Review

Recurrent implantation failure in assisted reproduction: how to counsel and manage. A. General considerations and treatment options that may benefit the couple

After his residency training in Obstetrics and Gynecology in the University of Hacettepe in Ankara, Dr Urman completed a 3-year fellowship programme in Reproductive Endocrinology and Infertility in Vancouver, Canada. He returned to Hacettepe University in 1991 and participated in the foundation of one of the first IVF clinics in Turkey. He worked as an Associate Professor until 1996 in the same institution. Dr Urman resigned from the university in 1996 and founded the Assisted Reproduction Unit of the American Hospital of Istanbul, one of the biggest IVF centres in the country. His major areas of interest are clinically assisted reproduction, laparoscopic and hysteroscopic surgery. He has published extensively in these fields, having over 70 articles published in renowned international journals.

Dr Bulent Urman

Bulent Urman[1], Kayhan Yakin, Basak Balaban
Assisted Reproduction Unit, American Hospital of Istanbul, Turkey
[1]Correspondence: Guzelbahce sok No. 20, Nisantasi, Istanbul. e-mail: burman@superonline.com

Abstract

Recurrent implantation failure is a distressing phenomenon, both for the infertile couple and for the physician responsible for their treatment. Aetiology is often not clear and treatment options are vague. Particularly when transferred embryos are of good quality, recurrent implantation failure may be attributed to less than optimal embryo transfer technique, pathological lesions of the uterine cavity, the presence of hydrosalpinges, fibroids and endometriosis. Poor embryo quality, especially when repetitive, is a major impediment to successful implantation and cannot be corrected at the present time. Molecular abnormalities at the endometrial level and abnormal embryo–endometrium dialogue may be responsible for some cases of recurrent implantation failure. Furthermore, there may be over- or under-expressed genes that may be related to successful implantation. At the present time, the physician confronted with a couple presenting with recurrent implantation failure should discuss openly the potential causes of this phenomenon, with special emphasis on correctable causes, and offer remedies that are evidence based.

Keywords: *assisted reproduction, embryo transfer, endometriosis, fibroids, hysteroscopy, implantation failure*

Introduction

The fertility specialist is often called upon to perform the unpleasant task of counselling an infertile couple following failed attempts at assisted reproduction. Frustration coupled with despair usually results from unrealized expectations, and is further augmented by the fear of a childless future. As assisted reproduction is considered to be the last step in the armamentarium of infertility treatments, the couple is faced with the cold reality of having tried everything and failed.

The aetiology of recurrent implantation failure in assisted reproduction is complex and ill understood. Embryonic aneuploidy, abnormalities of the uterine cavity, altered endometrial receptivity and a less than optimal transfer technique have been blamed (Damario and Rosenwaks, 2000).

What is recurrent implantation failure? Surely the older patient who fails to conceive with non-selective embryo transfers is different from the younger patient who fails to conceive despite the presence of multiple good quality embryos during each attempt. It is the latter patient that causes the greatest frustration. Recurrent implantation failure may be associated with good quality or poor quality embryos. In couples who repeatedly have poor quality embryos available for transfer the outcome is bleak, as many do not conceive despite multiple treatment attempts. The aetiology of poor embryo development is unclear. Many factors, mostly undefined, may be responsible for the generation of poor quality embryos. Older women seem to have more mitochondrial DNA mutations that can be responsible

for poor embryonic development (Bartmann *et al.*, 2004). The incidence of aneuploidy is increased in morphologically abnormal embryos. Ovarian stimulation protocols and in-vitro culture may also contribute to poor or retarded embryonic development (Check *et al.*, 1999; Van den Auwera *et al.*, 1999; Stouffer and Zelinski-Wooten, 2004). Softer stimulation protocols and currently used media that take into account the needs of the developing embryo will undoubtedly improve those aspects of embryo quality that may be affected by extrinsic factors (Gardner and Lane, 2003). However, most cases of poor embryo quality are due to embryonic aneuploidy, which is impossible to correct with current techniques (Gianaroli *et al.*, 1999; Munné, 2002).

Whether the phenomenon of recurrent implantation failure with good quality embryos is unexplained or undiagnosed is a matter of debate. Given that assisted reproduction treatment is complex, emotionally tiring and expensive, factors that are known to affect the outcome of treatment should be identified and corrected when possible. The couple is often not satisfied when their only option is to repeat the treatment because the scientific community has nothing better to offer. Unfortunately, centres are under pressure to offer treatment options that have not been proven beyond doubt to be of any benefit to the couple with recurrent implantation failure. Some of these are antithrombolytic medications, third-party lymphocyte sensitization, sequential embryo transfer, assisted hatching and endometrial co-cultures (Urman *et al.*, 2005). Furthermore, preimplantation genetic diagnosis for recurrent implantation failure is often informative but rarely beneficial (Munné *et al.*, 2003).

