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REVIEW

Management of Recurrent Implantation Failure of IVF-ET Cycle

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ABSTRACT

The main limitation of the successful in vitro fertilization-embryo transfer cycle is the low implantation rate of the in vitro embryos. The low implantation rate is the primary reason for the high cost of the procedure. Even though this problem can be overcome with repeated attempts or increasing the number of the transferred embryos, some cases are discouraged with the recurrent implantation failure. Many forms of management have been proposed to make a breakthrough. They are involved in searching for a specific cause, improving the uterine receptivity and embryo quality, and improving the technique of the embryo transfer.

Key words : in vitro fertilization and embryo transfer, recurrent implantation failure, oocyte donation, embryo co-culture, assisted hatching, immunotherapy

In the two decades since the first successful in vitro fertilization and embryo transfer (IVF-ET) in humans, assisted reproduction technology has been one of the fastest developing sciences. A lot of new knowledge and technologies, such as controlled ovarian (hyper) stimulation and micromanipulation of the gamete and embryo, have been introduced and helped clinicians and scientists make breakthroughs about the causes of infertility. Now, standard IVF centres can achieve a pregnancy and delivery rate per treatment cycle that is nearly as high as the rate for the natural conception cycle.[1] However, there is still an obstacle that makes the IVF cycle inferior to the natural cycle. That obstacle is the high rate of implantation failure in the transferred IVF embryos. From the current pregnancy rate and the average number of transferred embryos per cycle, it is estimated that the chance of successful implantation is about 15% per embryo.[2] The implantation failure of the IVF attempt is the primary reason for the high cost of the procedure. Some patients can compensate the low implantation rate with multiple IVF attempts, but others are still facing the problem of recurrent implantation failure which is devastating

and discouraging to patients. The causes of implantation failure can be divided into three main groups :

1. Embryo quality
2. Embryo transfer technique
3. Uterine receptivity

Although the transfer of poor grade embryos can result in successful pregnancy, only the good quality embryos can ensure the highest implantation rate. The quality of the embryo depends upon the quality of male and female gametes and the quality of the laboratory. The couples who provide the gametes, the clinician who stimulates and retrieves the gametes, and the scientist who handles the gametes and embryos are responsible for this part. The less traumatic and proper technique of embryo transfer depends mainly on the clinician. Finally, the uterine receptivity, the least understood part, depends on the patient with limited manipulation from the clinician. Many studies have tried to decode the enigma of the successful embryo implantation and to propose reasonable management for recurrent implantation failure cases.

Management of the recurrent implantation failure

Further investigations

Usually, the clinicians have already performed extensive investigations about the causes of infertility and implantation failure in the patients. However, there are some studies suggesting about further or special investigations in the problem cases :

Autoantibodies screening

Autoimmunity may play a role in implantation failure. Observation of the relationship between recurrent pregnancy loss and autoimmunity leads clinicians to investigate recurrent implantation failure cases. There have been many reports over the past few years about the incidence of autoimmunity in infertile patients and failed IVF cases. The autoantibody profile study includes the antiphospholipid antibodies - APAs [anticardiolipin (ACA), lupus anticoagulant (LAC), antibodies to phosphoserine, phosphoglycerol, phosphoethanolamine, phosphatidic acid, and phosphoinositol], antinuclear antibody (ANA), anti double-stranded DNA (dsDNA), rheumatoid factor (RF), activated partial thromboplastin time (PTT) and prothrombin time (PT). The incidence of circulating autoimmune antibodies in patients who previously had chemical pregnancies or failed to conceive following IVF-ET were 32-33 %.[3-4] The patients with organic pelvic pathology were prone to have positive autoantibodies. These autoantibodies, particularly antiphospholipids, may cause uteroplacental thrombosis and vasoconstriction which result from the binding of IgG to platelets and the endothelial membrane phospholipids, and may lead to a decrease in blood supply at the implantation site. The APAs may also interfere with the formation of syncytiotrophoblasts from cytotrophoblasts.[5] The immunological mechanism of implantation failure is the current topic of discussion in assisted reproduction.

