—Mini Review—

Therapeutic Strategies for Implantation Failure due to Endometrial Dysfunction

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Abstract: Dramatic advances in assisted reproductive technologies have greatly improved the pregnancy rate for infertile couples. Recently, however, the recent pregnancy rates and live-birth rates have not significantly increased, mainly because there are very few effective treatments available for implantation failure and subsequent early pregnancy loss. Successful implantation requires good embryo quality, appropriately timed and arranged endometrial receptivity, and the efficient crosstalk between the embryo and the receptive endometrium. It is thought that impairment of any one of these factors or biological processes may result in implantation failure. In this review article, I have focused on endometrial dysfunction as a cause of implantation failure, and discuss its possible etiologies and therapeutic strategies.

Key words: Implantation, Repeated failure, Endometrium, IVF-ET, Human

Introduction

Assisted reproductive technologies (ART) have evolved considerably since the first IVF birth in 1978, greatly contributing to the dramatic improvement in the pregnancy rate for infertile couples. Despite the advances in ART, however, pregnancy rates have not increased significantly in the last decade [1]. The lack of increase in pregnancy rate can be mainly attributed to implantation failure, the causes of which remain elusive, thus hindering the establishment of adequate treatments.

Human implantation is thought to involve a number of different steps, as suggested by studies of rhesus monkeys [2]. The blastocyst becomes polarized and correctly oriented when it approaches the endometrium prior to implantation. After the blastocyst completes the apposition with shedding of the zona pellucida, it contacts and adheres to the epithelial layer of the endometrium. Eventually it penetrates the endometrial surface and invades the stroma.

All of these spatiotemporal biological processes need to take place in a timed manner to achieve successful implantation and maintenance of pregnancy. The human endometrium undergoes cyclical changes including proliferation, differentiation, tissue breakdown, and shedding (menstruation) throughout a woman's reproductive life [3]. The postovulatory rise in ovarian progesterone induces profound remodeling and differentiation of the estrogen-primed endometrium [3]. In particular, human endometrium can achieve receptivity for blastocyst implantation for only ~ 4 days, starting the 6–8th day after ovulation [4]; this period is known as the implantation window. The importance of this timely arrangement of the endometrial environment for implantation has emerged from several studies [5, 6]. In addition to an impaired implantation window, many known and unknown causes result in embryonic loss, which occurs repeatedly during ART cycles. Indeed, poor embryo quality has also been identified as a major cause of implantation failure [7]. In this article, I will review the etiologies of implantation failure due to endometrial dysfunction in particular and highlight the possible treatment strategies.

What is the Definition of Implantation Failure?

It is generally accepted that repeated implantation failures are defined when a patient experiences failure to achieve pregnancy following multiple IVF cycles. The number of the unsuccessful IVF cycles required for further investigations and therapeutic interventions, however, varies across individual reproductive centers, institutions and hospitals, according to a survey in the

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Table 1. Assumed etiologies and suggested and/or possible methods of treatment for repeated implantation failure

Assumed etiologies	Estabilised or experimental diagnositc tools	Conventional or suggested treatments	Experimental or possible treatments
Decreased endometrial receptivity			
Uterine cavity abnormalities	US, HS, HSG, MRI	Transcervical resection, Myomectomy, Uteroplasty	
Thin endometrium	US, MRI		Low-dose aspirin, Sildenafil, Micronized estradiol, Pentoxyfyline, High-dose vitamine E
Dysregulated expression of endometrial receptive genes and proteins	Immunohistochemistry, DNA microarray (transcriptome)	Luteal support	Intrauterine administration of genes and/or drugs, Endometrial stimulation (biopsies)
Immunological factors	Immunohistochemistry, Flow cytometry, Blood test	Luteal support	Glucocorticoids, Low-dose aspirin, Intrauterine administration of autologous peripheral blood mononuclear cells
Thrombophilia	Blood/coagulation test	Low-dose aspirin, Heparin	
Defective embryonic development			
Genetic abnormalities (male/female/gamates/embryos)	PGS, CGH, FISH, DNA microarray (transcriptome)	Embryo selection	
Zona hardening	Microscopic examination of embryos	Assisted hatching	
Suboptimal culture conditions	Morphological and/or biochemical assesment of embryos or culture media (metabolome etc.)	Blastocyst transfer	Co-culture with fallopian or endometrial cells, ZIFT
Multifactorial effectors			
Endometriosis	Pelvic examination, US, CT, MRI	GnRH agonist, Operation for non-ovarian endometriosis	Danazol
Hydrosalpinx	US, HSG, MRI	Salpingectomy	
Subopitimal ovarian simulations	US, Hormonal examination, Morphological and/or biochemical assesment of embryos (metabolome etc.)	Tailoring the stimulation protocols	

CGH, comparative genomic hybridization; CT, computed tomograpy; FISH, fluorescence in situ hybridization; HS, hysteroscopy; HSG, histerosalpingography; MRI, magnetic resonance imaging; OC, oral contraceptives; PGS, preimplantation genetic screening; US, ultrasound sonography; ZIFT, zygote intra-Fallopian transfer.

UK [8]. On the basis of the survey results, the most common definition was three unsuccessful IVF cycles (range 2–6) [8]; however, there is no evidence-based consensus for this number. In addition to IVF cycles, implantation failure is believed to take place during natural cycles without any ART or ovarian stimulation, and the etiology of natural implantation failure will remain elusive until the discovery of novel diagnostic tools. It should be noted that the common definition of implantation failure as described above is not applied to implantation failure occurring during natural cycles.

What are the Etiologies of Implantation Failure?

