Reproductive BioMedicine Online (2014) **28**, 14–38

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REVIEW

Recurrent implantation failure: definition and management

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Abstract Recurrent implantation failure refers to failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. The failure to implant may be a consequence of embryo or uterine factors. Thorough investigations should be carried out to ascertain whether there is any underlying cause of the condition. Ovarian function should be assessed by measurement of antral follicle count, FSH and anti-Müllerian hormone. Increased sperm DNA fragmentation may be a contributory cause. Various uterine pathology including fibroids, endometrial polyps, congenital anomalies and intrauterine adhesions should be excluded by ultrasonography and hysteroscopy. Hydrosalpinges are a recognized cause of implantation failure and should be excluded by hysterosalpingogram; if necessary, laparoscopy should be performed to confirm or refute the diagnosis. Treatment offered should be evidence based, aimed at improving embryo quality or endometrial receptivity. Gamete donation or surrogacy may be necessary if there is no realistic chance of success with further IVF attempts.

KEYWORDS: failure, implantation, IVF, fibroids, hysterosalpingogram, embryo

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.08.011

Introduction

Implantation is a process whereby the embryo attaches itself to the luminal surface of the endometrium followed by migration via the luminal epithelium and invasion into the deep layer of the endometrium to become embedded into the deeper layer (**Figure 1**). Traditionally, implantation has been considered as a process involving only the embryo and the endometrium, but recent studies show that cumulus cell competency may also contribute to the process (Benkhalifa et al., 2012). While implantation is a process with a well-defined starting point, it is a gradual process which lasts for several weeks with no universal agreement on when the process is completed.

In clinical practice, implantation is often considered to be successful when there is ultrasonographic evidence of an intrauterine gestational sac. Conversely, implantation failure is considered to have occurred if there is a lack of ultrasonographic evidence of an intrauterine gestational sac. Implantation failure may occur very early on during the attachment or migration stages, with the result that there is no objective evidence of a pregnancy, i.e. negative urine or blood pregnancy test (human chorionic gonadotrophin, HCG). It may also occur later on, following successful migration of the embryo through the luminal surface of the endometrium, when HCG produced by the embryo may be detected in the blood or urine, but the process becomes disrupted prior to the formation of an intrauterine gestational sac. In this situation, it is clinically referred to as a biochemical pregnancy. In assisted conception treatment, implantation is considered to be successful when an embryo has produced an intrauterine gestational sac, detectable by ultrasonography, usually about 3 weeks after oocyte retrieval or about 5 weeks of gestation.

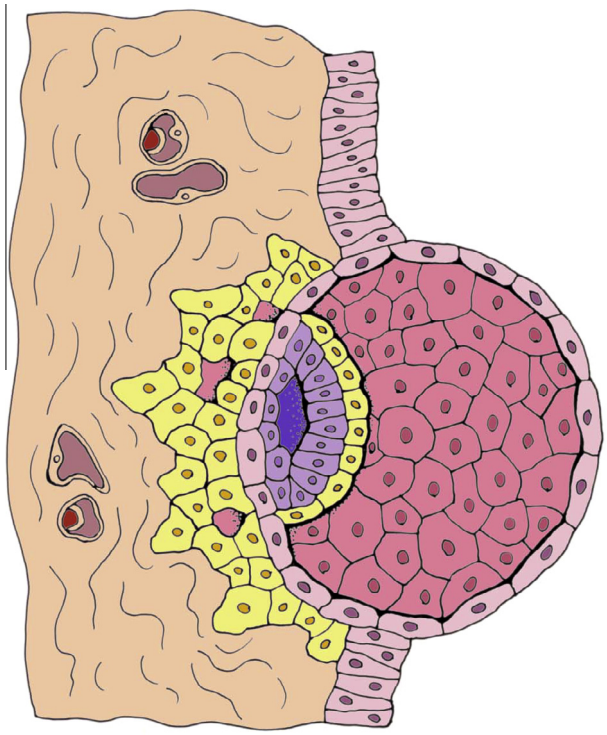

Figure 1 The initial stage of implantation, when the embryo is invading the epithelial layer of the endometrium to be embedded in the stroma compartment.

The implantation rate is defined as the number of embryos which have produced ultrasonographic evidence of an intrauterine gestational sac per the total number of embryos transferred into the uterine cavity (Zegers-Hochschild et al., 2009). In IVF—embryo transfer cycles, the implantation rate when day-2 or -3 embryos are transferred is about 25%, but the implantation rate when day-5 or -6 embryos are transferred is usually higher, about 40%.

Implantation failure refers to the failure of the embryo to reach a stage when an intrauterine gestational sac is recognized by ultrasonography. From the clinical point of view, it is worthy to note that the term 'implantation failure' refers to two different types of situation, those in whom there has never been evidence of implantation (no detectable HCG production) and those who have evidence of implantation (detectable HCG production) but it did not proceed to beyond the formation of a gestational sac visible on ultrasonography. Implantation failure may be a consequence of embryo or endometrial factors.

Definition of recurrent implantation failure

Recurrent implantation failure (RIF) is a clinical entity which refers to a situation when implantation has repeatedly failed to reach a stage recognizable by pelvic ultrasonography. There is as yet no universally accepted definition for RIF, despite many publications on this topic (Das and Holzer, 2012; Laufer and Simon, 2012; Penzias, 2012; Simon and Laufer, 2012a,b; Urman et al., 2005).

Because the probability for an embryo to successfully implant is only approximately 30%, the probability of it failing to implant is approximately 70%. However, following transfer of two embryos, the probability of both embryos failing to implant is $0.70^2 = 0.49$. Following the replacement of 3, 4, 5 or 6 embryos, the probabilities of all embryos failing to implant becomes $0.70^3 = 0.34$, $0.70^4 = 0.24$, $0.70^5 = 0.17$ and $0.70^6 = 0.12$, respectively. In a clinical setting, one has to decide when it becomes unusual for all transferred embryos to fail to implant. And then ask the question: why do all transferred embryos not implant?

The embryo

Quality

One important variable is clearly the quality of the embryo. If the quality of the embryo is poor, and assuming that the probability of successful implantation is reduced only to 0.10, following the transfer of 2, 3, 4, 5, 6 and 7 embryos, the probabilities of all embryos failing to implant is 0.81, 0.73, 0.66, 0.59, 0.53 and 0.48, respectively. In other words, there is still a 48% chance that all seven embryos will fail to implant. Hence, in arriving at a clinically useful definition, some investigators specified that good-quality embryos had been transferred (Margalioth et al., 2006). A good-quality embryo was defined as having the correct number of cells corresponding to the day of its development and day-5 embryos (blastocysts) were graded according to

expansion and quality of the inner cell mass and trophoectoderm Cutting et al. (2008). Other criteria included blastomeres of equal size and regular in distribution, even distribution of cytoplasm without granularity and less than 10% fragmentation (Cutting et al., 2008).

As well as the definition of a good-quality embryo, one needs to consider the current methods of embryo assessment, which are by no means perfect (Racowsky et al., 2000).

Number transferred

Some previous investigators proposed that RIF should be referred to as failure to achieve a clinical pregnancy after a total of 10 or more embryos had been transferred to the uterus (Stern et al., 2003). This might have been appropriate when the implantation rate was rather lower than what most IVF centres can now achieve, partly because the culture environments and the quality of culture media used are now improved. Moreover, the increasing use of blastocyst transfer has further improved implantation rates to approximately 40%. In this situation, the likelihoods of all 2, 3 or 4 embryos failing to implant (for 1 embryo being 0.60) is 0.36, 0.22 and 0.13, respectively. It seems appropriate, given the improved implantation rate achieved nowadays, to base the definition on the transfer of 4 or more embryos.

Stage of development

The implantation potential of a blastocyst is well recognized to be greater than that of the day-2 or -3 embryo, mainly because of natural selection of better quality embryos for further development.

Storage protocol

Some investigators believed that frozen embryo transfer cycles be excluded from the definition of RIF (Tan et al., 2005), almost certainly based on earlier data that the implantation rate of frozen–thawed embryos was inferior to that of fresh embryos. However, there is good evidence that the implantation rate of frozen–thawed embryos is similar to that of fresh embryos (Horne et al., 1997; Lieberman et al., 1992). Hence, the number of embryos transferred should include both fresh and frozen cycles in considering the diagnosis of RIF.

Comparative measures

It is a matter of debate whether the diagnosis of RIF be based entirely on the number of embryos transferred or on the number of embryo transfer cycles. Many investigators prefer to base it on the failure to achieve a clinical pregnancy after three transfer cycles (Margalioth et al., 2006; Tan et al., 2005), whereas others proposed to use the number of embryos transferred (Stern et al., 2003). There are pros and cons of each approach. The definition based on the number of embryos transferred is more scientific and logical, but the definition based on the number of transfer cycles is more pragmatic and easily understood by patients. At the Royal Hallamshire Hospital, both factors are considered and a diagnosis of RIF is based on the transfer of at least 4 embryos in a minimum of three transfer cycles.

Maternal age

Given that embryo quality is closely related to maternal age (Devroey et al., 1996; Spandorfer et al., 2000), the authors feel that the definition should incorporate an age limit of 40 years, although, strictly speaking, biological age is a more relevant consideration.

Uterine quality

The definition of RIF requires that good-quality embryos be transferred, but RIF may be also due to uterine factors (Demirol and Gurgan, 2004b; Richlin et al.,2002; Taylor and Gomel, 2008).

Distinction from recurrent IVF failure

RIF is not the same as recurrent IVF failure. The latter condition merely refers to the failure to achieve a pregnancy after several IVF attempts, a common cause being poor response to ovarian stimulation (Ferraretti et al., 2011). Suboptimal embryo quality, advanced maternal age and uterine factors are also relatively common causes for recurrent IVF failure. The term 'recurrent implantation failure' is a subgroup of recurrent IVF failure and should not be used to replace the latter.

Proposed definition

Based on the above considerations, this review proposes that RIF be defined as the failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. However, an internationally agreed consensus on the definition should be reached following further discussion, analogous to that of polycystic ovarian syndrome (Rotterdam ESHRE/ASRM – Sponsored PCOS Consensus Workshop Group, 2004).