The period during which the endometrium is receptive to the blastocyst is defined as the implantation window. This is characterized by the expression of various endometrial products such as pinopodes, integrins and leukaemia inhibitory factor (Bourgain and Devroey, 2003). Endometrial receptivity may be altered in stimulated cycles due to premature expression of pinopodes and integrins, resulting in precocious luteal transformation. In women with unexplained recurrent implantation failure with good quality embryos, natural cycle IVF may be an option that may result in enhancement of implantation (Kadoch, 2004).

Endometrial preparation in an assisted reproduction programme simply depends on controlled oestrogen–progesterone medication and monitoring is relatively crude, being performed with the measurement of endometrial thickness and echogenicity. However, implantation is a delicate process involving complex interactions of various factors derived either from the embryo or endometrium. Over the years, noteworthy progress has been achieved in the success rates of assisted reproductive techniques; however, embryo implantation still remains a major limiting factor. It is obvious that pathways leading to successful implantation have to be delineated prior to attaining higher pregnancy rates.

Implantation is a very complex mechanism involving many factors derived from the embryo, endometrium and the immune system. Any malfunctioning in this highly complex machinery may lead to failure of implantation. It is known that 85% of embryos transferred *in utero* fail to implant (Edwards, 1995). Even a top quality blastocyst has only a 60.9% implantation rate (Gardner *et al.*, 2004). Many of the aforementioned factors have clinical associations with infertility, but none has been proven to be an independent factor that may lead to implantation failure when deficient or malfunctioning. Implantation failure in knockout mice with a defective leukaemia inhibitory factor gene is the only clinically relevant piece of information to prove the net effect of lack of a cytokine on the implantation process (Arici *et al.*, 1995). It is not clear whether the abnormality of expression of a particular factor is the reason of implantation failure, or just a result or reflection of other abnormalities taking place in a complex cascade of events. The molecular mechanism of endometrial receptivity appears to be very complex, and identification of a single molecule as the likely cause of all unexplained implantation failures is highly unlikely. Further unravelling of molecules involved in the intricate mechanism of implantation is needed for better comprehension of the link between altered endometrial receptivity and implantation failure. For a comprehensive review of embryo implantation and its clinical implications, the reader is referred to the excellent review by Hoozemans *et al.* (2004).

What is the chance of conception following multiple failed assisted reproduction attempts?

The answer to this question is unfortunately not straightforward. Of couples undergoing IVF/intracytoplasmic sperm injection (ICSI), approximately 30% should attain a delivery per oocyte retrieval (SART/ASRM, 2004). It has been demonstrated that pregnancy rates do not change over the initial three treatment cycles, but decrease considerably after four or more failed attempts (Templeton and Morris, 1998). Approximately three in four couples conceive following four IVF attempts (Sharma *et al.*, 2002). Cumulative conception rates differ significantly between women <35 years of age and those >35 who have had five or more oocytes retrieved (83 versus 63%). When the number of retrieved oocytes was fewer than five, conception rates decreased significantly in both age groups. Even in younger women who responded poorly to ovarian stimulation and yielded fewer than five oocytes, cumulative conception rate over four treatment cycles was only 33%.

In older women (>40 years of age), ovarian reserve appears to significantly influence the chances of conception; however, despite apparently normal ovarian reserve, older-age women have a significantly lower chance of conception compared with younger women with a diminished ovarian reserve (van Rooij *et al.*, 2003). In women in the 43- to 45-year age group, pregnancy rates per cycle and embryo transfer were reported to be 6.6 and 9.4% respectively (Orvieto *et al.*, 2004). However, these seemingly optimistic pregnancy rates were counterbalanced by a 70% abortion rate. These findings indicate that the age of the woman is an independent and very significant factor affecting the success of assisted reproduction. Therefore, older women are more likely to fail multiple attempts at assisted reproduction, failure being most likely due to a low probability of conception to start with in this age group.

What is the reason for low implantation rates in older women? Why do older women have more aneuploid conceptions and why does embryonic aneuploidy lead to implantation failure?

Chromosome segregation in humans is controlled by the meiotic spindle, the components of which are supplied by the cytoplasm. Dysfunctional cytoplasmic factors may be responsible for structural abnormalities of the spindle that may lead to eventual malsegregation and poor implantation (Battaglia *et al.*, 1996). Older women and women with elevated FSH concentrations indicative of diminished ovarian reserve have been shown to be under an increased risk of Down's syndrome, lending support to the theory that ageing oocytes are at higher risk of being aneuploid (Dailey *et al.*, 1996; Freeman *et al.*, 2001; Salamanca-Gomez, 2001; Van Montfrans *et al.*, 2001). Mutations in the mitochondrial DNA of older women may lead to altered mitochondrial activity and decreased cytoplasmic ATP production, leading to altered spindle formation on the one hand and decreased free radical clearance on the other hand, which together may affect implantation via embryonic aneuploidy and cell damage (Bartmann *et al.*, 2004).

What are the risks associated with repetitive assisted reproduction treatment?