It is believed that successful implantation requires a good quality embryo, appropriately timed and arranged endometrial receptivity, and good communication between the embryo and the receptive endometrium. Impairment of any of these biological factors or processes can result in implantation failure. Thus, the etiologies for implantation failure can be divided into three categories: decreased endometrial receptivity, embryonic defects and combined multifactorial causes, as shown in Table 1 [9], which also shows the

corresponding diagnostic methods. Notably, there still remain diagnostic limitations due to the lack of tools to identify and evaluate some of these assumed causes.

Among the etiologies and the corresponding diagnostic methods listed in Table 1, bioinformatic molecular approaches for the evaluation of endometrial function have recently emerged. As a bioinformatic tool, DNA microarray techniques enable us to analyze the simultaneous expression of thousands of genes in a single sample of interest. These genomic approaches have revealed the alteration of expression of numerous endometrial genes during the menstrual cycle [10, 11]. Interestingly, some of these expression patterns seem to be inconsistent with histopathologic changes in the endometrium [10], raising the possibility that gene expression patterns may be not only a better marker for each biological phase of the menstrual cycle but also a more reliable predictor of endometrial receptivity than morphology.

Recently, several studies have investigated changes in endometrial gene expression during the receptive phase and all have reported a significant number of genes strongly up- and down-regulated during the implantation window [12–16]. These studies, however, provide the divergent results, which can be attributed to differences in study design and the software/statistics used in the analysis of the data [16]. Thus, standardization of methodology is required for meaningful conclusions to be made from genomic approaches. Nevertheless, all five studies reported that osteopontin is up-regulated when the endometrium is receptive [12–16], suggesting that osteopontin may be a potential maker of the implantation window and that it may play a role in the process of blastocyst implantation.

There are interesting microarray studies comparing natural and stimulated cycles indicating that controlled ovarian stimulation profoundly affects endometrial gene expression during the window of implantation [17, 18]. These findings provide evidence that the endometrial receptivity may be molecularly different between natural and stimulated cycles, therefore prompting us to reconsider the definition and entity of implantation failure as described above. Although a series of these transcriptomic approaches remain to be improved, a custom-made microarray harboring a set of the selected implantation window-associated genes may be applicable as a diagnostic tool for evaluating the endometrial receptivity of infertile patients with implantation failure.

How is Implantation Failure due to Endometrial Dysfunction Treated?

Table 1 summarizes the established, suggested, or experimental treatments for repeated implantation failure. In this section, I will discuss the possible strategies of treatments for endometrial dysfunction due to dysregulation of one or more endometrial receptivity-associated genes. Many bioactive substances including interleukin (IL)-12, IL-15, IL-18, IL-1β, IL-10, interferon-γ, $\alpha v\beta 3$ integrin and matrix metalloproteinases have been implicated as candidates for dysregulation of endometrial genes and proteins associated with repeated implantation failure [9]; however, to our knowledge, there have been very few clinical studies of treatments for implantation failure targeting the corresponding dysregulated endometrial factors.

We have previously reported that danazol treatment significantly increases the pregnancy rate together with up-regulation of $\alpha v\beta 3$ integrin in patients with repeated implantation failure [19]. Thus, danazol may have therapeutic potential for implantation failure, although whether danazol improves the endometrial receptivity by globally modulating the expression of endometrial genes or by specifically targeting $\alpha v\beta 3$ integrin remains to be elucidated. In this context, the orthodox strategy for the treatment of endometrial dysfunction is to supplement ovarian steroid hormones such as progesterone and/or their derivatives including danazol to normalize the intrauterine steroid hormone milieu. Interestingly, it has been demonstrated that, in addition to soluble factors, circulating blood cells contribute to maternal tissue remodeling and embryo-maternal cross-talk around the implantation period [20]. Based on this data, Yoshioka et al. administered autologous peripheral blood mononuclear cells into the uterine cavity of patients with repeated (four or more) implantation failures during IVF therapy, resulting in a significant improvement in the implantation rate [21].

A possible alternative and more tailored treatment modality is to supplement singly or multiply deficient factor(s) through direct introduction into the peritoneal and/or uterine cavity. Although such clinical studies have not yet been performed in humans, local administration of one or more deficient genes and/or proteins has been demonstrated to rescue the phenotype in mouse models of implantation failure [22, 23]. For instance, female mice lacking leukemia-inhibitory factor (LIF), in which blastocysts develop normally, are infertile; however, a single injection of LIF into these animals, on the day in which implantation

would normally occur (in mice, day 4 of pregnancy), is sufficient to initiate implantation and subsequent normal embryonic development to birth [24].

A number of studies have also reported successful gene transfer to the mouse uterus employing liposome-mediated gene transfection [25–28]. Nakamura et al. have demonstrated much higher transfection efficiency using HVJ-E (Hemagglutinating Virus of Japan envelope vector) than liposomes and their derivatives [29]. We recently reported *in vivo* gene transfer to the mouse uterus by microbubble-enhanced ultrasound (sonoporation) [30], the transduction efficiency of which was comparable to that of HVJ-E-mediated transfection and rather superior to that of liposome-based transfection [31]. Since microbubbles and ultrasound are usually used in clinical settings, sonoporation may be a more clinically accepted method than liposome- and viral envelope-based methods.

Conclusions

There exist many known and unknown reasons for implantation failure. We do not have the tools to diagnose the exact causes of repeated implantation failure for each case, and therefore there are very few effective evidence-based treatments. Currently, morphological markers of endometrial receptivity are unsatisfactory as predictors of pregnancy. Therefore, there is a need for *in vivo* methods to study and evaluate endometrial receptivity and the implantation process. New genomic and proteomic techniques are becoming available for examining endometrial changes prior to and during implantation. These approaches seem to be promising but still remain in their infancy with many improvements, including standardization, required. Nevertheless, the ultimate objective is to employ these tools in clinical settings to increase the implantation rate in artificial cycles and thereby improve the live-birth rate.

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