Causes of recurrent implantation failure

Gamete/embryo factors

Based on the definition proposed above, RIF is primarily due to uterine factors. However, as discussed, given that current methods used to assess embryo quality are subjective and not always accurate, there will inevitably be a proportion of cases due to gamete or embryo factors.

Oocyte quality

Compromised oocyte quality as a cause of RIF is often suspected when there is a poor response to ovarian stimulation (Ferraretti et al., 2011), with fewer numbers of oocytes retrieved, a high proportion of immature oocytes, a reduced fertilization rate and low embryo utilization rate. When the above features are associated with low antral follicle counts, high FSH and low anti-Müllerian hormone, it can be assumed that the underlying cause of RIF relates to poor oocyte quality. Age-related decline in oocyte quality is associated with increased chromosomal nondysjunction, resulting in

aneuploid embryos, decrease in mitochondrial membrane potential and increase of mitochondrial DNA damage (Wang et al., 2009). There is evidence to suggest that aggressive ovarian stimulation protocols may lead to the production of poor-quality oocytes and a higher rate of fertilization failure (Baart et al., 2007; Collins, 2009; Verberg et al., 2009).

It is now recognized that not only the oocyte but the cumulus cells play an important role in the implantation process. The cumulus oophorus is a mass of granulosa cells associated with the oocyte from the antral follicle stage to fertilization and until early embryo development (Hernandez-Gonzalez et al., 2006; Motta et al., 1995a,b; Nottola et al., 1991). Cumulus cells are a source of prostaglandins and express angiogenic factors (vascular endothelial growth factor) that may play a role in angiogenesis at the implantation site. Cumulus cell gene expression appears to correlate with oocyte quality, embryo competence and pregnancy outcome (Assou et al., 2010).

A recent prospective randomized trial showed that co-culture of embryos with cumulus cells produced improved implantation and pregnancy in women with repeated implantation failure compared with conventional culture without cumulus cells (Benkhalifa et al., 2012).

Sperm quality

Just as poor-quality oocytes produce poor-quality embryos, it is also possible that poor-quality spermatozoa may lead to the production of poor-quality embryos. It is widely accepted that conventional semen analysis parameters do not accurately reflect sperm quality. Genetic tests are more likely to be useful as genome and epigenome integrity is essential for fertilization, normal embryo development and successful implantation.

Several factors contribute to sperm DNA damage, including cigarette smoking, genital tract infection and previous chemotherapy or radiotherapy (Erenpreiss et al., 2002; Morris, 2002; Potts et al., 1999).

Sperm DNA damage is associated with poor embryo development (Fernandez-Gonzalez et al., 2008) and both animal and human studies have shown that it is associated with failure to achieve spontaneous (Evenson and Wixon, 2008) and assisted conception (Bungum et al., 2007; Duran et al., 2002; Muriel et al., 2006). A recent meta-analysis suggested that sperm DNA damage has a modest impact on pregnancy rates following IVF treatment (Collins et al., 2008). Consequently, there is increasing recent interest in the use of sperm DNA integrity testing in the evaluation of reproductive failure. DNA fragmentation may be associated with an increased risk of miscarriage (Absalan et al., 2012; Brahem et al., 2011) but its association with RIF has not yet been established.

Parental chromosomal anomalies

It is known that individuals with balanced translocations often produce gametes with chromosomal aberrations which may in turn result in various forms of reproductive failure, ranging from defective gametogenesis (Crosignani and Rubin, 1982) to recurrent spontaneous miscarriage (Campana et al., 1986). Stern et al. (1999) conducted a study to test the hypothesis that couples with a history of IVF failure, similar to those with recurrent miscarriage, have a higher than expected prevalence of translocations and found that couples with otherwise unexplained repeated IVF failures had a 2.5% chance of carrying a balanced chromosomal translocation, which was higher than that of the control population. A further study (Raziel et al., 2002) identified a high frequency of chromosomal aberrations in a selected group of couples with high-order implantation failures and recommended karyotyping as part of the work up for repeated implantation failure in assisted reproduction. The genetics and epigenetics of reproductive failure including RIF is attracting increasing scientific interest and is worthy of a separate review.

Uterine factors

Congenital uterine anomalies

Congenital uterine anomalies may affect endometrial receptivity manifesting as either infertility or recurrent pregnancy loss (Taylor and Gomel, 2008). The majority of uterine anomalies occur as a result of a defect in the development or fusion of the paired Müllerian ducts during embryogenesis. It is now recognized that Hox genes play a role in the regulation of Müllerian duct development (Du and Taylor, 2004). Nevertheless, a case—control study carried out in Thessaloniki found not one out of 30 women with Müllerian duct malformation had a plausible causative mutation in Hox A10 or Hox A11 genes (Liatsikos et al., 2010). In addition to their role in the development of the Müllerian tract in the embryonic period, two particular HOX genes, *Hoxa10* and *Hoxa11*, have been suggested as regulators of endometrial development in preparation for implantation (Daftary and Taylor, 2000; Taylor, 2000).

The septate uterus is the most common structural uterine anomaly. It has long been recognized that uterine septae are associated with adverse reproductive outcomes such as first- and mid-trimester miscarriages but also possibly infertility (Fedele et al., 1993; Pabuccu and Gomel, 2004; Raga et al., 1997). These poor outcomes are attributed not only to the disturbance of the uterine cavity but also to the inadequate blood supply to the septum (Fedele et al., 1996). There is preliminary evidence that the septate uterus may also contribute to RIF. In a study involving women with a septate uterus undergoing IVF treatment, untreated septate uteri had a poor outcome following IVF treatment in comparison to women who had undergone hysteroscopic metroplasty prior to IVF (Lavergne et al., 1996). Another study by Ban-Frangez et al. (2009) on the outcome of singleton pregnancy after IVF/intracytoplasmic sperm injection (ICSI) showed that the presence of a septum, whether large or small, was associated with a miscarriage rate of about 80%, which was reduced to 30% or so after surgical removal of the septum (Ban-Frangez et al., 2009).

The same does not apply to bicornuate uteri, which rarely require surgical treatment. This is a relatively common anomaly and most women have no difficulty conceiving (Grimbizis et al., 2001). The main risk for the woman with a bicornuate uterus is mid-trimester pregnancy loss and preterm birth (Grimbizis et al., 2001).

Acquired intracavity conditions

A number of acquired intracavity uterine pathologies, including submucous fibroids, endometrial polyps and intrauterine

adhesions, may contribute to RIF. The frequency of unrecognized intrauterine pathologies in patients with RIF varies between 25% and 50% (Makrakis and Pantos, 2010).

Submucous fibroids. There are several mechanisms by which fibroids can adversely affect implantation, including increased uterine contractility, deranged cytokine profile, abnormal vascularization and chronic endometrial inflammation (Buttram and Reiter, 1981; Donnez and Jadoul, 2002; Hunt and Wallach, 1974; Taylor and Gomel, 2008).

There is evidence to suggest that submucosal and intramural fibroids that distort the endometrial cavity are associated with decreased pregnancy and implantation rates in women who attempt to conceive spontaneously or who are proceeding with IVF treatment (Bernard et al., 2000; Farhi et al., 1995; Narayan and Goswamy, 1994; Varasteh et al., 1999). Several studies suggest that pregnancy rates improve following the resection of fibroids distorting the uterine cavity (Fernandez et al., 2001; Garcia and Tureck, 1984; Goldenberg et al., 1995; Pritts et al., 2009). Pritts et al. (2009) conducted a systematic literature review and meta-analysis of existing controlled studies and concluded that women with submucosal fibroids have decreased clinical pregnancy and implantation rates compared with infertile control subjects (Pritts et al., 2009). The authors concluded that removal of submucous myomas appeared to improve outcome (Pritts et al., 2009). Since then, one further randomized controlled trial investigated the effect of hysteroscopic resection of submucous fibroids in women with unexplained primary infertility. This study revealed that hysteroscopic resection of submucous fibroids alone appeared to double cumulative clinical pregnancy rates (Shokeir et al., 2010).

Intramural fibroids. There is controversy as to whether or not non-cavity-distorting intramural fibroids adversely affect IVF outcome. Some studies suggest an adverse effect of non-cavity-distorting fibroids on implantation and pregnancy rates in women undergoing IVF, particularly with large fibroids >4 cm, whereas others fail to demonstrate such an association (Eldar-Geva et al., 1998; Gianaroli et al., 2005; Hart et al., 2001; Klatsky et al., 2007; Oliveira et al., 2004; Stovall et al., 1998; Surrey et al., 2001; Wang and Check, 2004; Yarali and Bukulmez, 2002). There are three recent meta-analyses published on this particular subject (Metwally et al., 2011; Pritts et al., 2009; Sunkara et al., 2010). All three analyses concur that women with intramural fibroids appear to have reduced implantation rates compared with women without intramural fibroids. However, myomectomy did not appear to significantly increase the clinical pregnancy and live birth rates (Pritts et al., 2009) and the most recent meta-analysis cautioned that the available evidence is rather weak because of significant heterogeneity and methodological issues (Metwally et al., 2011).

Endometrial polyps. Endometrial polyps may also interfere with embryo implantation (Richlin et al., 2002). The removal of endometrial polyps has been found to result in improved spontaneous pregnancy rates in three nonrandomized studies (Shokeir et al., 2004; Spiewankiewicz et al., 2003; Varasteh et al., 1999). A more recent systematic review found that hysteroscopic removal of endometrial polyps resulted in doubling of the clinical pregnancy rate in women undergoing intrauterine insemination treatment (Bosteels et al., 2010). It seems likely that endometrial polyps contribute to RIF.

Intrauterine adhesions. The presence of adhesions within the uterine cavity may interfere with successful implantation at an early stage by preventing the embryos from attaching to the luminal surface of the endometrium. Intrauterine adhesions often occur following curettage of the gravid uterus to terminate an unwanted pregnancy or in cases of retained products of conception after a pregnancy or miscarriage. Intrauterine surgery or intrauterine infection of the nongravid uterus may also lead to the formation of intrauterine adhesions. Demirol and Gurgan (2004b) found that intrauterine adhesions occurred in 8.5% of women with RIF. The evidence available so far suggests that hysteroscopic removal of intrauterine adhesions improves fertility outcomes (Dawood et al., 2010; Katz et al., 1996; Pace et al., 2003; Yasmin et al., 2007; Zikopoulos et al., 2004).