Are there certain risks associated with multiple assisted reproduction treatments? This question is often asked of infertility specialists, as couples are concerned about risks such as premature depletion of the ovarian follicle pool and cancer. There is little indication in the literature that ovarian stimulation diminishes ovarian reserve, with the number of oocytes being maintained with repeated treatment attempts (Kelly *et al.*, 2003). Significant decrease in ovarian response, however, is evident with increasing female age. Pregnancy and live birth rates decline to a small degree only up to cycle 3 or 4, with increasing female age being the prime determinant. It is therefore advisable that couples in whom the female partner's age is a pressing issue undergo repeated treatment cycles without undue delay.

Assisted reproduction has exposed many women to potent drugs that are used for follicular stimulation. Follicular stimulation is associated with supraphysiological oestrogen, progesterone, and human chorionic gonadotrophin (HCG) concentrations. Drugs that are used to induce or potentiate ovulation have been associated with neoplasms of the ovary, particularly borderline and granulose cell tumours (Mosgaard *et al.*, 1998; Shushan *et al.*, 1999). However, previous data have been derived from population-based retrospective studies that were prone to bias. Medications used for ovulation induction were not clearly defined and were vaguely lumped into a single category called 'fertility drugs'. Current evidence does not indicate that ovarian stimulation leads to increased risk of ovarian malignancy (Venn *et al.*, 1999; Klip *et al.*, 2000; Dor *et al.*, 2002; Kashyap and Davis, 2003; Brinton *et al.*, 2004). In fact, infertility therapy may confer protection for those patients who conceive. Slight but non-significant elevations in risk for certain subgroups of users, however, support the need for continued monitoring of long-term risks (Brinton *et al.*, 2004). In women who had undergone multiple attempts at assisted reproduction without conception, follow-up with yearly vaginal sonograms and serum CA-125 measurements in their post-menopausal years for early detection of ovarian cancer appears to be prudent.

Ovarian stimulation that radically changes the hormonal milieu might affect the proliferation of epithelial breast cells and thus increase the risk of breast cancer (Shushan *et al.*, 1999). Women who have been exposed to fertility drugs with IVF seemed to have a transient increase in the risk of having breast cancer diagnosed in the 1st year after treatment, though the incidence overall was no greater than expected (Venn *et al.*, 1999). Ten Australian IVF clinics provided data for this study where women who had been referred for IVF before 1 January 1994 were analysed. The frequencies of invasive breast, ovarian, and uterine cancer were assessed by record linkage to population-based cancer registries and the national death index. For breast and ovarian cancer the incidence was no greater than expected [SIR 0.91 (95% CI 0.74–1.13) for breast cancer and 0.88 (0.42–1.84) for ovarian cancer in the exposed group and 0.95 (0.73–1.23) for breast cancer and 1.16 (0.52–2.59) for ovarian cancer in the unexposed group]. Analysis of cancer incidence within 12 months of exposure to fertility drugs with IVF showed that incidence was significantly higher than expected for breast and uterine cancer [1.96 (1.22–3.15) and 4.96 (1.24–19.8)].

Possible adverse effect of ovarian stimulation on implantation

Gonadotrophin preparations used for controlled ovarian stimulation are associated with supraphysiological concentrations of oestradiol that may perturb oocyte quality and endometrial receptivity (Forman *et al.*, 1988; Yang *et al.*, 2001). Endometrial receptivity may be altered in stimulated cycles due to premature expression of pinopodes and integrins, resulting in precocious luteal transformation. Very high oestradiol concentrations are usually associated with overstimulated polycystic ovaries. Assisted reproduction treatment in women with polycystic ovaries has been associated with decreased fertilization rates and poor embryo quality; however, these were compensated by the increased number of retrieved oocytes and a larger-than-normal pool of embryos to select from, therefore not affecting implantation and pregnancy rates (Urman *et al.*, 2004).

Most of the studies that evaluated the outcome of IVF, in relation to oestradiol concentrations on the day of HCG, failed to show any significant association (Mettler and Tavmergen, 1989; Dor *et al.*, 1992; Sharara and McClamrock, 2000; Papageorgiou *et al.*, 2002; Chen *et al.*, 2003). Some studies even showed a higher pregnancy rate in overstimulated women with high oestradiol concentrations (Chenette *et al.*, 1990; Gelety and Buyalos, 1995; Levi *et al.*, 2001). Only a few studies showed a negative correlation between oestradiol concentrations on the day of HCG and the outcome of IVF–embryo transfer (Simon *et al.*, 1995; Yu Ng *et al.*, 2000). It can be concluded according to data derived from the current literature that oestradiol concentrations on the day of HCG are not related to the outcome of assisted reproduction (Kosmas *et al.*, 2004). Furthermore, it is unlikely that ovarian stimulation and the associated hyperestrogenism are responsible for recurrent implantation failure. However, in couples where no other abnormality can be found responsible for recurrent implantation failure, natural cycle IVF can be considered (Kadoch, 2004).