Repeated hysteroscopy

Dicker et al[6] reported 18.2% positive evidences when they performed repeated hysteroscopy in cases of recurrent implantation IVF-ET failure. They found new uterine abnormalities, such as hyperplasia, polyps, endometritis, and synechiae, which were likely to cause implantation failure. Re-evaluation of these cases with the office hysteroscopy, which needs no anaesthesia, may be a good choice. However, this policy should depend on the time since the

first evaluation and clinical data of clinic visit.

Doppler ultrasound

The role of Doppler ultrasound is to study the uterine artery blood flow by detecting the abnormalities in the pulsatility index and the pattern of diastolic blood flow.[7] The impaired uterine blood flow, which may relate with autoantibodies and the imbalance of thromboxane and prostacyclin, is believed to be one cause of implantation failure.

Chromosome study

Normal embryo morphology does not mean normal karyotype. Sixty percent of clinical pregnancy losses after IVF were related to abnormal chromosomes.[8] This might also relate to peri-implantation loss. Chromosome study on the couple is advisable and preimplantation diagnosis in indicated cases may be justifiable.

These investigations offer some additional causes for infertility and implantation failure. Nevertheless, there are still a lot of controversies and a number of cases who have no further investigation to be offered.

Proposed treatments

Oocyte donation

The use of oocyte donation and hormonal replacement therapy in agonadal and amenorrhoeic patients resulted in a very interesting outcome. The implantation of the embryo was achieved at a rate of 20%, compared to 10-15 % in normal IVF patients. These success rates are not limited by the age of the patients. Virtually identical results were obtained from treatment in patients who were in their mid to late 40s and those treated after their menopause. Moreover, this therapy also helps to establish far more pregnancies in poor responders and in patients who suffer from very low implantation rates or repeated failure of IVF with their own oocytes.[2] There is no doubt that good quality oocytes are responsible for the success. They can develop into good quality embryos with a higher chance of implantation. Oocyte donation has become a choice of treatment for patients with recurrent implantation failure and after failed IVF-ET using their own gametes.

The success of oocyte donation also leads to a comparison of uterine receptivity in the superovulation cycle and the artificial cycle. Implantation rates seemed to be higher in the recipient than in the donor sharing the same cohort of oocytes. This suggested that the endometrial environment might be more favourable in the hormonal replacement cycle.[9] The histologic studies of the endometrium in cycles with induced ovulation revealed that supraovulative cycles might cause dyssynchrony in the morphological maturation of the endometrial gland and stroma, due to the supraphysiological hormone milieu, resulting in late luteal phase deficiency.[10] Furthermore, the high responders in stimulated cycles, defined by more than 15 oocytes obtained, were found to have a very high estradiol to progesterone ratio around the time of implantation and to relate negatively with the pregnancy and implantation rates.[11] To correct this defect in the stimulated cycle, some clinicians suggested the earlier progesterone supplement prior to oocyte retrieval, however, there was no significant improvement from the prospective randomised trials.[12] Artificial or hormonal replacement cycle has a minor role in IVF patients because of the decreased quality of the frozen embryos from the current technology. However, due to the risk of hyperstimulation and multiple pregnancies plus the development of technology in the in vitro oocyte maturation and

embryo cultivation, the natural cycle or artificial cycle IVF may become a standard practice in the next few decades.

Embryo co-culture

The limitation of in vitro fertilization and cultivation of human gamete/embryo results in a lower quality embryo than in the natural process. The in vitro growth of the embryo is usually arrested after 2-3 days of cultivation. In many centres, most culture media, whether prepared commercially or in their own labs, can achieve only a 20-25% blastocyst formation rate when culture conditions are optimal.[13] This forces the scientist to return the early-cleaved embryos back to the patient before they reach the proper uterine stage ; the blastocyst. No one knows exactly what happens to these embryos which are exposed to the uterine environment prematurely. They are expected to continue their development normally in the uterus and ultimately proceed to apposition and implantation. Unfortunately, implantation rates per embryo from most clinics do not confirm this assumption. Quality and stage of the embryo to be transferred are still a big problem in assisted reproduction.