Adenomyosis. There is literature evidence available to suggest that adenomyosis has an adverse effect on female fertility (Maheshwari et al., 2012; Sunkara and Khan, 2012). Transvaginal ultrasonography is useful for the detection of adenomyosis but is operator dependent. The prevalence of adenomyosis in women with RIF is likely to be underestimated as it may not always be detected by transvaginal ultrasonography. Magnetic resonance imaging provides superior soft tissue resolution and is probably the most accurate noninvasive diagnostic technique available (Ascher et al., 1994; Atzori et al., 1996; Bazot et al., 2002; Bromley et al., 2000; Reinhold et al., 1996). The condition may appear in two forms, diffuse and focal, and the posterior uterine wall appears to be predominantly affected (Atzori et al., 1996; Bazot et al., 2002; Fedele et al., 1992; Vercellini et al., 1998). Adenomyosis almost always affects the junctional zone of the uterus which is just beneath the endometrium and so may have a greater impact on implantation than intramural fibroids which are some distance away from the endometrium. Nevertheless, surgical intervention in the case of adenomyosis is technically more challenging than fibroids because there is no defined capsule, and the excision of adenomyosis often necessitates removal of part of the uterine wall.

Hydrosalpinges

Hydrosalpinx is a Greek word meaning a Fallopian tube filled with water or fluid (Bloechle, 1999). It is now recognized that the live birth rate of patients with hydrosalpinges undergoing IVF is only one-half that of women who do not have hydrosalpinges (Camus et al., 1999; Strandell et al., 1999; Zeyneloglu et al., 1998). Moreover, in a prospective, randomized multicentre trial in Scandinavia (Strandell et al., 1999), it was shown that in women who had hydrosalpinges and were randomized to have no intervention prior to IVF, the pregnancy rate was 23.9%, miscarriage rate was 26.3% and live birth rate was only 16.3%; however, in women who were randomized to have salpingectomy prior to IVF, the corresponding results were 36.6%, 16.2% and 28.6%, respectively. The live birth rate was significantly

($P < 0.05$) higher than the no-treatment group. In a subgroup of women in whom the hydrosalpinges were visible by ultrasonography, the difference in results appeared more significant (Strandell et al., 1999). There is, therefore, good evidence that salpingectomy prior to IVF in women with hydrosalpinges improves outcome.

The adverse impact of hydrosalpinges on implantation may be attributed to a direct embryotoxic effect, a mechanical effect whereby the accumulated fluid may flush the embryo out of the uterus, as well as a negative effect on endometrial receptivity. A study by Seli et al. (2005) showed that the expression of leukaemia inhibitory factor, a cytokine essential for successful implantation, was reduced in the presence of hydrosalpinges, but the expression was restored to normal after salpingectomy (Seli et al., 2005). A further study showed that removal of hydrosalpinges may improve endometrial receptivity by restoring normal $\alpha v\beta 3$ integrin expression (Bildirici et al., 2001).

Immunological factors

The molecular and immunological aspects of implantation failure is an interesting area and is worthy of a separate in-depth review (Koot et al., 2012; Makrigiannakis et al., 2011). Of particular interest is the differentiation of endometrial stromal cells, a process called decidualization, which is considered critical for the establishment and maintenance of pregnancy. The decidualized stromal cells acquire the ability to regulate trophoblast invasion and to dampen local maternal immune responses (Blois et al., 2011; Gellersen et al., 2007). There is much conflicting evidence in the literature on the value of various immunological investigations and treatments in women with RIF (Clark and Stricker, 2006; Stephenson and Fluker, 2000). There is no consensus on whether or not immunological investigations are useful and whether immunological treatment is of benefit.

Thrombophilic conditions

Many clinicians are attracted to the hypothesis that thrombophilic conditions contribute to the cause of RIF, mainly because antiphospholipid syndrome has been shown to be an important cause of recurrent pregnancy loss and that treatment of this condition with aspirin and heparin significantly improves the outcome. However, there is controversy regarding the association between antiphospholipid antibodies and IVF failure, with some studies describing a significant association (Birkenfeld et al., 1994; Coulam et al., 1997; Kaider et al., 1996; Qublan et al., 2006; Stern et al., 1998), whereas others could not confirm it (Hill and Scott, 2000; Hornstein et al., 2000; Kowalik et al., 1997). In addition, unlike the situation with recurrent miscarriage, the value of treatment in women with RIF and tested positive for the antibodies has not been confirmed. Similarly, in the case of heritable thrombophilia, while several studies have observed increased prevalence of conditions in women with RIF (Azem et al., 2004; Grandone et al., 2001; Qublan et al., 2006), a relatively large case–control study of 468 women undergoing IVF did not show any association between maternal thrombophilia and IVF failure; however, the study population did not refer specifically to recurrent IVF failure (Martinelli et al., 2003). A further study investigated thrombophilia and its relationship with repeated IVF failure (Simur et al., 2009), which concluded that factor V Leiden, methylene tetrahydrofolate reductase mutations and prothrombin gene mutations do not have a significant role in IVF–embryo transfer implantation failure. Overall, it remains to be determined whether thrombophilic conditions are a cause of RIF.

Investigations

Gamete and embryo factors

Ovarian function tests

Women with RIF should be offered ovarian reserve tests such as basal FSH, anti-Müllerian hormone and antral follicle counts to exclude any significant compromise of ovarian function associated with RIF, which may help in the counselling process.

Sperm DNA integrity testing

Several laboratory tests are available to measure sperm DNA fragmentation. They include TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL; Gorczyca et al., 1993), Comet (Hughes et al., 1996), CMA3 (Manicardi et al., 1995), in-situ nick translation (Tomlinson et al., 2001), DNA breakage detection fluorescence in-situ hybridization (Fernandez et al., 2000) and sperm chromatin dispersion (Fernandez et al., 2003). The most extensively studied, sperm chromatin structure assay, measures the stability of sperm chromatin in acid media by measuring DNA susceptibility to denaturation following exposure to mild acid with acridine orange (Erenpreiss et al., 2006; Evenson, 1990; Evenson et al., 2002). Flow cytometric analysis determines the proportion of spermatozoa with fragmented DNA and is expressed as a DNA fragmentation index (Sakkas and Alvarez, 2010). Earlier studies indicate that an index value of >27% is associated with pregnancy failure in assisted reproductive technology (Larson et al., 2000; Larson-Cook et al., 2003), although, recent studies have questioned the predictive value of this test (Bungum et al., 2004, 2007; Gandini et al., 2004). Some clinics have already introduced sperm DNA integrity testing in the partners of women with RIF, which may well be premature. At present, sperm DNA integrity testing should only be offered to couples with RIF as part of a research programme.

Karyotyping

Although only a small proportion of couples with RIF have abnormal karyotype results (2.5%; Stern et al., 1999), the rate is higher than that of the general population, suggesting an association between the two conditions. The test should be considered in couples with RIF.

Uterine factors

In women with RIF, thorough investigations must be carried out to exclude any uterine pathology contributing to the clinical problem.

Ultrasonography

Pelvic ultrasonography is an integral part of IVF treatment as a means to monitor follicle growth and endometrial development. It is often assumed that significant uterine pathology such as large intramural fibroids would have been detected during the course of IVF treatment. Transvaginal ultrasonography may also detect some cases of hydrosalpinges, especially if they are large and persistent. However, it is necessary to confirm whether careful evaluation of the uterine anatomy has ever been carried out by an experienced ultrasonographer, and if not, it ought to be arranged.

Hysterosalpingography

Hysterosalpingography (HSG) is a useful test in RIF mainly because of its usefulness in the detection of hydrosalpinges. Its value in the detection of intrauterine pathology is limited. It is not a particularly sensitive test as some subtle lesions such as adhesions may be missed from time to time. Moreover, HSG has a high rate of false-positive results as air bubbles, mucus and debris may all mimic filling defects.

Sonohysterography

Sonohysterography (SHG) involves the use of contrast media, for example saline, along with transvaginal ultrasonography and is thought to improve the visualization of the uterine cavity (Ayida et al., 1997). It has clear advantages over the use of HSG in that the use of radiation and iodine contrast is avoided and it is less invasive than hysteroscopy. A recently published study involving 64 patients investigated the use of SHG as a first-line evaluation for uterine abnormalities in women with RIF (Shokeir and Abdelshaheed, 2009). A radiologist performed transvaginal ultrasound, SHG and then HSG prior to hysteroscopy, which was performed by a surgeon. All patients had a minimum of two unsuccessful IVF cycles in which two or more reasonable embryos were transferred per procedure. This study found that there was no statistically significant difference between the radiological methods in terms of diagnostic accuracy. In this particular study, SHG detected all uterine abnormalities except for a single, small endometrial polyp. The authors concluded that compared with hysteroscopy, SHG offered similar diagnostic capabilities, was less invasive and incurred less costs (Shokeir and Abdelshaheed, 2009). However, as in the case of ultrasound and HSG, subtle small intrauterine lesions may not always be detected (Soares et al., 2000). A recent prospective study compared transvaginal ultrasound, SHG and diagnostic hysteroscopy in the evaluation of endometrial pathology and concluded that diagnostic hysteroscopy was significantly more accurate in the diagnosis of intracavitary lesions than SHG and transvaginal ultrasound (Grimbizis et al., 2010).

Hysteroscopy

Hysteroscopy is one of the most important investigations in women with RIF. It allows reliable visual assessment of the cervical canal and uterine cavity. It is considered to be the gold standard to diagnose intrauterine pathology and has minimal intraoperative and post-operative morbidity.

Two prospective, randomized controlled studies confirmed the value of hysteroscopy in women with RIF demonstrating significantly increased clinical pregnancy rates (Demirol and Gurgan, 2004a; Rama Raju et al., 2006). It is concluded that, in women with RIF, even if the hysterosalpingogram was normal, hysteroscopic evaluation should be offered. Current evidence suggests that the incidence of abnormal hysteroscopic findings in women with recurrent IVF failures varies between 25 and 50% (Makrakis and Pantos, 2010). From time to time, women with RIF may already have had a hysteroscopy in the past, often prior to the commencement of infertility treatment. The question may then be: should it be repeated? It should be repeated if the hysteroscopic assessment was conducted more than 2 years ago or if the patient has since had a further intrauterine surgery (e.g. removal of products of conception after miscarriage).