Impact of endometriosis on implantation

How important is the presence of endometriosis in women undergoing assisted reproduction, to what extent should one carry out non-invasive and invasive diagnostic tests, and should endometriosis lesions be treated prior to instituting assisted reproduction treatment? The answers to these questions are unfortunately vague due to lack of evidence derived from well-designed studies. Endometriosis is known to be associated with infertility (Strathy *et al*., 1982; Jansen, 1986; Rodriguez-Escudero *et al*., 1988). What is not clear, however, is whether treatment of endometriosis restores fertility and alters the natural course of the disease. As endometriosis is a common occurrence in women of reproductive age, it is not surprising that many women undergoing assisted reproduction for indications other than endometriosis may harbour the disease. Endometriosis may cause infertility due to mechanical factors, cellular and humoral alterations of the intraperitoneal environment, adverse effects on gamete interaction, gamete transport and embryo implantation (Lessey *et al*., 1994; Lebovic *et al*., 2001; Genbacev *et al*., 2003; Kao *et al*., 2003). Furthermore, the luteal phase has been shown to be abnormal in women with endometriosis (Schenken *et al*., 1984).

There have been recent reports focusing on the potential adverse effect of endometriosis on oocyte development (Barnhart *et al*., 2002; Mahutte and Arici, 2002). Granulosa cell apoptosis, which may affect oocyte quality, has been shown to be increased in women with endometriosis (Barnhart *et al*., 2002; Mahutte and Arici, 2002).

Simon *et al*. analysed the outcome of patients with endometriosis compared with tubal factor controls and showed decreased pregnancy rate per transfer and implantation rate in the former group (Simon *et al*., 1994). When the authors analysed their oocyte donation cycles, they found that in women with endometriosis, donor oocytes obtained from women without the disease implanted as efficiently as in other recipients. Recipients who received oocytes from donors with endometriosis had significantly lower implantation rates. The same group in a later study evaluated the performance of donated sibling oocytes in recipients with and without advanced stage endometriosis (Diaz *et al*., 2000). The results of these two studies indicate that endometriosis affects gamete quality rather than endometrial receptivity.

Impaired implantation due to decreased endometrial receptivity has been proposed as one of the mechanisms by which endometriosis causes infertility. Recently, the molecular basis of implantation failure has been investigated using microarrays to uncover genes or gene candidates that are aberrantly expressed during the window of implantation in women with endometriosis (Guidice *et al*., 2002). Paralleled gene expression profiling was applied using high-density oligonucleotide microarrays to investigate differentially regulated genes in endometrium from women with and without endometriosis (Kao *et al*., 2003). The investigators showed dysregulation of select genes, leading to an inhospitable environment for implantation, including genes involved in attachment, embryo toxicity, immune dysfunction, and apoptotic responses.

Contrary to data from preclinical studies, most data from IVF cycles failed to show any difference between implantation rates in women with and without endometriosis (Inoue *et al*., 1992; Dmowski *et al*., 1995; Olivennes *et al*., 1995; Pal *et al*., 1998; Bukulmez *et al*., 2001; Dmowski *et al*., 2002; Hickman, 2002). Only a few studies showed statistically significant lower implantation and pregnancy rates with IVF in women with early as well as late stages of the disease (Matson and Yovich, 1986; Simon *et al*., 1994; Arici *et al*., 1996; Azem *et al*., 1999). A recent meta-analysis of 22 studies evaluated the outcome of IVF–embryo transfer in women with endometriosis (Barnhart *et al*., 2002). The chance of achieving pregnancy was significantly lower for endometriosis patients compared with tubal factor controls (odds ratio, 0.56; 95% CI, 0.44–0.70). Multivariate analysis revealed significant decrease in the number of oocytes retrieved, fertilization, and implantation rates. Pregnancy rates in patients with severe endometriosis were significantly lower than for women with mild disease.

The physician is often confronted with the patient who has failed IVF treatment and has ovarian endometriosis. Should endometriomas be removed prior to a subsequent attempt? Does surgery before assisted reproduction improve outcome? The answers to these questions are unfortunately not straightforward. In two recent studies, surgical treatment (laparoscopic excision) of ovarian endometriosis did not improve the outcome of subsequent IVF treatment when treated patients were compared with a control group (Surrey, 2003; Garcia-Velasco *et al*., 2004). It should be noted that there are no randomized studies evaluating the outcome of IVF/ICSI in women with treated versus non-treated ovarian or for that matter peritoneal endometriosis. Although retrospective studies show no benefit of treatment, the subset of patients with recurrent implantation failure may be totally different from patients undergoing assisted reproduction treatment for the first time (Surrey, 2003; Garcia-Velasco *et al*., 2004). In the recurrent implantation failure patient, given the molecular association of endometriosis with implantation, laparoscopic removal of ovarian lesions is recommended prior to embarking on a subsequent IVF attempt. However, whether laparoscopy should be performed to delineate peritoneal lesions after multiple failed IVF attempts is debatable.