The co-culture system has been applied to human IVF with the expectation that it would mimic the in vivo environment and nurse the embryo to develop better. Many kinds of "helper" cells have been used as a feeder layer for the embryo. They are the trophoblastic, oviductal, uterine, cumulus, and granulosa cells from the genital organs of humans and other mammals. The experiments were expanded to other cell lines from bovine fibroblast, Vero cells (kidney epithelial cells from African monkeys) and human platelets.[14] The results of embryo culture on the feeder layer seemed to be impressive. In a mouse model, the co-culture system increased the rate of development, blastocyst formation, hatching rate, and the total cell number of the trophectoderm and inner cell mass.[15] Many control studies in humans also reported very high blastocyst formation rates (41-83%), compared to the control system (3-20%).[13,16] They extended the period of in vitro culture to 3-5 days and transferred the blastocyst stage embryos back to the uterus. Surprisingly, the positive effects of the feeder layer are not limited by the type of cell, i.e. somatic or epithelial cell ; origin of cell, i.e. from the reproductive tract or other systems ; or even species, i.e. bovine, monkey, or human. The co-culture system increased the number of viable embryos, rescue medium to poor quality embryos, aid in the embryo repair process after micromanipulation procedures, aid in the cryopreservation programme, and provided enough time and enough cells for genetic diagnosis.[14] Many mechanisms are thought to facilitate the actions of these helper cells, both in an autocrine effect (trophoblast) and a paracrine effect.

The helper cells in the feeder layer may remove some toxic compounds from the culture medium and supply metabolites such as glutathione and taurine to allow for the normal metabolic process of the embryo. They may also provide some growth factors, such as transforming growth factor -b (TGF-b), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), and platelet derived growth factor (PDGF), which are all essential for embryo development and are not supplied by common culture media.[16] A further application is using a reconstituted basement membrane preparation (Matrigel) to supply the growth factors to the embryo. Matrigel could give positive results in a mouse experiment similar to co-culture with Vero cells.[17]

However, there are still a lot of controver-

sies about the clinical results of the co-culture system. The patients with recurrent implantation failure seem to benefit from co-culture according to some control studies.[13,16] The clinical pregnancy rate and the implantation rate were improved in these patients. But for the patients attempting IVF for the first time, the clinical pregnancy rate was not significantly improved. Instead, the multiple pregnancy rate was reduced due to the limited number of the blastocysts that were transferred. Many clinics showed a good clinical pregnancy rate with normal culture system.

The co-culture system needs the development of more expertise and technology. Scientists should have some experience in cell or tissue culture. Even though the commercial Vero cells are available and very convenient, they still need good care and culture technique to grow. Other cell types may be risky depending on the pathogen cleansing which must occur before use. Finally, no one can ensure the safety of using animal cells to feed human embryos. Co-culture is not an ideal system for embryo cultivation, but it is a good model for studying the requirements of the embryo. Scientists have to develop good culture media and proper in vitro conditions for the adequate supply of vital factors and normal development of embryos. The conditioned culture medium might offer a success rate similar to the co-culture system. Synthetic serum, growth factors, and the Matrigel are being studied and applied to human embryos. They are easy to use and have no disadvantages of the co-culture system.

Assisted hatching

Another laboratory technique for improving the implantation rate of the embryo is making an opening on the zona pellucida (ZP) before the embryo is transferred. This technique is done on the 4- to 12- cell stage embryo by mechanical methods, i.e. partial zona dissection (PZD) ; chemical zona drilling or thinning with acid Tyrode's solution ; or with Er:YAG Laser. Before the implantation occurs, the blastocyst undergoes cycles of contractions and expansions that cause a decrease in the thickness of the ZP until it becomes almost invisible - a process in which the embryo lysin may also have a role.[18] Abnormal thickness of the zona (>12 μm) has been observed frequently in older patients (> 38-40 years) and patients with elevated follicle stimulating hormone (FSH) concentration.[13] The zona hardening and thickening seems to relate to patient factors and prolonged in vitro culture which affects the property of the glycoprotein. It may impair the normal process of implantation and cause a low implantation rate in these patients. The assisted hatching is supposed to allow the embryo to complete hatching on time with little energy consumed. It may also promote the embryo-endometrium synchronization in the stimulated cycle by facilitating embryo-maternal communication.[19]