Hysteroscopy is not only a diagnostic tool; it also allows therapeutic procedures to be carried out at the time of diagnosis. It is useful to time the hysteroscopy to take place in the luteal phase of the cycle preceding IVF treatment as hysteroscopic-directed endometrial biopsy (scratch) may also be performed at the same time to improve the implantation rate (Coughlan et al., in press). However, when hysteroscopy is performed in the mid-luteal phase, the possibility of disturbing a spontaneously occurring pregnancy should be explained and women advised to consider protected sexual intercourse in the treatment cycle. On the other hand, hysteroscopy performed in the follicular phase has an advantage that the endometrium is thinner and the visibility is better.

Combined laparoscopy and hysteroscopy

In women suspected to have a congenital uterine anomaly on the basis of ultrasonography or HSG, further investigation is required to confirm the diagnosis. These tests include three-dimensional ultrasonography, magnetic resonance imaging or combined hysteroscopy and laparoscopy. The latter is considered to be the gold standard as it allows for direct visualization of the internal and external contour of the uterus and enables the clinician to diagnose and treat concurrently.

Hydrosalpinges

It is advisable to exclude hydrosalpinges as a cause of RIF, regardless of the initial infertility diagnosis leading to IVF treatment. A HSG should be arranged unless one has been performed recently. Ultrasound examination should not be relied upon to rule out hydrosalpinges as it may not always be visualized by ultrasonography. If the HSG is inconclusive, laparoscopic examination should confirm or refute the diagnosis beyond doubt.

Management

A multidisciplinary approach should be adopted in the management of a couple with RIF. It should involve not only an experienced fertility specialist but also a senior embryologist and, where appropriate, a reproductive surgeon or a counsellor.

Couples with RIF should be reviewed by an experienced fertility specialist as there are inevitably many questions to be answered and important clinical decisions to be made. Patients need to be reassured that treatment is under the

supervision of an experienced clinician. The couple should be offered ample time for their questions to be addressed and a clear treatment plan agreed. The appointment should not be just another 'routine' review. It ought to be a thorough review of the diagnosis of the underlying cause of infertility, the investigation results, the treatment protocol, the response to ovarian stimulation, the quality of the oocyte and embryos and possible explanation as to why they have not produced a successful pregnancy. The couple should have explained to them that any treatment plan recommended would be discussed and confirmed in a multidisciplinary team meeting and the final decision confirmed in writing.

Secondly, there ought to be an agreed local protocol as to how couples with RIF should be further investigated and managed. This is particularly important as there is still no universally agreed protocol for the investigation and management of this condition. The protocol ought to be updated regularly to take into consideration the findings of recent studies. The protocol should contain sufficient details to ensure that patients and staff clearly understand the plan of action and the rationale behind any decisions made.

Appropriate counselling of the couple with RIF is of the utmost importance prior to proceeding with further treatment. The couple should be advised as to the likelihood of success in future cycles and advised not to pursue further treatment if their prognosis is poor (i.e. $<$5%). The service of an independent counsellor should be offered at these difficult times. If it is deemed reasonable to pursue further treatment, it is beneficial to instigate appropriate investigations and review previous unsuccessful IVF treatment cycles with a view to modifying or changing the treatment protocol if indicated.

Lifestyle changes

In addition to a review of investigations and treatment to date, clinicians should discuss and advise as to lifestyle changes which could improve the likelihood of treatment success.

Smoking

Women who smoke should be advised to stop as there is evidence that smoking is associated with an increased gonadotrophin requirement for ovarian stimulation, fewer oocytes retrieved, higher numbers of cancelled cycles, lower implantation rates and more cycles with failed fertilization in those undergoing IVF treatment (Cooper et al., 1995; Feichtinger et al., 1997; Hughes and Brennan, 1996; Klonoff-Cohen et al., 2001; Sterzik et al., 1996; Van Voorhis et al., 1996).

Male partners of women with RIF should also be advised to abstain from smoking due to its adverse effect on sperm counts and motility, increase in abnormal sperm morphology and sperm DNA damage (Potts et al., 1999).

Body mass index

Underweight women (body mass index $<$19 kg/m^2) should be encouraged to gain weight and obese women (body mass index $>$29 kg/m^2) should be advised to lose weight prior to further attempts at IVF treatment.

For obese women, the first-line treatment is diet modification and regular exercise. A multidisciplinary approach is often necessary. It has been shown that women participating in structured weight-loss programmes involving a behavioural modification component are more successful than those who attempt weight loss on their own (Wadden and Foster, 2000). In addition to lifestyle changes, pharmacotherapy such as orlistat may also be beneficial. In women with morbid obesity refractory to conventional measures, bariatric surgery may be considered but pregnancy is not recommended in the first year following the surgery as this is the time when the majority of weight loss occurs (ASRM, 2008b). Studies have shown that previous bariatric surgery is not associated with an increased risk of adverse perinatal outcomes (Marceau et al., 2004; Printen and Scott, 1982; Sheiner et al., 2004), but the incidence of anaemia due to iron, folate, vitamin B12 and nutritional deficiencies may be increased (ASRM, 2008b).

Alcohol consumption

It is recognized that alcohol consumption in pregnancy is associated with increased risks of spontaneous miscarriage, premature birth and low birthweight (Mukherjee et al., 2005; Netherlands, 2007, 2007/19E; Windham et al., 1992). Women with RIF should be advised to reduce consumption to one or two units once or twice a week when trying to become pregnant (NICE guideline, 2004) or abstain from alcohol altogether.

Ovarian stimulation protocol

The ovarian response to gonadotrophin stimulation should be reviewed. If the response is deemed satisfactory, it is not necessary to change the stimulation protocol.

In a small proportion of women who are deemed to have suboptimal response to ovarian stimulation, the dose of gonadotrophin may be increased. There is no firm evidence that antagonist protocol is better than agonist protocol or vice versa. There is some evidence to suggest that poor responders to FSH stimulation in down-regulated cycles may benefit from the addition of LH (Phelps et al., 1999; Surrey and Schoolcraft, 2000). Evidence also points to a possible benefit from the addition of LH to the cycles of women older than 35 years of age (Balasch et al., 2001; Marrs et al., 2004; Phelps et al., 1999).

In women with endometriosis and adenomyosis, the use of ultra-long protocol involving the administration of gonadotrophin-releasing hormone (GnRH) agonists for a few months prior to IVF or ICSI may increase the pregnancy rate (Sallam et al., 2006a,b; Tremellen and Russell, 2011).

Sperm DNA fragmentation

In recent years, there has been increasing interest in sperm DNA fragmentation and its effect on fertility. Sperm DNA integrity testing has been proposed as a test with promising potential as compared with the standard semen analysis (Aitken and De Iuliis, 2007; Barratt et al., 2010).

When suboptimal spermatozoa are considered to be a contributory cause of RIF, supported by an increased amount of sperm DNA fragmentation, several treatment options may

be considered. First, medical treatment may be used to improve sperm quality (Isidori et al., 2006). In particular, oral antioxidant treatment has been shown to reduce the incidence of sperm DNA fragmentation (Greco et al., 2005b).

Secondly, it is possible to select spermatozoa with low levels of DNA damage from the ejaculated semen samples (Sakkas and Alvarez, 2010). A number of techniques have been proposed, including the use of annexin-V columns which has been shown to significantly reduce the percentage of spermatozoa with DNA fragmentation as measured by the TUNEL test and a sperm selection method incorporating sperm hyaluronic acid binding (Jakab et al., 2005; Said et al., 2005, 2006). Other techniques proposed include the use of confocal light absorption scattering spectroscopy (CLASS) technology and the use of high-magnification ICSI to identify spermatozoa devoid of surface vacuoles (Bartoov et al., 2003). Intracytoplasmic morphologically selected sperm injection (IMSI) is considered to be a refined form of ICSI as it utilizes spermatozoa selected under high-power magnification with a defined set of morphological criteria. A recent meta-analysis comparing ICSI and IMSI outcome demonstrated a statistically significant improvement in implantation and pregnancy rates and a significant decrease in miscarriage rates with use of IMSI (Souza Setti et al., 2010). However, a further study involving 200 couples with a minimum of two prior unsuccessful ICSI cycles demonstrated no statistically significant difference between the two groups in terms of fertilization, implantation and pregnancy rate (Oliveira et al., 2011). Further randomized controlled studies are required to confirm the superiority of IMSI over ICSI.

Thirdly, based on the observation that sperm DNA damage is lower in the seminiferous tubules as compared with the cauda epididymis and ejaculated spermatozoa (Greco et al., 2005b; Steele et al., 1999; Suganuma et al., 2005), it has been proposed that men with high levels of DNA damage in ejaculated spermatozoa have spermatozoa removed surgically from the testis for ICSI (Greco et al., 2005a). The use of testicular spermatozoa in couples with repeated implantation failure associated with high sperm DNA fragmentation in semen has been reported to result in a significant increase in pregnancy rate (Greco et al., 2005b; Weissman et al., 2008) and reduction of miscarriage rate (Borini et al., 2006), but further studies are required to confirm the benefit.

Improving embryo quality and selection

Even though RIF refers to those who fail to achieve a clinical pregnancy despite the transfer of good-quality embryos, embryo factors still play a part because the currently used methods of embryo selection are not always reliable. A careful review of recent investigations including age of the woman, antral follicle count, basal FSH measurement, anti-Müllerian hormone concentration, number of follicles produced in response to stimulation, number of oocytes retrieved, the proportion of immature oocytes, fertilization rate, the proportion of good-quality embryos and the total number of good-quality embryos transferred should be noted.

Blastocyst transfer

Several studies have suggested that extending embryo culture to day 5 or 6 in order to transfer the embryo at the blastocyst stage increases the implantation rate (Cruz et al., 1999; Gardner et al., 2000, 2004; Guerif et al., 2004; Levitas et al., 2004; Machtinger et al., 2006; Marek et al., 1999). A recent Cochrane review by Blake et al. (2007), supported the rationale that blastocyst transfer improves implantation rates by enabling better selection of embryos and with better synchronicity between the embryo and endometrium (Blake et al., 2007). Since the meta-analysis, a further report demonstrated significantly improved live birth rates after blastocyst transfer (Papanikolaou et al., 2008). In women with RIF, blastocyst transfer ought to be considered if not performed in previous treatment cycles.