Women with endometriosis were shown to have higher pregnancy rates in IVF when amenorrhoea was induced for 3 months with gonadotrophin-releasing hormone (GnRH) analogues prior to commencement of the treatment (Surrey *et al*., 2002). Edwards *et al*. (1997) found higher pregnancy rates in women who were previously amenorrheic, irrespective of age and number of embryos transferred. Whether inducing long periods of amenorrhoea prior to treatment with IVF in women with endometriosis will improve outcome, and whether this should be the standard of care in these women, however, should be evaluated in larger randomized trials.

Pathological lesions of the uterus, endometrial cavity, and Fallopian tubes that may affect implantation

There may be physical impediments to embryo implantation that ideally need to be corrected prior to initiation of treatment. These may have been missed or not attributed adequate weight

during the initial evaluation phase. It should, however, be stressed that the adverse effect of most perceived abnormalities such as minor endometrial pathologies, intramural fibroids, and hydrosalpinges that are not visible on ultrasound is far from clear. Randomized studies are lacking, and in the future will be difficult, if not impossible, to perform.

Intracavitary lesions

There appear to be three prerequisites for successful implantation: good quality embryos, a functional and intact uterine cavity, and an optimal embryo transfer technique. In couples who fail to conceive with multiple assisted reproduction attempts despite the transfer of good quality embryos, integrity of the uterus should be questioned. The uterine cavity should be free of lesions such as adhesions, polyps, fibroids and septae. As treatment of these lesions in otherwise infertile women improves the success of spontaneous conception, it is logical to assume that they should be attended to prior to IVF/ICSI (Mastrominas *et al*., 1996; Varasteh *et al*., 1999).

Most clinics carefully evaluate the uterus and the uterine cavity prior to embarking upon a costly and emotionally tiring process such as assisted reproduction. An integral part of this evaluation is high resolution transvaginal ultrasound, which depicts abnormalities such as fibroids, Mullerian anomalies, and intracavitary lesions. When transvaginal ultrasound is performed in the preovulatory phase of the cycle, intracavitary lesions can be seen more clearly and thickness and echogenicity of the endometrium can be evaluated. In women with intrauterine adhesions preovulatory endometrium is usually thin with interruption of the endometrial line at various localizations.

Three-dimensional ultrasound appears to be particularly useful for delineating the contours of the uterus. The depth of a uterine septum can be accurately measured with this technique. However, its accuracy is decreased when the uterus is retroverted or axial in position.

Hysterosalpingography (HSG) is usually performed during the course of infertility investigation. Hysterosalpingography, however, lacks sufficient accuracy in demonstrating tubal patency and intrauterine lesions. Further evaluation by sonohysterography or hysteroscopy may be necessary when HSG suggests abnormality.

Sonohysterography is a simple and accurate means of assessing the uterine cavity both in the infertile patient and in the patient with post-menopausal bleeding (Breitkopf *et al*., 2003; Nass *et al*., 2003; Berridge and Winter, 2004). Sonohysterography accurately depicts intrauterine lesions such as fibroids and polyps (Becker *et al*., 2002; Alcaizar *et al*., 2004). Mullerian anomalies can also be detected and differential diagnosis between the bicornuate and the septate uterus can be made with hysterosonography (Alborzi *et al*., 2002).

Office hysteroscopy, which may be performed under sedation, enables intracavitary lesions to be accurately diagnosed and treated simultaneously. General anaesthesia is not necessary, as the procedure is tolerated very well by the patient (Betocchi *et al*., 2004). Hinckley *et al*. performed office hysteroscopy prior to IVF treatment in 1000 consecutive infertile patients (Hinckley and Milki, 2004). Of these, 62% had a normal uterine cavity and 32% had endometrial polyps. Other pathology included submucous fibroids (3%), intrauterine adhesions (3%), polypoid endometrium (0.9%), septum (0.5%) retained products of conception (0.3%), and bicornuate uterus (0.3%). These findings suggest that evaluation of the uterine cavity with office hysteroscopy or at least sonohysterography should be undertaken in all women scheduled to undergo treatment with assisted reproductive techniques.

In women who fail to respond to assisted reproduction treatment, evaluation of the uterine cavity is strongly recommended if this had not been performed previously. Oliviera *et al*. performed hysteroscopic assessment of the uterine cavity in women who repeatedly failed to conceive despite the transfer of good quality embryos (Oliveira *et al*., 2003). All women had a previous normal hysterosalpingography. Of the evaluated 55 patients, 45% had abnormal hysteroscopic findings that were corrected during the same session. All patients underwent a subsequent IVF cycle where patients who were treated for uterine abnormalities achieved higher implantation (19 versus 5.5%) and pregnancy rates (50 versus 20%) compared with patients who had normal uterine cavities. The findings of this study suggest that a considerable proportion of patients who repeatedly fail to conceive with assisted reproduction have intracavitary abnormalities that should be corrected prior to a subsequent attempt.

