CAUSES AND TREATMENT OF IMPLANTATION FAILURE

Atsushi Yanaihara*, Shinji Iwasaki, Takashi Okai
Department of Obstetrics and Gynecology, Showa University School of Medicine, Tokyo, Japan.

SUMMARY
In recent years, a high fertilization rate has been achieved via the rapid evolution of assisted reproductive technology. However, the overall pregnancy rate remains the same. Implantation failure appears to be one factor. In preparation for implantation, the human endometrium undergoes dramatic proliferation and differentiation during the menstrual cycle in response to the rise and fall of ovarian steroids. The characteristic restructuring of the endometrium is thought to be effected not only by steroids but also by locally released factors. Elucidation of the implantation mechanism and treatment of implantation failure may improve the low pregnancy rate in in vitro fertilization-embryo transfer. In this review, the cause of and treatment for implantation failure are summarized. [Taiwanese J Obstet Gynecol 2005;44(1):26-30]

Key Words: implantation, implantation failure, treatment of implantation failure

Physiology of Implantation
When the trophectoderm, which is adjacent to the inner cell mass of the embryonic pole, attaches to the endometrial epithelium, an intrauterine pregnancy will progress. Preparation for implantation occurs between the embryo and the endometrium prior to implantation and various factors including cytokines, which are controlled by sex steroids produced by both the embryo and endometrium, participate in the complicated implantation cascade. Trophinin and tastin appear to form a cell adhesion molecule complex that potentially mediates initial attachment of the blastocyst to the uterine epithelium at the time of implantation. These substances are probably controlled by sex steroids in the mouse [1]. It has been suggested that trophinin and its related factors, such as bystin, may be involved in implantation, particularly by facilitating adhesion. L-selectin, which was recently discovered in trophoblasts, is related to implantation [2]. In the cow and sheep, interferon-τ is a signal substance that trophoblasts secrete, and it has been suggested that this is controlled by interleukin (IL)-3 and granulocyte-macrophage colony stimulating factor, which are produced by the endometrium [3,4].

The maintenance and support of the union of two columnar epithelial layers requires additional factors, and the integrin family appears to play an important role in adhesion of a cell to the external cell matrix. Expression of integrin αvβ3 is suppressed in the endometrium of patients with unexplained infertility who have suffered recurrent in vitro fertilization-embryo transfer (IVF-ET) failures. Therefore, integrin αvβ3 appears to be essential for connection of the embryo to the mother [5,6]. Cytokines IL-1, IL-6, IL-11, and leukemia inhibitory factor (LIF) and its receptor appear to be involved in implantation [7–11]. IL-1 reinforces the action of integrin. LIF mRNA concentration peaks in human endometrium at the time of implantation, when the blastocyst contains mRNA for the receptor [12]. Recently, we reported that Janus kinase 1 (JAK1) mRNA is expressed in the human endometrium under the influence of progesterone [13]. Cytokine binding induces receptor dimerization, activating the associated JAK, which phosphorylates the receptor. These receptor families include substances such as gp130 and interferon. JAK forms a JAK-STAT passageway by activating a STAT protein. Thus, it plays an important role in the transfer of a cytokine signal to the receptor, which does not have tyrosine kinase activity. Furthermore, it has been reported that substances, such as heparin-
binding-epidermal growth factor (HB-EGF), calcitonin, homeobox gene (HOXA)-10, HOXA-11, and insulin-like growth factor binding protein-1 (IGFBP-1), are expressed at the time of implantation as local factors [14–17]. Although the relationship of these cytokines to implantation appears to be quite important, the role in humans remains unknown.

Large granular lymphocytes, which do not appear in peripheral blood, are abundant in the endometrium. Most of these lymphocytes are natural killer (NK) cells, and it is thought that the function of the NK cell differs from that of the conventional killer cell. The cytokine network generated by these lymphocytes is thought to influence implantation. Moreover, the Th1/Th2 cytokine paradigm from the helper T-lymphocyte and human leukocyte antigen (HLA) are involved in implantation. It is thought that an extracellular matrix-related molecule is indispensable to the invasion phenomenon that occurs after adhesion. It is very difficult to identify all the factors responsible for implantation and their relative importance; however, implantation must occur via an intricate interaction of these factors (Figure).

Concept and Categorization of Implantation Failure

The concept of implantation failure has existed since the 1960s, and the diagnostic definition has become clear with IVF-ET technology. Briefly, implantation failure secondary to gross disease, such as a leiomyoma, is common. Cases in which conception does not occur despite the transfer of good-quality embryos (in our hospital, three or more attempts are often made) are considered implantation failures in a narrow sense. The causes of implantation failure can be divided into three types: embryonic factor, maternal factor, and embryonic–maternal interaction factor.

Embryonic factors

The heterochromosome rate is 15% in sperm and 25% in ova. Fragmentation of DNA reaches 5% in ova and 80% in sperm. Ultimately, some 45% of embryos have genetic disorders. In a recent study of embryos that reach the blastocyst stage, chromosomal abnormalities were found in about 40% [18]. If these heterochromatic blastocysts are transferred, the implantation rate is low.

Conditions such as cytoplasmic denaturation of the ovum prior to fertilization, sperm abnormalities, and focal granulosis of the oocyte decrease the likelihood of embryonic development.

Implantation failure may be due to a hard zona pellucida in embryos frozen for some time.