Assisted hatching

Hatching of the blastocyst plays an integral role in the implantation process. Failure to hatch (due to intrinsic abnormalities in either the blastocyst or zona pellucida is a possible cause of implantation failure. Assisted hatching involves the artificial thinning or breaching of the zona pellucida and has been proposed as one technique to improve implantation and pregnancy rates following IVF (ASRM, 2008a). The assisted hatching process itself is not without complications, including damage to individual blastomeres or to the embryo and as a result may compromise embryo viability. Studies have suggested that artificial manipulation of the zona pellucida is associated with an increased risk of monozygotic twinning (Hershlag et al., 1999; Schieve et al., 2000).

A comprehensive review and meta-analysis identified 23 randomized controlled trials involving 2572 women undergoing assisted hatching during assisted reproduction treatment (Edi-Osagie et al., 2003). Clinical pregnancy rates were evaluated in 19 trials (722 clinical pregnancies, 2175 women). An improvement in clinical pregnancy rates following assisted hatching was identified (OR 1.63, 95% CI 1.27—2.09), although significant heterogeneity was noted. Subgroups of patients who demonstrated the greatest improvement in clinical pregnancy rates were those with prior failed cycles (OR 2.33, 95% CI 1.63—3.34) and older women. It is an inherent weakness in this review and meta-analysis that only six of the studies included in the analysis (involving 523 women) reported live birth rates with and without assisted hatching. Taking into account that the study populations were heterogeneous, the live birth rates in the two groups were not different (OR 1.26, 95% CI 0.82—1.78).

Two further meta-analyses also confirmed that the live birth rates were not significantly higher than control subjects (Das et al., 2009; Seif et al., 2005) and a third systematic review and meta-analysis of randomized controlled trials could not draw a proper conclusion regarding miscarriage and live birth due to the small sample evaluated by the pool of included studies (Martins et al., 2011). There are two possible explanations for these observations. First, it may be that assisted hatching facilitates the production of suboptimal embryos, which subsequently miscarried. Secondly, the total number of cases available for analysis was relatively small, which does not produce sufficient power to detect a small difference. Interestingly, the miscarriage rate was not higher in the assisted hatching group, suggesting that the production of suboptimal embryos by assisted hatching is a less likely possibility (Seif et al., 2005).

It is possible that the beneficial effect of assisted hatching depends on the selection of subjects. In a study by Petersen et al. (2005), it was found that assisted hatching produced significantly higher implantation rates in women with repeated implantation failure, but not in women with only one previous implantation failure. A further study reported that the beneficial effect of assisted hatching in RIF was more significant in women younger than 38 years of age (Ghobara et al., 2006).

The American Society of Reproductive Medicine published recommendations regarding assisted hatching in 2008 (ASRM, 2008a). The Practice Committee suggested that available evidence does not support the routine application of assisted hatching in all IVF cycles. However, it proposed that assisted hatching may be clinically useful in patients with a poor prognosis, including those with a history of two or more unsuccessful IVF cycles, poor embryo quality and older women ($\geq$38 years of age) Mansour et al., (2000). In summary, it does seem that assisted hatching is worth considering in women with RIF.

Preimplantation genetic diagnosis

The value of preimplantation genetic diagnosis (PGD) in RIF is controversial. There is no evidence to suggest that the embryos produced by women with RIF are more likely to be abnormal. The frequency of aneuploidy (67%) in embryos from women with RIF (Pehlivan et al., 2003) was rather similar to the frequency (64%) in women without the condition (Baart et al., 2006).

Two randomized trials on the value of PGD for aneuploidy screening in women with RIF showed no evidence of benefit (Gianaroli et al., 1999; Werlin et al., 2003). A recent review by Donoso et al. (2007) also concluded that PGD should not be implemented in women with RIF on a routine basis and highlighted mosaicism of blastomeres as the major source of misdiagnosis in PGD. Chromosomal mosaicism, in which different blastomeres have a different chromosomal complement, is well documented (Harper et al., 1995; Munné et al., 1997; Voullaire et al., 2000) and affects up to 50% of early human embryos. This means that the blastomere biopsied for PGD may not be representative of the remainder of the embryo. Mosaicism exists in embryos, cannot be corrected and is an inherent limitation when a single blastomere is analysed (Wilton et al., 2009). In an effort to detect mosaicism, some laboratories analyse 2 cells from each embryo. However, removal of 2 cells from the early embryo may pose a threat to its viability (Cohen et al., 2007; Goossens et al., 2008).

In recent years, there is increasing interest in providing a more detailed characterization of blastocyst cytogenetics using methods such as comparative genomic hybridization (CGH) and single-nucleotide polymorphism microarrays (Fragouli et al., 2008; Northrop et al., 2010) with a view to detecting and preferentially transferring euploid normal embryos. Early studies suggest that an approach combining blastocyst biopsy and comprehensive chromosome screening using CGH or microarray CGH may represent the optimal approach for PGD (Fragouli et al., 2010; Schoolcraft et al., 2010). A recent study evaluated the accuracy and efficiency of CGH and microarray CGH for trophectoderm analysis using 52 blastocysts (Fragouli et al., 2011). This study found both CGH and microarray CGH trophectoderm analyses to be accurate aneuploidy detection tools (Fragouli et al., 2011). A further study examined comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure and identified higher implantation and pregnancy rates in those patients receiving blastocyst analysis, suggesting that comprehensive chromosome screening may assist patients with RIF capable of producing blastocysts in achieving successful pregnancies (Fragouli et al., 2010). Further studies are required to confirm whether or not routine PGD using microarray CGH is beneficial in women with RIF.

Metabolomics

For many years, the selection of embryos for transfer into the uterine cavity is based on the visual assessment and scoring of the embryos at various stages of development. To improve the selection, metabolomic changes in the culture medium of embryos and oocytes (exometabolomics) may be measured determining what the embryo consumes or secretes (e.g. amino acids, proteins and oxygen consumption) and these parameters have been shown to correlate with embryo viability (Brison et al., 2004; Conaghan et al., 1993; Gardner et al., 2001; Gott et al., 1990; Hardy et al., 1989; Houghton et al., 2002; Lopes et al., 2007; Sakkas and Gardner, 2005; Sallam et al., 2006a,b; Scott et al., 2008). It may improve the embryo selection process, thereby improving the implantation rate. Its application in women with RIF has yet to be confirmed.

Embryo transfer

Embryo implantation has been found to be dependent on embryo quality, endometrial receptivity and transfer efficiency (Paulson et al., 1990). In women with RIF, the details of previous embryo transfers should be reviewed, paying particular attention to any technical difficulties encountered. In the absence of any particular difficulty encountered in previous attempts, there is no evidence that a change of embryo transfer technique will improve the implantation rate. However, if there had been difficulty with previous embryo transfers, identified as a procedure taking longer than usual, causing significant pain or requiring change of catheter, cervical dilatation or use of a tenaculum, it is accepted that the pregnancy rate would be lower (Mains and Van Voorhis, 2010; Sallam et al., 2003; Tomas et al., 2002). Difficult embryo transfer may be due to cervical stenosis or acute anteversion/retroversion or acute anteflexion/retroflexion of the uterus. Several techniques may be considered in women with a history of difficult embryo transfer.

Ultrasound guidance

The transfer should be performed under ultrasound guidance (Brown et al., 2010). A systematic review and meta-analysis of prospective, randomized, controlled trials comparing ultrasound with clinical touch methods of embryo catheter guidance concluded that ultrasound-guided embryo transfer significantly increases the chance of live birth and ongoing and clinical pregnancy rates (Abou-Setta et al., 2007).

Trial embryo transfer

A trial embryo transfer should be considered where a prior embryo transfer has been described as difficult or where difficulty may be anticipated ,such as previous LLETZ.

Transfer tips

Filling the bladder in women with acute anteversion or anteflexion is a simple measure which may sometimes be useful, but will not be helpful in cases of acute retroversion or retroflexion where an empty bladder is preferable (Sharif et al., 1995). The application of a tenaculum to the anterior lip of the cervix and applying traction gently downwards may help to straighten an acutely flexed uterus but may compromise pregnancy rates by inducing uterine contractions (Lesny et al., 1999; Mains and Van Voorhis, 2010; Wood et al., 1985). The use of a rigid catheter may help to negotiate the cervix if difficulty is encountered with the use of a soft catheter (Abou-Setta et al., 2005).

Transfer method

Alternative methods to transcervical embryo transfer include transmyometrial and tubal transfer but should be reserved for cases which are extremely difficult or impossible (Sharif et al., 1996; Yang et al., 1992).

Irrigation and aspiration of cervical mucus

Irrigation and aspiration of cervical mucus has been proposed to improve pregnancy rates but not all studies concur with this hypothesis (Glass et al., 2000; McNamee et al., 1997). The removal of cervical mucus is thought to improve pregnancy rates by preventing or minimizing bacteriologic contamination of the endometrial cavity and preventing cervical mucus occluding the catheter tip but it remains to be determined as to whether this practice improves pregnancy rates (Letterie et al., 2003). There is insufficient evidence to show that bed rest after transfer improves outcome (Bar-Hava et al., 2005).

Sequential embryo transfer

Sequential embryo transfer has been proposed as a means of improving implantation rates (Goto et al., 2003). The concept behind this strategy is to overcome the problem of embryo—endometrium asynchronicity as a potential cause of implantation failure.

However, a case—control study (Ashkenazi et al., 2000), which evaluated the consecutive transfer approach of early embryos and blastocysts, did not show any clinical benefit; the authors proposed that a second transfer may have an adverse effect on the implantation process because a second insertion of a catheter through the cervix may cause trauma to the endometrium or stimulate the secretion of prostaglandins that could produce uterine contractions. It might also introduce more mucus or additional microbial contamination to the uterine cavity and both these factors may disturb the implantation process and decrease the pregnancy rate (Egbase et al., 1996).

