVF, 19.2% of all oocyte aspirations for intracytoplasmic sperm injection (ICSI), and 14.7% of all thaws were performed in women above 40 years of age. The delivery rates were 10.1% and 7.6% for IVF and ICSI fresh cycles, respectively, and 10.6% for frozen cycles. Counseling this age group should be based not only on the prognosis of further IVF cycles, but also on the fact that the delay in pregnancy is followed by adverse pregnancy outcomes related to advanced maternal age. Many epidemiological studies have clearly shown that advanced maternal age, above 35 years, is associated with intrauterine growth restriction, preeclampsia, placental abruption, preterm birth, gestational diabetes, admission to the intensive care unit, stillbirth, and cesarean section. The incidences of most of these adverse pregnancy outcomes have been found to be similar regardless of whether donated or their own oocytes have been used for IVF treatment.

A prospective study in the UK included 156,947 women who received 257,398 IVF ovarian stimulation cycles and calculated the live-birth rates within the first and subsequent cycles and finally the cumulative live-birth rates using the Kaplan–Meier method. The authors performed various estimates of the cumulative live-birth rates based on assumptions about the cumulative live-birth rate in women who discontinue IVF if they had continued with further IVF cycles (Figure 19.1). These included the optimal estimate (assumes that the cumulative live-birth rate in these women would have been equal to the rate in women who continued to have further IVF cycles), the previous oocyte yield-adjusted estimate (assumes that in these women, the cumulative live-birth rate...
would have been equal to the rate in women who had the same oocyte yield in the immediately previous IVF cycle and who continued to have further IVF cycles), the prognostic-adjusted estimate (assumes that 30% of women who discontinued IVF did so because of poor prognosis and would have had a live-birth rate of zero if they had continued further IVF cycles), and the conservative estimate (assumes that in these women the cumulative live-birth rate would have been zero if they had continued with further IVF cycles). Among all these estimates, the prognostic-adjusted estimate provides the most realistic value. In the age group below 40 years, 133,379 women; in the age group of 40–42 years, 15,561 women; and in the age group above 42 years, 4420 women, started treatment cycles. However, in the last group, a low number of cases were recorded to undergo more than five cycles.

This study showed that, for women aged 40–42, in the sixth cycle the cumulative prognosis-adjusted live-birth rate was 31.5% (95% CI 29.7–33.3), with no further increase following more IVF attempts. The live-birth rates were 12.3%, 10%, 8.6%, 7.8%, 5.3%, and 6.9% for cycles one through six, respectively.

For women older than 42 years, the live-birth rates were 3.7%, 3.3%, 3.3%, 1.2%, and 4.5% for cycles one through five, respectively. At the fifth cycle, the cumulative prognosis-adjusted live-birth rate was 10.7% (95% CI 8.2–13.2), while the low number of cases undergoing more than five cycles could not provide reliable estimates.

For women below 40 years, the live-birth rates were 32.3%, 27.1%, 24.3%, 21.4%, 19%, 17%, 17.3%, 19.1%, and 19.6%, for cycles one through nine, respectively. At the seventh, eighth, and ninth cycle, the cumulative prognosis-adjusted live-birth rates were 69.8% (95% CI 69.1–70.4), 70.9% (95% CI 70.1–71.6), and 71.6% (95% CI 70.8–72.5), respectively. The estimated natural fecundity rate of the general population is about 20% per month, and the cumulative rates of conceiving naturally are 45%, 65%, and 85% after 3, 6, and 12 months. It is obvious that in younger women undergoing IVF treatment, their chances for live birth are similar to the natural conception probability with high-rank IVF cycles, suggesting that in such women it is worth extending the IVF attempts even up to nine. However, these data originate from a general IVF/ICSI population with repeated IVF failures, which may not be identical to the RIF population. The “repeated IVF failure” population

![Cumulative live-birth rates across all cycles using different estimates.](image)