Some good randomised control studies showed the benefit of assisted hatching in the recurrent implantation failure cases, old aged and elevated FSH concentration patients. The implantation rates per embryo in these patients were increased from 3-6% to 16-22% and the clinical pregnancy rates per embryo transfer were increased from 6-7% to 23-28%.[13,18] However, no significant difference was found in the general, unselected cases.[20] Moreover, the zona thinning method was found unsatisfactory. Tucker et al[21] explained that the human ZP had a more compact but resilient inner layer. Removal of the less dense, easily digestible outer layer did not affect the hatching process. Combination of the assisted hatching and co-culture provided higher

implantation and clinical pregnancy rates than using assisted hatching alone.[22]

Assisted hatching has created a lot of controversies as well, not only in the results but also in the safety of the method. Premature openning of the only barrier to the embryo might increase the risk of the immune cells attacking. Therefore, many studies included antibiotics and/or corticosteroid treatment on the day of oocyte retrieval for their patients. Another risk of assisted hatching is the embryo being trapped at the slit on ZP that may result in implantation failure or monozygotic twins. Assisted hatching needs more and larger randomised control studies to prove its benefit and safety.

Improve embryo transfer techniques

Improper embryo transfer technique is one cause of implantation failure. Traumatic insertion of the catheter can result in the release of prostaglandins from the cervix causing an increase in the myometrial activity, thus squeezing the embryo out. High insertion of the catheter may result in ectopic pregnancy and rapid withdrawal of the catheter may cause a hydraulic effect resulting in immediate expulsion of the embryo after transfer. Not only problems with technique, but also problems with the uterus can make the procedure difficult. Mock embryo transfer in the previous cycle, or immediately before the real transfer in the treatment cycle, was shown to improve the success of the procedure.[23] Performing the embryo transfer with a full bladder or in knee-chest position can ease the procedure by changing the posture of the uterus.

In some cases, it is impossible for transcervical transfer technique due to an abnormal cervix or the unfavourable posture/position of the uterus. The transmyometrial embryo transfer with ultrasonographic guidance has shown improved implantation rates. A 16-gauge spinal needle or a "Towako" ET catheter is used.[24-25] In addition, a special "glue" component (fibrin sealant or Tissucol) was shown to enhance the success of embryo implantation. The "glue" was supposed to seal the embryo on the endometrium and to disappear before the time of implantation.[26] Nevertheless, these special techniques still need further prospective control studies to prove their values. Most of the previous reports were small studies or case demonstrations without good control.

Apart from the embryo transfer technique, it has been suggested that the number of transferred embryos should be increased to six or more in the older patients, when poor quality embryos are obtained, and in recurrent IVF-ET failure cases. Azem and his colleagues[27] showed higher results in the clinical pregnancy rate with the transfer of six or more embryos compared to the transfer of five embryos (56% VS 29%) without significant increase in the multiple gestation rate. However, this policy may not be feasible in some countries because of their regulations. Larger studies are needed to assess the risks of multiple pregnancies.

Improve uterine perfusion with low dose aspirin therapy

Impaired uterine blood flow detected by Doppler ultrasound has challenged clinicians for a long time. Recently, Wada et al[7] used a low dosage of aspirin (150 mg per day) in frozen embryo transfer patients who showed a pattern of impaired uterine perfusion. They reversed the pattern and improved the clinical pregnancy rate of the patients. Aspirin, in low doses, might shift the balance of thromboxane and prostacyclin towards more prostacyclin production. Good

randomised control studies are the answer for the value of this treatment and should be expanded to the fresh embryo transfer cycle as well.