In contrast, Almog et al. (2008) demonstrated that interval double transfer did improve the outcome in women with repeated IVF—embryo transfer failures and postulated that the reinsertion of the catheter may affect the endometrial cavity in a positive manner by inducing factors which enhance implantation, a view supported by Barash et al. (2003). Loutradis et al. (2004) also found that double-embryo transfer for women who had had three or more implantation failures with the transfer of good-quality embryos had a beneficial effect.

Overall, there appears to be preliminary evidence to suggest that double-embryo transfer may be of benefit but carefully designed randomized controlled trials are required to confirm its value, if any, in women with RIF.

Transfer into the Fallopian tube

During natural conception, zygotes come in contact with numerous growth factors and cytokines in the tubal fluid and, as a result, are thought to attain to the uterus with greater synchronization, which is thought to contribute to the development of the early embryo and enhance implantation potential (Jansen, 1984).

Zygote intra-Fallopian transfer (ZIFT) as a method of treatment for patients with repeated IVF failure was reported by Levran et al. (1998) in a case—control study. The pregnancy and implantation rates in the ZIFT group were found to be significantly higher than in the control group: 34.2% (24/70) and 8.7% (29/333) versus 17.1% (12/70) and 4.4% (13/289), respectively. The authors speculated that ZIFT allowed early embryonal growth in the natural tubal environment rather than the uterine cavity. The initial enthusiasm regarding ZIFT was later curtailed by the results of a series of prospective, randomized studies that failed to demonstrate any difference in implantation rates in IVF—ZIFT as compared with standard IVF—embryo transfer (Fluker et al., 1993; Toth et al., 1992; Tournaye et al., 1992). A meta-analysis reported similar pregnancy and implantation rates in ZIFT and IVF—embryo transfer groups (36.5% versus 31.4% and 15% versus 12%, respectively; Habana and Palter, 2001). The authors of this meta-analysis concluded that, with the advent of improvements in culture techniques in the IVF laboratory, intrauterine transfer remains the technique of choice.

Moreover, there are a number of disadvantages to the use of either zygote or embryo intra-Fallopian tubal transfer. These include the need for general anaesthetic, laparoscopy, theatre time and surgical equipment. From the financial viewpoint, it is also an expensive procedure. Furthermore, it has been reported to be associated with the increased risk of ectopic gestation (Habana and Palter, 2001).

Nevertheless, several recent studies have renewed the interest in ZIFT as it seems to be of particular value in couples with RIF (Levran et al., 2002; Weissman et al., 2007, 2013). Further, large, prospective, randomized studies are required to further evaluate the role of ZIFT in RIF.

The uterus

Hysteroscopy

There is convincing evidence that hysteroscopy improves the outcome of women with RIF (Demirol and Gurgan, 2004b). It may be performed as an outpatient procedure and small lesions may be removed at the same time, but more significant pathology may need to be dealt with later under general anaesthesia.

Intracavity lesions

Submucous fibroids. A recent meta-analysis showed that submucous fibroids significantly reduced the implantation rate, clinical pregnancy rate and live birth rate and significantly increased the miscarriage rate (Pritts et al., 2009). The presence of a submucous fibroid in women with RIF, regardless of the size, should be removed as it was shown in the meta-analysis that removal of submucous fibroids improves clinical pregnancy rates (Pritts et al., 2009).

Prior to the surgery, the size and number of fibroids and the depth of intramural extension should be carefully assessed. Resection of a solitary submucous fibroid less than 5 cm in diameter and with little intramural extension should not pose significant difficulties. However, a submucous fibroid more than 5 cm in diameter or more than 50% embedded in the intramural part of the uterus may require removal in two stages. In the case of multiple submucosal fibroids, there is an increased risk of intrauterine adhesion formation after the procedure. Some surgeons advocate the removal of the anterior wall and posterior wall fibroids on separate occasions to reduce the risk of intrauterine adhesions.

Endometrial polyps. Similarly, endometrial polyps in women with RIF ought to be removed. It has been shown that the removal of endometrial polyps in women undergoing intrauterine insemination resulted in doubling of the clinical pregnancy rate (Bosteels et al., 2010). In women with multiple endometrial polyps, as in the case of multiple submucous fibroids, the possibility of the procedure being complicated by intrauterine adhesions should also be borne in mind.

Uterine septum. In women with RIF, uterine septae should be removed, regardless of the size. Ban-Frangez et al. (2009) found that small and large septae had similar adverse impact, with significant increase in miscarriage rate in women undergoing IVF/ICSI treatment and that removal of these septae produced similar improvement in results (Ban-Frangez et al., 2009). The various techniques used to remove uterine septae have been reviewed (Homer et al., 2000).

Intrauterine adhesions. It is accepted that intrauterine adhesions would interfere with the implantation process and adversely affect the implantation rate and so, if present in women with RIF, should be removed (March, 2011). Nevertheless, there are as yet no firm literature data to confirm that removal of intrauterine adhesions improves the implantation rate. Furthermore, intrauterine adhesions often recur after surgical removal and there is a high rate of complication (10% or more) in cases of severe intrauterine adhesions resulting in partial or complete obliteration of the cavity.

The procedure should be carried out by an experienced reproductive surgeon under ultrasound guidance with a view to minimize complications. Special measures including the use of anti-adhesion barrier, intrauterine balloon, antibiotic therapy and high-dose oestrogen in the post-operative period to promote regeneration of the endometrium should be considered. The various surgical techniques used to remove intrauterine adhesions and to prevent reoccurrence of adhesions have been reviewed (March, 2011; Yu et al., 2008).

Myometrial pathology

Intramural fibroids. While women with RIF should have submucous fibroids removed, the possible contribution of intramural fibroids which are not distorting the uterine cavity to RIF is far from clear. There is no consensus on whether or not intramural fibroids in women with RIF should be removed. Many clinicians would recommend removal of intramural fibroids if they are more than 4 cm in diameter. There is a lower threshold to removing an intramural fibroid if it is situated in the anterior lower uterine segment as it may pose problems in delivery of the fetus, especially if Caesarean section is required. The pros and cons of myomectomy should be carefully explained in each case.

Couples should be aware of the possible complications of myomectomy, including the likelihood of blood transfusion, a small risk (1%) of hysterectomy and a relatively high risk of adhesion formation over the uterine scar as well as a small but serious risk of scar rupture during the ensuing pregnancy. On the other hand, the couples should also understand that intramural fibroids may not only cause implantation failure but also a number of other problems including miscarriage (both first and second trimester), red degeneration, preterm delivery, placental abruption, fetal growth restriction, malpresentation, difficulty with delivery and intrapartum and post-partum haemorrhage.

In the authors' view, the final decision must be individualized, and the involvement of a reproductive surgeon in the decision-making process is recommended. The techniques of myomectomy have recently been reviewed (McIlveen and Li, 2005). Uterine artery embolization is a possible alternative to myomectomy but is not preferred because of a small but worrying risk of compromise to the ovarian blood supply and because there is only a modest reduction in size, but not complete resolution of the fibroid. A recent study suggested that magnetic resonance-guided focused ultrasound surgery is a possible noninvasive therapy for intramural fibroids as the pregnancy outcome after the treatment appeared encouraging (Rabinovici et al., 2010).

Adenomyosis. The role played by adenomyosis in reproductive failure is receiving increasing attention and is now recognized to be a cause of RIF (Tremellen and Russell, 2011). Unlike intramural fibroids, adenomyosis is not usually amenable to surgical treatment, but Tremellen and Russell (2011), reported on four cases of RIF associated with adenomyosis, all successfully treated with an ultra-long pituitary downregulation protocol.

Thin endometrium

RIF may sometimes be associated with a thin endometrium ($<$7 mm) noted at the time of ultrasound examination on the day of HCG administration or embryo transfer. The observation suggests that the endometrium is not optimally responding to oestrogenic stimulation. There are several possible underlying causes. It may be congenital, associated with Turner's syndrome or a T-shape uterus. It may also be acquired, as a consequence of previous radiotherapy to the

pelvis or iatrogenic damage to the endometrium following intrauterine surgery or infection. From time to time, the underlying cause may not be obvious.

In this particular clinical situation, hysteroscopic examination of the uterine cavity is recommended to rule out intrauterine adhesions or Asherman's syndrome. Any intrauterine adhesion should be carefully removed with minimal thermal injury, under ultrasound guidance, by an experienced reproductive surgeon. The main challenge of intrauterine adhesiolysis is the prevention of recurrence of the adhesions. The various methods available to prevent recurrence have been recently reviewed (Yu et al., 2008).

Modified long protocol with exogenous oestrogen therapy

In the absence of any surgically correctable underlying pathology, there ought to be a strategy to improve endometrial growth by increasing the duration of oestrogenic priming prior to HCG trigger. The Royal Hallamshire Hospital employs a modified long protocol, which as yet has not been subjected to clinical trials. In essence, GnRH agonist is commenced in the mid-luteal phase of the cycle preceding IVF treatment. Two days after menstruation has started, usually a week after the initiation of GnRH therapy, high-dose oestrogen therapy in the form of oestradiol valerate 6—8 mg/day or oestradiol transdermal patch 400 μg/day is commenced. Endometrial thickness is monitored with serial ultrasonography after 7 days of oestrogen therapy and thereafter every 3 or 4 days until the endometrium has grown to more than 5 mm. At this stage, gonadotrophin may be commenced to stimulate the ovary to grow follicles, while the oestrogen therapy continues to ensure ongoing growth of the endometrium. The oestrogen therapy may stop on the day of HCG administration, when endogenous oestrogen concentration is often well over 5000 pmol/l.

In women who fail to respond to the treatment outlined above or fail to achieve implantation again, the Hospital's policy is to repeat the treatment protocol in a further cycle, collect the oocytes, but not transfer any embryo. Luteal support should be started after oocyte retrieval as usual. An endometrial biopsy should then be obtained 7 days after oocyte retrieval for histological evaluation. If there is evidence of satisfactory secretory transformation, embryo transfer may proceed in a subsequent artificial cycle with high-dose oestrogen therapy. If, however, there is no evidence of secretory transformation, this suggests that the endometrium is unable to support implantation and the couple should be advised to consider surrogacy.