**Figure 19.1** Cumulative live-birth rates across all cycles using different estimates.
may include cases with suboptimal embryo quality, poor response to ovarian stimulation, repeated fertilization failure, and repeated difficult transfers. Although the real prevalence of RIF is not clear, it has been suggested that the frequency of this condition in the general IVF population is at least below 20%.17,18

ATTEMPTS FOLLOWING THE EVALUATION AND TREATMENT OF RIF

Assumed etiologies for RIF include decreased endometrial receptivity, embryonic defects, and factors with combined effect. With appropriate investigations, the underlying cause for the repeated failures may be identified and the suggested treatment should be targeted to the abnormality detected. Therefore, in clinical practice, once the diagnosis of RIF is established, usually meaning that three unsuccessful cycles have already been performed, and the diagnostic evaluation has identified the abnormality or malfunction that contributes to implantation failure, the couple should be treated properly before the initiation of the next cycle. It has to be stressed that some of the conditions related to RIF usually are managed before starting the first IVF cycle rather than expecting two or three failed attempts. These include smoking cessation, weight loss in obese patients, hydrosalpinx removal, and hysteroscopic removal of endometrial polyps, submucosal fibroids, and uterine septum. However, some couples may be reluctant to have these conditions managed before their first IVF cycle and may choose instead to directly undergo IVF treatment.

Today there are many studies investigating the various etiologies of RIF and that have shown that following treatment there are significant improvements in implantation in the subsequent IVF cycle. However, the levels of evidence for many of these treatments range from high to empirical. A significant problem, regarding management and counseling couples with RIF, is the absence of data on cumulative clinical pregnancy and live-birth rates following various treatments. Moreover, an unanswered question is the cumulative success rate of alternative or combined treatments for a specific implantation abnormality in subsequent cycles since there are no relevant studies in the literature.

Another important issue that has to be considered is that RIF may be a condition ranging between a lower than normal chance of implantation and a definite failure of implantation. Poor endometrial preparation or even excessive estradiol levels during superovulation may have detrimental effects on implantation, which however may be corrected in subsequent cycles.

Therefore, a rational approach in treating RIF couples may be based on the anticipation that after correction of the underlying cause of RIF, the couples will enter a new treatment strategy assuming that they have probabilities of pregnancy more or less equal to couples undergoing the first treatment cycle, with similar infertility characteristics but no RIF. Since the prevalence of RIF in the general infertility population is estimated to be below 20%, it is not expected to exert a rather strong effect on the estimation of cumulative pregnancy rates in large databases. Based on these assumptions, the information from appropriate large studies may be used to counsel RIF couples specifically on the appropriateness of proceeding with further IVF attempts.