Demirol and Gurgan (2004) evaluated the effect of hysteroscopic diagnosis and treatment of intrauterine pathologies on the outcome of assisted reproduction in women with two or more previous implantation failures. Patients who underwent hysteroscopy and subsequent treatment of depicted intrauterine pathology attained higher pregnancy rates compared with patients who never had a hysteroscopy. The authors recommended that all patients with recurrent implantation failure should be evaluated by hysteroscopy, despite normal hysterosalpingography.

In a recent study, Barash *et al*. (2003) performed an endometrial biopsy during the luteal phase of the previous cycle in patients who repeatedly fail to conceive with IVF. Implantation and pregnancy rates were significantly higher in biopsied patients compared with patients who were not subjected to this procedure. The authors speculated that local injury induced by the biopsy procedure might have increased the release of growth factors and cytokines that may render the endometrium more favourable to implantation. Hysteroscopic treatment of minor uterine abnormalities may act in the same manner. Although it is difficult to recruit patients, a future study should include patients with minor uterine abnormalities randomized to hysteroscopic treatment versus diagnostic hysteroscopy and endometrial biopsy. Only then one can conclude beyond reasonable doubt that treatment of these minor intracavitary abnormalities has any value.

Intrauterine adhesions may result from previous curettage, hysteroscopic myomectomies and polypectomies. They may also be due to tuberculous endometritis especially in Third World countries. When mild–moderate, intrauterine adhesions can be treated easily and successfully by hysteroscopy. Office hysteroscopy is particularly useful in this setting. Intrauterine adhesions should be suspected in women who conceived but miscarried in their first IVF cycle but failed to conceive in their

subsequent cycles. These women should be evaluated with office hysteroscopy and treated accordingly.

Fibroids

One of the most controversial issues in women scheduled to undergo assisted reproduction treatment is the presence of fibroids. Uterine fibroids are the most common benign tumour of the genital tract and may be encountered in a substantial proportion of women being treated with IVF/ICSI. Fibroids have long been regarded as innocent bystanders, provided that they do not encroach on the uterine cavity. There is general agreement on the necessity to treat intracavitary (submucous) fibroids because they act as space occupying lesions in the uterine cavity where the embryos are destined to implant. Furthermore, they may induce a local inflammatory reaction or change the secretion of implantation related cytokines (Surrey, 2003). The impact and possible mechanism of action of intramural lesions, which do not clearly alter the contour of the endometrial cavity, however, remain controversial (Bajekal and Li, 2000; Donnez and Jadoul, 2002). Alterations in uterine artery blood flow may have an impact on implantation, although conflicting results have been reported (Ng *et al*., 2005). Other recent studies have evaluated alterations in gene expression and local cytokine release, which may also play a role (Surrey, 2003). While some studies demonstrated significantly reduced implantation and conception rates (Eldar-Geva *et al*., 1998; Hart *et al*., 2001; Check *et al*., 2002) in women with intramural fibroids other studies failed to show any adverse effect (Seoud *et al*., 1992; Jun *et al*., 2001; Surrey *et al*., 2001; Yarali and Bukulmez, 2002). Different outcomes may be partly attributable to differences in patient inclusion criteria and inconsistencies in analyses of precise fibroid location and size. One prospective case control study showed that intramural fibroids even when <5 cm and not distorting the uterine cavity halved the pregnancy rate in couples undergoing IVF compared with a control group without fibroids (Hart *et al*., 2001). Although the group with fibroids was older (36.4 versus 34.6 years) significance persisted after logistic regression analysis that controlled for age and the number of embryos transferred. Only patients undergoing their first treatment cycle and patients who had intramural fibroids <5 cm were included in the study. Therefore, inferences regarding patients with larger fibroids and couples with recurrent implantation failure cannot be made. Furthermore, there is no evidence concerning the effect of myomectomy on the outcome of subsequent treatment cycles.

Fibroids may co-exist with other pelvic pathologies such as endometriosis and adhesions from prior myomectomies that may also negatively affect the chances of conception. When only couples undergoing ICSI for male factor were considered, Yarali and Bukulmez (2002) failed to show any negative effect of intramural and subserosal fibroids measuring from 0.5 to 10 cm in size. However, a more recent study that evaluated the impact of intramural fibroids that did not distort the uterine cavity found significant decrease in pregnancy and increase in abortion rates when the size of the fibroid exceeded 4 cm (Oliveira *et al*., 2004).

The couple with recurrent assisted reproduction failures where the female partner has a fibroid should be counselled carefully in light of the available literature, and if there is no other negative factor besides the fibroid/s myomectomy should be considered.

Hydrosalpinx

The effect of hydrosalpinges and for that matter severe tuboperitoneal disease on the outcome of assisted reproduction has been intensely debated over the last decade. Severe pelvic adhesions may be associated with poor ovarian response and difficult oocyte retrieval procedures. However, there is no study that evaluated the effect of adhesiolysis and restoration of normal pelvic anatomy on the success of assisted reproduction. A recent Cochrane review concluded that the role of surgery for tubal disease in the absence of a hydrosalpinx is unclear and merits further evaluation (Johnson *et al*., 2001).