Immunotherapy

A lot of new knowledge has been found to confirm the role of the immune system in the success of embryo implantation. Immune cells, particularly lymphocytes and macrophages, as well as their cytokines were shown to be present in the luteal phase during the time of implantation. Some cell types may play a special role in the uterine receptivity. Michel et al[28] reported that cells with large cytoplasmic granules (> = 1 μm) were abundant in the group of on going pregnancies whereas cells with smaller granules (< 1μm) that were similar to the large granular lymphocyte were more abundant in the biopsy specimens from the failed pregnancy group. These immune cells may secrete some cytokines that act as embryo growth-development factors or local immunosuppressive factors, such as TGF-b.[29] The TGF-b and other cytokines are believed to inhibit maternal cytotoxic cells potentially able to reject the embryo. Due to the placental semi-allograft, the local immune response is likely to be modulated by the mechanisms of local immunosuppressive factors and/or the blocking antibodies. In the opposite way, these immune cells may be harmful to the embryos if the introduction of the ET catheter into the uterus is traumatic. This knowledge encourages the clinician to try immunotherapy with the expectation that this intervention could overcome the problem of implantation failure.

Immunosuppression

Cohen and his colleagues[30] reported higher implantation rates in the PZD embryos when they gave a low dose of methylprednisone to the patients. Although the study was small and not well-controlled, the report triggered similar interventions in other IVF-ET cases in following studies. However, the collected results are very controversial. Most of the studies were very small and were not randomised control trials (RCT). Most of them used low dose glucocorticoid (methylprednisone 16-60 mg per day) on the day of oocyte retrieval and continued for several days. Polak de Fried et al[31] demonstrated improved implantation rates (18.9% VS 3.4%-in control group) in the cases of previously failed IVF-ET and similar high success rate in the first attempt group. In contrast, other studies performed in the IVF cycles with and without micromanipulation could not demonstrate any benefits of treatment.[32-34] Steroids may have some effects on local immunosuppression and cause adequate secretion of some cytokines. However, in a laboratory which normally gets good implantation and clinical pregnancy rates, the steroid therapy may not be useful. In some "compromised" situations such as pre-existing autoimmune diseases or inflammatory process in the uterus, below standard embryo laboratory, and poor ET technique, steroids may play a permissive role to achieve a higher clinical pregnancy rate.[32]

In cases of positive autoantibodies, it is reasonable to try treatment with steroid and/or heparin or aspirin. These drugs were used with low doses starting two weeks prior to the treatment cycle and, if successful, continued through late pregnancy. The clinical pregnancy rates per ET were improved from 0-16% to 46-49%.[3,33] Heparin may inhibit binding of APAs with phospholipids and protect the trophoblast from injury. Aspirin may exert an anti-thromboxane effect and inhibit platelet aggregation resulting in improved uteroplacental blood flow.

Immunopotentiation

The clinicians who believe in the hypothesis of blocking antibodies and the immunosuppressive role of some immune cells have been studying the effect of paternal leucocyte immunization and intravenous immunoglobulin treatment. This active and passive immunopotentiation originates from success in the recurrent pregnancy loss cases. The studies, not RCTs, showed improved implantation rates and clinical pregnancy rates (17-18% from 0-7% and 33-56% from 8-24% respectively) in the previous IVF failure cases,[35] and better embryo development.[36] The immunopotentiation is supposed to support the embryo development by enhancing cytokines and the growth factors secretion,[37] enhancing suppressor T cells function, down regulation of B cells function, and/or reduction of activation of complement components.[35]

Immunotherapy is also a controversial issue due to lack of good studies. The reason for supporting the treatment is still a hypothesis and many studies cannot repeat the same results. In addition, immunomodulation in the patient is not without risk. The clinician must be very careful and monitor his patient closely if he wants to perform a trial.

Conclusion

Many forms of management have been proposed for recurrent implantation failure cases. The aims of the management are to discover any organic causes, to improve the embryo quality and transfer technique, and to enhance the uterine receptivity. Even though the embryo quality is limited by our current technology, the simpler co-culture system and the developing conditioned culture media may be promising and available in the near future. Little is known about the physiology of the endometrium during the implantation "window" period. It is likely that the contemporary controlled ovarian stimulation is not ideal for the uterine receptivity and the immune cells may take a major role in implantation. All of the proposed treatments have to be proven and supported with further RCT studies and more scientific evidence. Management of implantation failure and improving the current implantation rates are still controversial issues which are awaiting scientific breakthroughs.

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