The study by Smith et al. is a large prospective study that provides important information for counseling and decision-making. Based on these data, it seems rationale to suggest that women with three failed cycles below 40 years, after having their underlying condition treated, should extend the number of subsequent cycles up to nine. Women aged 40–42 years with RIF, should be counseled that up to six more cycles may be of benefit, but this strategy should be accomplished in a short period, since the delay is followed by aging-related detrimental effects on both pregnancy rates and, in the case of success, on pregnancy outcome. For women older than 42 years with RIF, the live-birth rates per cycle are very low, below 5%, up to fifth cycle, with severe aging-related adverse effects on both pregnancy rates and on pregnancy outcome. Older women with RIF attributed to aging oocytes, should be counseled that they should not delay to enter an oocyte donation program, since even with donated oocytes, they are at high risk for severe adverse pregnancy outcomes.
Another approach for counseling the RIF couples may derive from a recent mathematical challenge of the RIF definition.20 The authors' approach was based on the fact that IVF treatment has a relatively low success rate, nearly 30% in women without RIF.21 They calculated the expected pregnancy rates per cycle and the cumulative chances of pregnancy after various numbers of cycles, postulating that RIF has a prevalence between 5% and 25%. It was found that for women not affected by RIF and for a constant pregnancy rate of 30% per cycle, the chances of pregnancy decrease gradually with increasing number of treatment cycles. In this population, the pregnancy rates are 27%, 26%, 25%, 23%, 20%, and 18% at the first, second, third, fourth, fifth, and sixth cycles, respectively. Furthermore, the cumulative pregnancy rates increase slowly with increasing number of cycles, being 27%, 46%, 59%, 68%, 75%, and 79% after the first through sixth cycles, respectively. It was calculated that in this population with no RIF cases included, the false-positive rate of an RIF diagnosis was 86%, 81%, 75%, 68%, 60%, and 51%, respectively. However, these figures are dependent on the success rate of IVF and on the frequency of RIF in the studying population. Therefore, when the rate of IVF success is as high as 50%, the rate of false-positive diagnosis of RIF decreases from 88% to 69% after two cycles, from 87% to 53% after three cycles, and from 83% to 12% after six cycles. Similarly, when the rate of RIF in the population increases up to 25%, the rate of false-positive diagnosis of RIF decreases from 90% to 60% after two cycles, from 87% to 51% after three cycles, and from 69% to 26% after six cycles. This mathematical analysis showed that the rate of false-positive diagnosis of RIF, defined as three failed IVF cycles, is above 50% in all sensitivity analyses performed, suggesting that RIF diagnosis seems more reliable after six failed cycles. The authors proposed a possible definition of RIF based on the prognosis of the patient, and in very good prognosis patients (50% expected rate of success), RIF could be diagnosed after three failed IVF cycles, or after six failed cycles in a normal IVF population with a 30% expected rate of IVF success.

In another study,22 the embryo-uterus model was used, which included intercycle correlations for the embryo and uterine submodels, and estimated these effects in a large, multicenter UK database. This database of 12,480 embryo transfer cycles from 8768 IVF patients had sufficient information to allow reliable estimation of these effects.23 Empirical Bayes estimates were used to predict the prognosis of continuing IVF attempts based on previous cycle failures. The results suggested that in excess of 10 treatment failures are required to reduce the chances of subsequent success by half of the initial cycle. Even more cycles are required in single embryo transfer (SET) cycles. In support of our suggested approach in counseling RIF couples, this study22 implies that a treatment failure should be considered as statistical misfortune, and the policy of offering patients only a small number of IVF attempts has weak clinical justification.

The above suggestions are theoretical or based on mathematical models. There are few studies investigating the cumulative success rate following treatment of specific conditions related to RIF. In one study, 1174 RIF couples were treated with supportive lymphocyte immunotherapy (LIT) in Germany.24 After LIT treatment, for a time period of two years, the RIF women had a mean of 1.5 (1–8) oocyte retrievals and a mean of 1.7 (1–10) embryo transfers, resulting in a 28.4% delivery rate. Some patients had a high number of attempts, with the highest overall amount of oocyte retrievals leading to birth was 14, and the highest number of transfers was 17. The results showed that within two years, the birth rate per fresh embryo transfer and per couple showed a tendency, although not statistically significant, to decline after the third to the seventh IVF cycle. When frozen embryo transfers were included, the delivery rate per oocyte retrieval was about 20%. The average birth rate per fresh embryo transfer was 17.3%, per frozen transfer 8.2%, and 18.6% per oocyte retrieval, cumulating fresh and frozen transfers. The delivery rates were dependent on female age, and the two-year cumulative delivery rates were, for women below 30 years 39%, from 30 to 39 years 25%–30%, and above 39 years 17%.