Sildenafil

Sildenafil citrate has also been proposed in the treatment of women with RIF associated with a thin endometrium. Sildenafil, a phosphodiesterase-5 inhibitor, augments the vasodilatory effects of nitric oxide. The hypothesis behind the use of sildenafil is that it increases endometrial blood flow, which then leads to an increase in endometrial function. There have been two small observational studies on the use of sildenafil (Viagra): one involving four women with RIF (Sher and Fisch, 2000) and the other involving two women with Asherman's syndrome (Zinger et al., 2006). In addition, there has been a cohort study involving 105 infertile women with at least two consecutive prior IVF failures attributed to inadequate endometrial development (Sher and Fisch, 2002). Sildenafil was administered as a vaginal suppository at a dose of 25 mg four times a day for 3—10 days. Among the 73 subjects who attained an endometrial thickness of 9 mm or more, the implantation rate (29%) and pregnancy rate (45%) were significantly higher than the those observed among 32 subjects whose endometrial thickness was less than 9 mm (implantation 2%, pregnancy 0%, respectively) (Sher and Fisch, 2002). While the authors concluded that sildenafil appeared to benefit about 70% of subjects with inadequate endometrial development, the observation has not yet been confirmed by any randomized controlled trial.

Luteal support with GnRHa

In a recent randomized, placebo—control study involving 120 women with thin (7 mm or less) endometrium, women who received GnRHa on day of oocyte recovery, on the day of embryo transfer and 3 days later appeared to have significantly higher oestradiol and progesterone concentrations, thicker endometrium and higher implantation and pregnancy rates than those who received placebo (Qublan et al., 2008). However, it is unclear whether GnRHa has any significant benefit over other commonly used forms of luteal support such as progesterone or HCG.

Endometrial perfusion with granulocyte colony-stimulating factor

A recent study reported on the successful use of endometrial perfusion with granulocyte colony-stimulating factor in four women with inadequate development previously resistant to the use of oestrogen and vasodilators (Gleicher et al., 2011). The novel approach requires further investigation to confirm its usefulness.

Removal of hydrosalpinges

Salpingectomy

There is now good evidence that the removal of hydrosalpinges improves the implantation and live birth rates in women undergoing IVF. However, as salpingectomy is a surgical procedure which incurs extra cost, it opens up a debate on whether or not it is cost-effective to routinely remove all hydrosalpinges prior to IVF. Strandell et al. (2005), analysed the cost-effectiveness of salpingectomy prior to IVF, for up to three IVF cycles (Strandell et al., 2005). They found that the cost per live birth in the salpingectomy group was 22,823 Euros, which was significantly lower than the cost per live birth in the control (no salpingectomy) group (29,517 Euros). The observation suggests that it is more cost-effective to routinely remove hydrosalpinges prior to IVF treatment. There are conflicting reports on whether or not salpingectomy compromises ovarian response to stimulation during subsequent IVF treatment. It is prudent, when carrying out salpingectomy, to diathermize and incise as close to the Fallopian tube as possible and as far away from the ovary as possible to avoid disruption to the ovarian blood supply (**Figure 2**).

Salpingostomy

Salpingectomy is not the only surgical treatment option for women with hydrosalpinges contemplating IVF treatment.

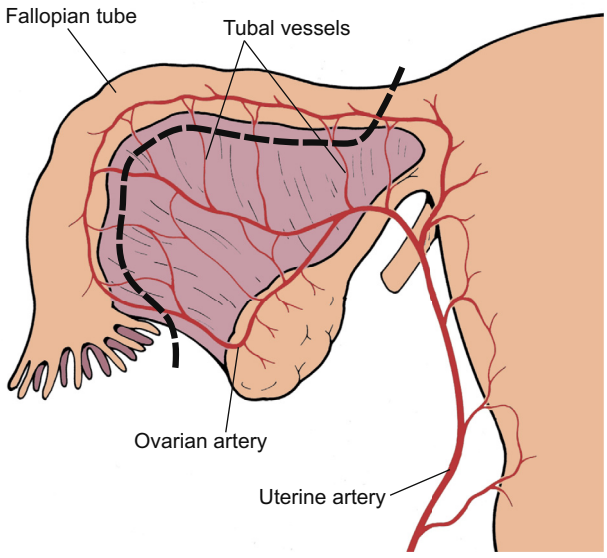

Figure 2 Salpingectomy for hydrosalpinx to improve the implantation rate. Diathermy and incision should be made as close as possible to the under surface of the tube and as far as possible from the ovary to avoid compromising the ovarian supply, which may in turn reduce ovarian response to stimulation during IVF treatment.

Salpingostomy may be a possible alternative as it not only 'removes' the hydrosalpinges but also produces the possibility of natural conception. The intrauterine pregnancy rate following salpingostomy has been reported by a number of investigators to be over 30% (Canis et al., 1991; Dubuisson et al., 1994; McComb and Paleologou, 1991; Mettler et al., 1990; Singhal et al., 1991). The results are likely to be higher if the damage to the Fallopian tubes is minimal (Winston and Margara, 1991).

It seems logical, therefore, to recommend that in women with hydrosalpinges and Fallopian tubes with minimal damage salpingostomy should be considered whereas tubes which are severely damaged (especially for those with intraluminal adhesions) ought to be removed (salpingectomy). The drawback of this approach is the possible recurrence of hydrosalpinges after salpingostomy, which than necessitates a further procedure to remove the tube, further delaying the treatment and incurring extra cost.

Other alternatives

Ultrasound-guided surgical drainage of hydrosalpinx has also been examined as a possible alternative to salpingostomy and salpingectomy. In a retrospective analysis, Sowter et al. (1997) showed that drainage of a hydrosalpinx did not improve the live birth rate. In a more recent randomized controlled trial, Hammadieh et al. (2008) found that ultrasound-guided hydrosalpinx aspiration increased the biochemical pregnancy rate but not the clinical pregnancy rate.

Transvaginal aspiration of hydrosalpinges is not recommended for a number of reasons. First, the fluid may rapidly reaccumulate as the underlying pathology has not been altered. Secondly, there is a risk of introducing infection. In addition, the efficacy of such a treatment modality has not been proven.

Alternatively, occlusion of the proximal part of the tube to prevent the hydrosalpingeal fluid from discharging into the uterine cavity has been proposed (Sagoskin et al., 2003). It has the possible advantages of being a simpler operation and less likely to disturb ovarian blood supply and hence compromise ovarian response to stimulation by gonadotrophins during IVF treatment. A recent trial showed indirectly that proximal tubal occlusion and salpingectomy result in similar improvement in IVF outcome in patients with hydrosalpinges (Kontoravdis et al., 2006). A further review provides evidence that laparoscopic tubal occlusion is an alternative to laparoscopic salpingectomy in improving IVF pregnancy rates in women with hydrosalpinges (Johnson et al., 2010).

However, occlusion of the proximal part of the tube leaves behind tubes blocked at both proximal and distal ends, with fluid trapped within, thereby increasing the risk of future infection (pyosalpinx) and persistent pain and the likelihood of a further surgical procedure to remove the diseased tube. For those patients with severe pelvic adhesions in whom surgery is expected to incur increased risks such as bowel injury, the use of Essure may be considered. It involves the occlusion of the Fallopian tubes hysteroscopically and avoids the need for either laparoscopic or open surgery. A number of studies have reviewed the use of Essure in the treatment of hydrosalpinges prior to IVF (Galen et al., 2011; Mijatovic et al., 2010, 2012; Thebault et al., 2012), but its use should be reserved for cases with extensive pelvic adhesions.

For now, therefore, the treatment of hydrosalpinges in women with RIF should be either salpingectomy or salpingostomy, with proximal tubal occlusion reserved for cases with severe/dense tubo-ovarian adhesions when the surgical morbidity in such cases is significantly increased.

Endometrial scratch

Barash et al. (2003) explored the possibility that local injury of the endometrium in the cycle preceding IVF treatment increases the success rate of implantation in a prospective study involving 130 patients who failed to conceive after one or more IVF treatment cycles. Forty-five out of 134 subjects were randomized by consent to have repeated endometrial biopsy on days 8, 12, 21 and 26 of the cycle immediately before the IVF treatment cycle. They found that the treatment resulted in a significant improvement (approximately double) in the rates of implantation, clinical pregnancy and live births (27.7%, 66.7% and 48.9%, respectively), compared with control subjects who did not have endometrial biopsies (14.2%, 30.3% and 22.5%, respectively).

It is not exactly clear why the endometrial biopsies helped the implantation rate. Barash et al. (2003) speculated that the healing process following endometrial biopsy may release cytokines and growth factors which facilitate the process of implantation. However, it is not certain whether four endometrial biopsies are required or only one is necessary to produce the observed beneficial results. Similarly, a further study identified a favourable influence of local injury to the endometrium in ICSI patients with high-order implantation failure (Raziel et al., 2007).

Subsequently, there have been two randomized controlled trials which examined the value of endometrial biopsy or endometrial scratch in the luteal phase on IVF outcome in women who have repeated failures (Karimzadeh et al., 2009; Narvekar et al., 2010). The pooled live birth rate in these two randomized controlled trials was significantly higher, more than double that of the control subjects. On the other hand, the pooled data from two randomized controlled trials on endometrial scratch carried out in the follicular phase of the index cycle showed no convincing evidence of benefit (Karimzade et al., 2010; Zhou et al., 2008).

The overall conclusion of these studies suggests that endometrial scratch is of benefit in women with RIF but it should be carried out approximately 7 days prior to the onset of menstruation, immediately before the start of ovarian stimulation for IVF treatment. However, all couples should be advised regarding the importance of protected intercourse in the month of the endometrial scratch because, when carried out in the luteal phase of the cycle, there is the risk of performing the procedure in the presence of an early pregnancy.

Endometrial injury or scratch could simply be performed by the use of a pipelle endometrial biopsy sampler (Laboratoire CCD, Paris; **Figure 3**). After the pipelle sampler is introduced into the endometrial cavity, the inner shaft is withdrawn to create a negative suction after which the pipelle sampler is gradually rotated as it is moved up and down the endometrial cavity several times to produce the 'scratching' action. In some centres, mock embryo transfers are routinely carried out prior to IVF treatment. In this situation, endometrial scratch and mock embryo transfer may be carried out at the same time.