Maternal factors

Conditions in which the endometrial cavity is distorted, such as fibroids, endometrial polyps, endometrial adhesions, and congenital anomalies, impact fertility. Hysterosalpingography and hysteroscopy are effective diagnostic modalities. Although it is often difficult to evaluate the impact on implantation based on the grade and state of disease, surgical correction should be taken into consideration in patients experiencing implantation failure.

It is generally held that not only IVF treatment but also luteal dysfunction can result in implantation failure. Decidualization of the endometrium by progesterone is a phenomenon indispensable to implantation. When using clomiphene for a long duration, particularly for luteal dysfunction, one must consider its effect on endometrial development. Incomplete endometrial development is a side effect of medical treatment; thus, the endometrium should be examined. When the endometrial thickness is 6 mm or less, the likelihood of pregnancy is quite low [19]. Although the underlying mechanism for inadequate endometrial development is currently unknown, abnormalities in secretion, such as in steroid receptors and cytokines (growth factors), have been implicated [20–22].

It has been reported that self-antibodies, which respond abnormally in autoimmunity such as that found in systemic lupus erythematosus, prevent implantation. Assisted reproductive technology (ART) treatment, low-dose aspirin, and prednisone are effective in promoting implantation in such cases. Although low-dose aspirin affects neither endometrial thickness nor uterine artery resistance, it improves the endometrial environment. It has been reported that aspirin administration results in higher pregnancy rates and better endometrial patterns in patients with a thin endometrium [23].
Embryonic–maternal interaction factors
Some abnormalities in embryonic–maternal interaction, such as functional disorders and signal transfer obstacles, may lead to implantation failure. However, the aspects of this have not yet been clarified. Basic research is needed to develop effective treatment for this type of implantation failure.

Improvement of Pregnancy Rates in ART

Preimplantation diagnosis
In embryo biopsy, a portion of the embryo is extracted and subjected to genetic analysis. Although this has been used clinically in Western countries for about 15 years [24], many ethical problems are unresolved in Japan.

Blastocyst transfer
From fertilization until the eight-cell stage, the fertilized ovum is cultured in a solution with a glutamine base and ethylenediamine tetraacetic acid (EDTA); subsequently, glucose is added as an energy source. Because of refinements in sequential culture media, embryos readily develop to the blastocyst stage, which is the optimal point for embryo transfer because the heterochromosome rate is low [25]. An implantation rate of 35% with day 3 embryos has been reported; the rate rose to 55% with day 5 embryos [26–28]. However, these investigators reported that the frequency of monozygotic twinning was high with blastocyst transfer. The frequency of monozygotic twinning is about 0.4% in natural pregnancies; however, it has been reported that the rate of monozygotic twinning with conventional day 3 transfer is about 2%, and blastocyst transfer has a rate of about 5%. This phenomenon should be fully explained to the patient prior to treatment [29].

Frozen embryo transfer
This technique is indispensable to ART; it avoids multiple gestations as well as ovarian hyperstimulation syndrome by using surplus embryos that have been preserved. Its applications and adaptations have expanded. Although concern exists in regard to deterioration of the embryo, it has been reported that the birth defect rate is no higher than in natural pregnancies.

The zona pellucida hardens as a result of freezing, but this problem has been resolved using assisted hatching (AH). For implantation to occur, the embryo and the endometrium must be synchronized. Often, synchronization is not present during the stimulus cycle; thus, frozen embryos can be transferred during a subsequent, synchronized cycle. In cases of a thin endometrium, the lining can be enhanced in subsequent cycles prior to transfer.

Two-step transfer
The rationale of this transfer method is based on the fact that the endometrium is optimal for implantation when the embryo is transiting the oviduct [30,31]. The implantation rate increases from 30% with transfer of cleavage-stage embryos on day 2 to 59.7% with transfer of blastocyst-stage embryos on day 5, which is statistically significant [32].

Other factors than the embryo may influence the endometrial immune environment for implantation. One problem with the two-step transfer method is an increased risk of multiple gestations. Furthermore, this technique cannot be used when a small number of ova are harvested. Further investigation of this technique, comparing two-step transfer with blastocyst transfer, is necessary.

Assisted hatching
The zona pellucida, which surrounds the ovum, is influenced by protease; it becomes soft and thin during the process of implantation (hatching). It is thought that thickening and hardening of the zona pellucida result in a decreased implantation rate. Some or all of the zona pellucida is removed either chemically or mechanically in AH, which was adopted for ART after being reported by Cohen in 1990 and has become commonplace [33]. Mechanical AH entails boring a small hole in the zona pellucida using a laser and a micromanipulator (partial zona dissection). Chemical AH uses an acid enzyme solution and is carried out to three levels: partial zona thinning, circumferential zona thinning, and zona removal.

The rate of monozygotic twins and ectopic pregnancies with AH is unusually high, according to recent reports [34–36]; thus, informed consent is indicated. Furthermore, while success with AH has been reported for elderly patients, others question its value for these women [37,38]. Further modification of AH is required.

Prospective View
When studying implantation, the subject of ectopic implantation arises; this situation strongly supports an embryonic factor resulting in implantation. Global genetic analysis has further clarified the implantation mechanism. The protein-array procedure, which analyzes many proteins simultaneously, develops a microarray
that has potential clinical usefulness. If the genes and proteins involved in implantation failure are identified, detection and correction of implantation defects can be attained.

References