In another prospective study in 421 RIF patients,25 it was found that according to the histopathologic/immunohistochemical examinations, 33.7% had chronic endometritis (CE). With the first-line doxycycline treatment, the histopathologic cure rate was 92.3%, and after the second-line
When should patients abandon treatment?

metronidazole/ciprofloxacin treatment, the overall cure rate was 99.1%. In this study, the live-birth rate in the first ET cycle and the cumulative live-birth rate after three ET cycles following antibiotic treatment in the cured RIF group were compared with RIF patients that did not have evidence of CE. For the cured RIF group, the live-birth rate in the first cycle and the cumulative rate were 32.8% and 38.8%, respectively, while for the RIF patients with no CE, the respective values were 22.1% and 27.9%, which both were significantly lower. However, the majority of patients in this study had cryopreserved ET cycles. It is important to underline that in the two groups of RIF patients, the live-birth rates at the sixth cycle were 32.8% and 22.1%, while in the study of Smith et al.,[16] for women below 40 years of a general infertility population, the live-birth rate at their sixth cycle was only 17%. Therefore, it seems rational to attempt more cycles in RIF patients similarly to the general infertile population. Although, there are no relevant data in the literature, such an approach may be applied in unexplained RIF cases.

The RIF couples, after unsuccessfully completing the appropriate number of cycles, according to the above approach, should be informed that alternative treatments may be offered as final options for childbearing. These options include gamete donation and surrogacy. Based on the history of the repeated reproductive failures and laboratory investigations, if sperm or oocytes are considered compromised, with no improvement after relevant treatments, then sperm or oocyte donation should be advised. If, however, there is evidence of severely impaired endometrial receptivity, such as Asherman syndrome, that has not improved despite pharmacological or surgical treatment, then surrogacy should be the realistic option for reproductive success.

REFERENCES

Proposed management of patients with recurrent implantation failure and directions for future research

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INTRODUCTION

The purpose of any management algorithm is to guide clinicians in the most appropriate and efficacious evidence-based management of patients with a certain condition. Such an algorithm requires a clearly defined population and a good understanding of the potential etiologies of the condition as well as their relative prevalence. It is also imperative that good-quality evidence exists to support the selection of the most effective treatment depending on the underlying cause.

Unfortunately, as outlined in the preceding chapters, recurrent implantation failure (RIF) remains a loosely defined clinical entity and high-quality evidence regarding the therapeutic management of RIF is not currently available. For this reason, in this chapter we will identify the research priorities around RIF and propose a management algorithm based on the best available evidence, acknowledging that this evidence is frequently of very low quality.

IS THE PATIENT SUFFERING FROM RECURRENT IMPLANTATION FAILURE?

As clearly outlined in Chapter 1, there is currently significant heterogeneity in the definition of RIF by different clinicians and researchers. This creates uncertainty in what RIF truly is, but also renders the interpretation of the relevant research problematic. Furthermore, during the last 10 years, there have been significant changes in the laboratory practices and the way in vitro fertilization (IVF) is practiced, such as implementation of blastocyst culture, adoption of single embryo transfer, and replacement of slow freezing by vitrification. The European Society of Human Reproduction and Embryology (ESHRE) Preimplantation Genetic Diagnosis (PGD) Consortium has suggested that RIF can be diagnosed after the unsuccessful transfer of more than 3 high-quality embryos or more than 10 embryos in multiple embryo transfers.

However, since a failed implantation is one of the two expected outcomes after an embryo transfer, the other being the achievement of pregnancy, one should be cautious when defining RIF in order to avoid overdiagnosis. Accounting for the probability that RIF might be attributed to pure chance, one could allow for a more objective diagnosis, limiting unnecessary and expensive diagnostic testing while also avoiding the iatrogenic stress accompanying the attachment of the RIF label to a patient.