When women with tubal disease with and without hydrosalpinges were compared in a meta-analysis, significantly poorer outcomes were recorded in the former group (Camus *et al*., 1999). The study examined nine published retrospective comparative series and five series published as abstracts for which additional information was obtained. A total of 5592 patients were studied (1004 with hydrosalpinx and 4588 with tubal infertility without hydrosalpinx). Pregnancy rates were significantly lower in the presence of hydrosalpinges: 31.2% for the tubal factor group without hydrosalpinges compared with 19.7% for the group with hydrosalpinges (odds ratio: 0.64; 95% confidence interval: 0.56–0.74). Similarly, implantation and delivery rates in the hydrosalpinx group were significantly decreased (implantation: 8.5 and 13.7%; delivery: 13.4 and 23.4% respectively). The incidence of early pregnancy loss was also higher in the group with hydrosalpinges (43.7%) than in the control group (31.1%).

Why do hydrosalpinges reduce the chances of pregnancy and what is the appropriate intervention prior to embarking upon IVF or after failed treatment attempts? There are several hypotheses regarding the mechanism/s of hydrosalpinx-associated implantation failure. Embryotoxic effects, mechanical flushing of the embryos due to intermittent regurgitation into the uterine cavity of the hydrosalpinx fluid, and diminished endometrial receptivity due to disturbed expression of the cytokine cascade, which is essential for implantation are some of the theories that have been proposed (Ajonuma *et al*., 2002; Strandell and Lindhard, 2002). Chronic inflammation associated with chlamydial infections lead to hydrosalpinx formation and accumulation of hydrosalpinx fluid. Inflammatory mediators may act upon the Fallopian tube epithelial cells to increase permeability through secondary messengers. During inflammation, cystic fibrosis transmembrane conductance regulator in endosalpingeal cells may be continually activated, leading to increased fluid secretion and decreased fluid absorption (Ajonuma *et al*., 2002).

Should hydrosalpinges be removed prior to assisted reproduction treatment? The answer to this question can be found in recent studies and meta-analyses (Johnson *et al*., 2001, 2002; Strandell *et al*., 2001; Zeyneloglu, 2001). Compiling data from the published studies in the literature, Zeyneloglu *et al*. (2001) showed lower pregnancy and implantation rates in women with hydrosalpingees. The effect of salpingectomy on the outcome of IVF was examined in a prospective randomized multicentre Scandinavian trial. Laparoscopic salpingectomy results in higher pregnancy and live birth rates in women undergoing their first or repeat IVF cycle (Strandell *et al*., 1999, 2001). Clinical pregnancy rate per included patient was 36.6%

in the salpingectomy group and 23.9% in the non-intervention group (P = 0.067). A subgroup analysis revealed significant differences in favour of salpingectomy, for implantation rates in patients with bilateral hydrosalpinges (25.6 versus 12.3%, P = 0.038) and for clinical pregnancy rates (45.7 versus 22.5%, P = 0.029) and delivery rates (40.0 versus 17.5%, P = 0.038) in patients with ultrasound-visible hydrosalpinges. The delivery rate was increased 3.5-fold following salpingectomy in patients with bilateral hydrosalpinges visible on ultrasound (P = 0.019).

The removal of ultrasound-visible unilateral or bilateral hydrosalpinges in women who fail to conceive with assisted reproduction is recommended, despite the transfer of good quality embryos. Although non-significant, reduced pregnancy rates associated with hydrosalpinges that are not visible on ultrasound mandate a serious discussion with the couple regarding their removal particularly following failed IVF attempts.

Concern has been voiced regarding ovarian implications of salpingectomy (Lass, 1999). Salpingectomy may impair ovarian circulation and compromise future assisted reproduction treatments through the attenuation of follicle reserve particularly in the ipsilateral ovary (Lass *et al.*, 1998). More damage may be inflicted when salpingectomy is bilateral. Salpingectomy, therefore, should not be an indiscriminate intervention, but rather be tailored according to the perceived outcome of the individual couple. The result of the Scandinavian multicentre study on salpingectomy prior to IVF has promoted a discussion on whether there is a risk of unnecessary salpingectomies being performed. In the couple undergoing ICSI for male factor infertility, bilateral hydrosalpinges should be removed. However, in the couple scheduled to undergo IVF for distal tubal occlusion, laparoscopy should be complemented by salpingoscopy and evaluation of the tubal mucosa. In women with severe distal tubal occlusion (dense tubo-ovarian adhesions, rigid tubal wall, hydrosalpinx diameter >3 cm, and severely damaged endosalpingeal mucosa) salpingectomy is prudent whereas in milder cases salpingostomy may be attempted.