Apart from the use of a pipelle endometrial sampler, endometrial biopsy may also be carried out at the time of hysteroscopy. Two randomized controlled trials also showed evidence of benefit when endometrial biopsy and hysteroscopy were carried out in the luteal phase of the cycle preceding IVF treatment (Demirol and Gurgan, 2004b; Rama Raju et al., 2006).

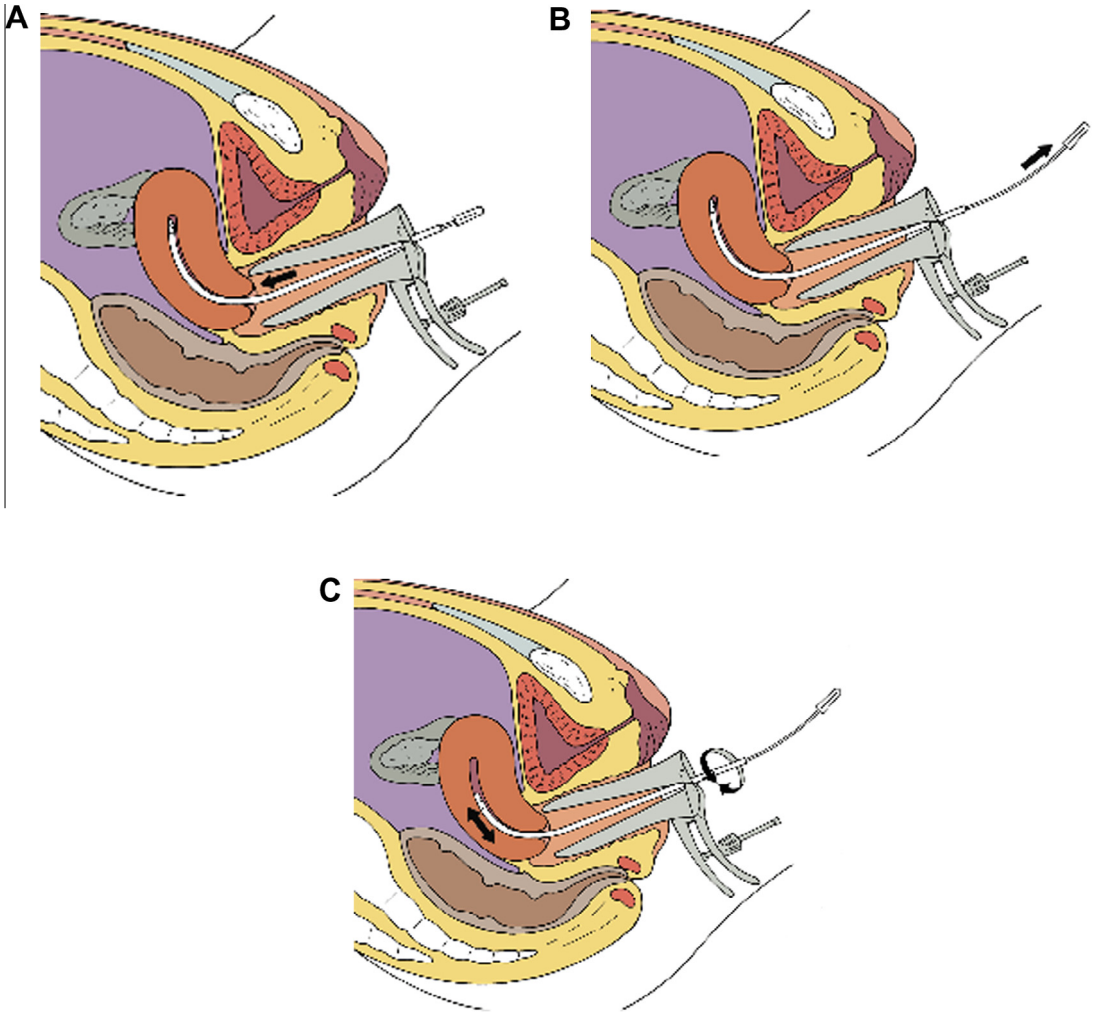

Figure 3 Endometrial scratch to improve implantation rate in women with recurrent implantation failure. (A) First, the pipelle sample is inserted until it reaches the fundus. (B) The inner plunger is withdrawn to apply a suction force to the endometrial cavity. (C) Endometrial scratch of the superficial layer of the endometrium is performed with the use of a 'hoovering' movement, combining a rotational and in-and-out movement of the pipelle sampler several times.

Table 1 Investigation of recurrent implantation failure.

Recommended investigations	*Investigations of research value*
Hysteroscopy	Hereditable/acquired thrombophilia
Hysterosalpingography	Sperm DNA fragmentation
Pelvic ultrasonography	
Parental karyotype	
Ovarian reserve and function: FSH, anti-Müllerian hormone, antral follicle count	

Table 2 Hierarchy of evidence as adopted from Green-top Guidelines published by the Royal College of Obstetricians and Gynaecologists.

Evidence level	*Type of evidence*
1++	High-quality meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with a very low risk of bias
1+	Well-conducted meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with a low risk of bias
1−	Meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with a high risk of bias
2++	High-quality systematic reviews of case–control or cohort studies or high-quality case–control or cohort studies with a very low risk of confounding, bias or chance and a high probability that the relationship is causal
2+	Well-conducted case–control or cohort studies with a low risk of confounding, bias or chance and a moderate probability that the relationship is causal
2−	Case–control or cohort studies with a high risk of confounding, bias or chance and a significant risk that the relationship is not causal
3	Non-analytical studies: e.g. case reports, case series
4	Expert opinion

Source: www.rcog.org.uk/guidelines.

Taken together, these trials on endometrial scratch suggest that it may be performed either with the use of a pipelle sampler or during hysteroscopy. Two recent systematic reviews and meta-analyses of the available literature showed a beneficial effect of local endometrial injury in RIF but advised that further robust randomized trials are required (El-Toukhy et al., 2012; Potdar et al., 2012).

Empirical therapies

Aspirin

Some centres offer empirical use of aspirin or heparin in women with RIF. A recent systematic review and meta-analysis on the use of low-dose aspirin showed no benefit of its use in IVF programmes (Gelbaya et al., 2007). A subsequent prospective, randomized, double-blind, placebo-controlled trial involving 201 couples concurred with the conclusion of the earlier meta-analysis (Dirckx et al., 2009). There is good evidence to suggest that aspirin should not be used in women with RIF.

Heparin

A recent randomized, double-blind, placebo-controlled trial of heparin and aspirin in women who had RIF (defined in study as $\geq$10 embryos transferred without achieving a pregnancy) and who were tested positive for either antiphospholipids or antinuclear or β_2-glycoprotein I antibodies, showed that the treatment did not improve implantation or pregnancy rate (Stern et al., 2003). The Practice Committee of the American Society for Reproductive Medicine advises that the assessment of antiphospholipid antibodies is not indicated among couples undergoing IVF and therapy is not justified on the basis of existing data (Practice Committee of the American Society of Reproductive Medicine, 2008).

Clinical trials

Women with RIF are desperate to seek a treatment which will lead to a successful outcome. Many of them would have been browsing the internet looking for a 'new' treatment on the horizon. They may not be able to judge for themselves the scientific credibility of such claims and they often seek the advice of their specialist to confirm whether certain treatments are worth trying. Clinicians should be able to judge whether a certain treatment has been proven to be of value. If not, treatment should not be initiated or the treatment should be offered only as part of a clinical trial. In this situation, prior ethics approval for the trial should have been obtained and national research governance guidelines followed. Written consent from each patient should be obtained.

Gamete donation and surrogacy

Couples with RIF need guidance on the appropriateness of proceeding with further IVF attempts. If implantation fails to occur despite repeated treatment attempts or if the prognosis of further IVF treatment is considered poor, alternative treatment options ought to be explored. If the likely source of the problem lies with the embryo, gamete donation should be advised. On the other hand, if the problem lies in the uterus, for example multiple small fibroids or Asherman's syndrome which has failed to respond to surgical treatment, surrogacy ought to be discussed.

Summary

To conclude, RIF should be defined as the failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. Women with RIF should be offered appropriate investigations to rule out an underlying cause for the repeated failure (**Table 1**). The main treatment strategy in couples with RIF is to improve the quality of the embryos transferred and the receptivity of the endometrium.

The following recommendations should be considered in the management of couples with RIF. The levels of evidence available in the literature to support each recommendation are given in accordance with the guidelines published by the Royal College of Obstetricians and Gynaecologists (www.rcog.org.uk/guidelines; **Table 2**).

Hysteroscopy should be carried out to exclude any intracavity uterine pathology; it has been shown to improve outcome (evidence level 1+).

Appropriate investigations should be carried out to exclude hydrosalpinx as it has been shown to reduce implantation rate, increase miscarriage rate and reduce live birth rate; removal of hydrosalpinges has been shown to improve the outcome (evidence level 1++).

Submucosal fibroids have been shown to reduce implantation, pregnancy and live birth rates; removal of submucosal fibroids improves implantation rate (evidence level 1+).

Endometrial polyps should be removed; although there is no data on its impact on women undergoing IVF, it has been shown to improve outcome in women undergoing intrauterine insemination (evidence level 1−).

Endometrial scratch should be considered in the luteal phase of the cycle immediately preceding IVF treatment; it improves implantation rate and outcome in women with unexplained RIF (evidence level 1−).

Uterine septum increases miscarriage rate; its removal improves outcome (evidence level 2+).

The use of ultra-long protocol may improve outcome in women with endometriosis and adenomyosis (evidence level 3).

Intramural fibroid of more than 5 cm should be removed (evidence level 3).

Intrauterine adhesions are a recognized cause of thin endometrium not responding to ovarian steroid stimulation; if present, intrauterine adhesions should be removed (evidence level 4).

A multidisciplinary approach should be adopted in the management of RIF (evidence level 4).

Empirical therapies should, whenever possible, be considered only in the setting of carefully conducted clinical trials (evidence level 4).

Acknowledgement

The authors would like to thank Patrick Elliott who kindly provided the figures for this manuscript.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 13 November 2012; refereed 5 May 2013; accepted 6 August 2013.