If we accept that at least three embryo transfers are required to diagnose RIF and that 15% or more is a reasonable probability of failure due to chance, then we could estimate the cumulative number of embryos transferred required to diagnose RIF. For example, assuming that the probability of implantation of a euploid day five embryo is around 50%, the probability that three consecutive, single-embryo transfers of day five euploid embryos would fail due to chance is \((1-0.5)^3 = 0.125\).
Pretreatment phase

(i.e., 12.5%). Hence, patients with three or more failed transfers of a single day five euploid embryo could be diagnosed as RIF patients. On the other hand, if a good-quality day five embryo has 50% probability of being euploid for a woman who is 35 years old, then seven such embryos need to fail to implant for that probability to be <15% due to pure chance. Hence, these patients would need to have at least seven embryos transferred over at least three embryo transfers for RIF to be diagnosed. The selection of 15% as a cutoff for the RIF definition is arbitrary and could be revised in the future based on appropriate evidence. Furthermore, such a definition means that the diagnostic threshold will be determined by the probability of implantation of individual embryos, which can vary depending on their developmental stage, the age of the woman, and the clinic.

There is an urgent need for the scientific community to agree on the definition of RIF. This is crucial for any future research in the field. Using a universally accepted definition will allow for the proper estimation of the prevalence of RIF as well as the identification of relevant risk factors. Most importantly, it will allow for the proper evaluation of diagnostic and therapeutic modalities in this population.

PSYCHOSOCIAL SUPPORT

Failed embryo transfers could have a negative impact on the emotional well-being of the couple (or woman). Although most couples (or women) with multiple failures seem to emotionally adapt over time, it is clinically sensible to ensure that these patients are psychosocially supported by the staff of the fertility clinic and that they are aware that formal counseling services are available in case they feel the need for them.

It is also critical to discern between couples or women that have reached a stage in which they have very low chances of success using assisted reproduction technology using their own oocytes (i.e., women of very advanced reproductive age or women with premature menopause). These individuals might benefit from counseling and relevant psychosocial support on discontinuing treatment or pursuing oocyte donation or adoption.

PRETREATMENT PHASE

Lifestyle factors

A number of lifestyle factors have been linked with a negative effect on implantation. Studies performed specifically in RIF patients are lacking, and more research is required to ascertain the relative contribution of lifestyle factors in the etiology of RIF and, furthermore, the potential therapeutic value of such lifestyle modifications in these patients. However, considering the overall health benefits, it is sensible to recommended that patients optimize their weight, cease smoking, and avoid excessive alcohol consumption. Furthermore, avoiding exposure to endocrine chemical disruptors such as bisphenol A (BPA), phthalates, polychlorinated biphenyls (PCBs), and heavy metals that are known to adversely affect implantation, should be advised in patients with otherwise unexplained RIF.

Genetics

In addition to naturally occurring aneuploidy due to advanced female age, structural chromosomal aberrations (such as inversion and translocation) either in the male or female gametes can lead to recurrent implantation failures or recurrent miscarriages. There is some evidence suggesting higher prevalence of such chromosomal abnormalities in patients with RIF. For this reason, couples with RIF should be karyotyped, although the number of patients that will be affected is probably small (2%–3%).

Endocrine disorders

A number of endocrine causes have been implicated in failed IVF cycles, and these are described in Chapter 4. The evidence originates from studies performed mostly in the general IVF population, and, hence, the prevalence of these conditions in RIF patients and the degree to which they might contribute to RIF have not yet been estimated.
Nevertheless, patients presenting with RIF should probably be investigated for hypo- or hyper-thyroidism and thyroid autoimmunity. In most cases, these would have been investigated at an early stage of the couple’s fertility journey. RIF patients should also be investigated for diabetes mellitus, hyperinsulinemia, and insulin resistance, conditions that have also been suggested to negatively affect endometrial receptivity.

Vitamin D has also been shown to exert a positive role in implantation, and hence, vitamin D deficiency should be investigated and treated in any patient commencing their fertility journey, including RIF patients.

As previously discussed, evidence linking these endocrine disorders specifically with the occurrence of RIF and subsequent randomized controlled trials (RCTs) demonstrating efficacy of these interventions are lacking and, thus, urgently warranted. Until such data become available and considering the overall health benefits associated with treating most of these disorders, it is advisable for these conditions to be investigated and treated.