Suboptimal embryo transfer as an explanation for recurrent implantation failure

Although embryo transfer is the final and most important step of assisted reproduction treatment, interestingly it is the least studied. All the efforts on the part of the couple and the assisted reproduction team can be spoiled by a less than an optimal embryo transfer technique. There are no randomized studies that compare different aspects of embryo transfer, mainly because the physicians hold strong beliefs that are mostly based on personal experiences and are reluctant to change.

Difficult embryo transfer is generally accepted to be a bad prognostic factor for pregnancy (Goudas *et al.*, 1998; Noyes *et al.*, 1999; Tomas *et al.*, 2002). A recent meta-analysis of controlled studies concluded that for patients with difficult transfers the pregnancy rate was 22.3% compared with 31.6% for patients with easy transfers (OR: 0.74; 95% CI 0.64–0.87) (Sadek *et al.*, 2004). The ease of transfer, however, is a clinical perception that is highly subjective. A recent study that evaluated the endometrial effects of embryo transfer via office hysteroscopy failed to show a significant correlation between the perceived ease of transfer and endometrial trauma (Murray *et al.*, 2003).

When there is difficulty in negotiating the embryo transfer catheter through the endocervical canal, when there is blood at the catheter tip, and when the patient feels cramping the procedure is usually regarded as difficult. The above notwithstanding, Tur-Kaspa *et al.* (1998) failed to show any difference in pregnancy rates when they compared easy and difficult transfers. The latter included those patients with uterine manipulation or cervical dilatation and patients who had one or more of their embryos retained in the transfer catheter.

It is always wise to perform a trial transfer preferably during the initial patient visit (Mansour *et al.*, 1990; Urman *et al.*, 2000). If a soft catheter cannot be negotiated through the cervix, a stiffer catheter with a malleable inner stylet can be tried. If the cervix is small with a pigeon eye appearance and the cervix–corpus angle is acute, a difficult transfer can be anticipated and this information should be shared with the couple. If the woman has a history of difficult embryo transfer during previous treatment cycles, a cervical dilatation can be undertaken prior to initiation of controlled ovarian stimulation (Abusheikha *et al.*, 1999). Cervical dilatation during oocyte retrieval was associated with easier transfers; however, the pregnancy rate was disappointingly low (Groutz *et al.*, 1997). If cervical dilatation has already been attempted without success, dilatation with hygroscopic rods (Serhal *et al.*, 2003) or hysteroscopic shaving of the endocervical canal may be other alternatives (Noyes, 1999). In patients with previous difficult embryo transfers who failed to conceive, placement of hygroscopic rods intracervically 4 h prior to gonadotrophin stimulation facilitated the subsequent transfer procedure in almost 80% of the cases giving rise to a clinical pregnancy rate of 55% (Serhal *et al.*, 2003).

Every attempt should be made to render the embryo transfer as easy and as atraumatic as possible. The physician should avoid touching the uterine fundus not to evoke uterine contractions. Recent evidence indicates that embryo transfer under ultrasound guidance and deposition of the embryos lower in the uterine cavity yield higher pregnancy rates (Pasqualini and Quintans, 2002; Buckett, 2003; Sallam and Sadek, 2003; van de Pass *et al.*, 2003; Frankfurter *et al.*, 2004; Pope *et al.*, 2004). Anderson *et al.* compared the results of transvaginal ultrasound guided embryo transfer in 129 women who failed to conceive in previous IVF–embryo transfer cycles where ultrasound guidance was not used (Anderson *et al.*, 2002). The authors concluded that ultrasound guided embryo transfer is responsible for successful IVF cycles in patients who had previously failed to conceive as all other parameters that were compared were similar.

It can be concluded from the available data in the literature (Frydman, 2004) that: ultrasound guidance should be used for embryo transfer; cervical mucus should be aspirated; the tip of the catheter should be placed 1–2 cm below the uterine fundus; touching the fundus should be avoided; a soft catheter that is inserted directly should be used whenever possible; and a trial transfer is useful, as it assists the physician during the actual procedure.

Cervical dilatation (mechanical or hygroscopic) or hysteroscopic cervical shaving should be considered in women with repeated difficult embryo transfers, especially when a trial transfer is unsuccessful.

Conclusions

In couples with recurrent implantation failure, maximum effort should be deployed to isolate the potential correctable factors that may be responsible for this phenomenon. Particularly when transferred embryos are of good quality, recurrent implantation failure may be attributed to less than optimal embryo transfer technique, pathological lesions of the uterine cavity, the presence of hydrosalpinges, fibroids and endometriosis. Poor embryo quality especially when repetitive is a major impediment to successful implantation and appears to be uncorrectable at the present time. Molecular abnormalities at the endometrial level and abnormal embryo–endometrium dialogue may be responsible for some cases of recurrent implantation failure. Furthermore, there may be over- or under-expressed genes that may be related to successful implantation. At the present time, the physician confronted with a couple presenting with recurrent implantation failure should discuss openly the potential causes of this phenomenon with special emphasis to correctable causes and offer remedies that are evidence based.

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