Anatomical causes

Some congenital uterine anomalies (e.g., septate uterus, uterus didelphys, bicornuate, and unicornuate uterus) have been linked with a decrease in the probability of pregnancy. The association though with the achievement of pregnancy after IVF is questionable and well-designed prospective studies in RIF patients have not been performed. Most of these congenital uterine anomalies have been linked to first or second trimester pregnancy loss and inferior obstetric outcome. For this reason, women with RIF should be investigated for the presence of congenital or acquired uterine anomalies, preferably with three-dimensional ultrasound, in the context of a thorough evaluation of the uterine anatomy. If a uterine septum is identified, then hysteroscopic resection should be considered.

Furthermore, the presence of primarily submucosal fibroids and potentially intramural fibroids can have a negative impact on implantation, and for this reason, surgical excision should be considered. Similarly, hydrosalpinges, intrauterine adhesions, endometrial polyps, and chronic endometritis have been shown to significantly reduce the probability of implantation. Appropriate investigation (ultrasonography and/or hysteroscopy and/or laparoscopy) and treatment is recommended.

Finally, adenomyosis has also been shown to be associated with reduced implantation rate after IVF, and for that reason, its presence should be investigated. Although it can be diagnosed using pelvic ultrasound, MRI of the pelvis is considered more specific. Surgical treatment of focal adenomyosis and/or administration of gonadotropin-releasing hormone agonists pretreatment should be discussed with these patients, especially in the absence of other factors that can explain RIF.

Immunological causes

An immunological cause of RIF has been hypothesized for many years. It is not surprising that there is number of cohort or case-control studies investigating differences in the immunological profile of patients with and without RIF. Despite these intense research efforts, our understanding of the various immunological mechanisms that contribute to implantation remains limited. A number of immunological parameters (NK cells, ANA, T cells and genetic polymorphisms affecting immunological responses) have been suggested to be associated with RIF, although the evidence is not robust. Similarly, a number of immunomodulating treatments have been proposed. These include administration of corticosteroids, intravenous immunoglobulin G (IVIG), intralipid, G-CSF, and endometrial scratching. From these, endometrial scratching appears to have the largest evidence base with systematic reviews and meta-analyses supporting its therapeutic effect in RIF patients. However, a recent well-designed large RCT supports no benefit in patients with ≥2 previous failed embryo transfers (ETs).

Other immunomodulating treatments such as corticosteroids, IVIG, and intralipid have a much weaker evidence base, and for that reason, their use should be performed in the context of well-designed RCTs.
Thrombophilia

Although the prevalence of thrombophilia has been reported to be higher in patients with failed IVF cycles, the evidence supporting the use of either low-molecular-weight heparin (LMWH) or aspirin is lacking. For this reason, investigation of thrombophilia in RIF patients and subsequent use of LMWH or aspirin is justified only in the context of well-designed clinical studies.

SPERM
Sperm DNA fragmentation

Increased sperm DNA fragmentation has been suggested to impact negatively on the outcome of IVF. Nevertheless, there is a paucity of data regarding its contribution in the prevalence of RIF. Furthermore, the potential therapeutic effect of antioxidants is still highly debated since the studies suggesting a potential beneficial effect are small and of very low quality.

Apparently, there is an urgent need for a proper assessment of the prevalence of high sperm DNA fragmentation in RIF patients. Equally, RCTs are required to prove whether or not administration of antioxidants or utilization of other laboratory techniques (such as intracytoplasmic morphological sperm injection, IMSI) can improve the chance of IVF success in these patients.

TREATMENT PHASE
Optimizing endometrial receptivity

There is a large body of evidence showing the detrimental effect of elevated serum progesterone on endometrial receptivity following a stimulated IVF cycle. On the other hand, currently available evidence does not support an association between serum estradiol or LH with pregnancy rates after embryo transfer. However, these associations have rarely been investigated in RIF patients.

Considering that these factors are not relevant in the frozen-thawed cycle and that almost all patients today undergo routinely frozen-thawed ET (FET) cycles, it is unlikely that they have a substantial contribution to the etiopathogenesis of RIF. Nevertheless, for patients who are repeatedly failing fresh IVF cycles and have not yet been exposed to FETs, freezing all embryos and deferring the ET for a naturally or artificially prepared endometrium could be explored.

Genetically testing the embryos

Preimplantation genetic testing of the embryos for aneuploidy (PGT-A) embryos can be an important diagnostic and therapeutic tool in RIF patients. Although specific data in RIF patients have not been produced, it can be assumed that PGT-A can ensure that a common cause of implantation failure, such as naturally occurring embryo aneuploidy due to advanced female age, is addressed. It can also help diagnose cases with unexpectedly high incidences of embryonic aneuploidy since it has been shown that even if the parental karyotypes are normal and the patients are not of advanced reproductive age, oocyte- or sperm-specific defects can lead to the creation of aneuploid embryos in an abnormally high percentage of cases. However, the prevalence of this phenomenon in RIF patients has not been assessed and is expected to be low.

Finally, ensuring through PGT-A (using a trophectoderm biopsy) that the embryo transferred is euploid will likely increase the probability of implantation per embryo transfer and allow clinicians to focus on other potential etiologies of RIF.

Optimizing embryo transfer technique

It is unlikely that poor ET technique is the sole reason for RIF. However, in these patients, it is important to ensure that the ET technique is optimal and does not compromise the implantation potential of the embryo. Although there are no studies performed specifically on RIF patients, available evidence suggests that embryo transfer should be performed using soft catheters, under ultrasound guidance, and placing the embryo about 15–20 mm from the uterine fundus.
WHEN EVERYTHING ELSE FAILS

**Oocyte and sperm donation/surrogacy**

As previously discussed, RIF can be expected in patients who, either due to advanced female age or other genetic reasons (e.g., chromosomal aberrations), produce a very high proportion of genetically abnormal oocytes that lead to embryos with very limited implantation potential.

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**Figure 20.1** Proposed management algorithm for patients with RIF: (a) before treatment.
In these cases, and when PGT-A is not indicated or effective, oocyte donation could represent an alternative option. Needless to emphasize, no RCTs are currently available on this strategy, and hence its efficacy is mostly based on retrospective data from oocyte donation cycles.

On the other hand, the quality of the sperm could also be a major contributor in the quality of the resulting embryos. Hence, despite the absence of high-quality data from RCTs, sperm donation could be argued as an option in cases in which the sperm are believed to compromise the quality of the embryos. Unfortunately, the specific indications for which sperm donation is most effective are not currently clear, and this should be the focus of future research.

Finally, in cases where the cause of RIF is suspected to be the maternal interface and this either cannot be effectively treated (e.g., severe Asherman Syndrome) or clearly identified, then surrogacy might be indicated and should be discussed with the patients.

**Treatment discontinuation**

Despite adequate clinical management, some patients will continue experiencing RIF. The possibility of treatment discontinuation has to be discussed with these patients, especially when the probability of pregnancy achievement in future cycles is expected to be very low. There is currently no specific cutoff, neither in terms of the number of failed cycles already performed nor in terms of future prognosis (e.g., <5% of success in a future cycle), that has been universally adopted as the starting point for initiating the discussion around treatment discontinuation. Cost-effectiveness analyses could in the future also aid in the construction of such decision-making models by taking into account society’s willingness to pay for a new live birth in patients with RIF. Until such studies become available, the duty of care dictates that the clinician ensures that patients understand that IVF is not always successful and treatment discontinuation is the conclusion of the fertility journey for a proportion of couples.

For the management algorithms proposed, see Figure 20.1.

**Figure 20.1 (Continued)** Proposed management algorithm for patients with RIF: (b) during treatment, and (c) after treatment.
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