Contribution of immunology to implantation failure of euploid embryos

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Outcomes in assisted reproduction have seen marked improvement. With increased ability in the embryology laboratory to use extended embryo culture which in turn enables other selective techniques, such as trophectoderm biopsy and comprehensive chromosome screening, the chance of success per embryo transfer is increased. However, even the selection of a euploid blastocyst, which selects out many embryonic factors, does not yield successful implantation and ultimately delivery in all cases. Among the factors that affect implantation failure of apparently reproductively competent embryos, the immune system has been perhaps both the most plausible and the most debated. There are data on T-helper cells, in particular the T_H1-T_H2 balance, peripheral and uterine natural killer cells, and autoantibodies, all of which have been shown to have variable effects on implantation. Many investigators have developed and used a wide range of immune tests and treatments aimed at manipulating the milieu to favor implantation. Although it is certain that the immune system plays a role in implantation, our understanding of the physiology, let alone the pathophysiology, remains incomplete. It is imperative that we gain more clear evidence of causes and test and implement treatment paradigms. In the meantime, immune testing or empirical treatment with the use of immune modulators must be approached with caution. (Fertil Steril® 2017;107: 1279–83. ©2017 by American Society for Reproductive Medicine.)

Key Words: Implantation failure, immune system, natural killer cell, KIR

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It is clear that human embryonic aneuploidy has a great effect on reproductive competence (1). Furthermore, when validated techniques are applied to embryonic chromosomal screening, we have class I data demonstrating improved pregnancy rates (2–4). These technologies have improved clinical and sustained implantation rates through the process of embryo selection in patients with normal ovarian reserve. However, accurate selection of a chromosomally normal embryo alone does not yield entirely optimized pregnancy rates; in more than 30% of cases, a chromosomally normal embryo does not result in a live birth. Although additional work with next-generation sequencing with the analysis of subchromosomal abnormalities and embryonic mosaicism may yield further precision, it is clear that chromosome analysis alone is not the answer (5, 6).

Another factor affecting reproductive competence that has long been hypothesized and debated when studying recurrent implantation failure is the immune system (7). It should be noted when considering the immune system that the thought process behind recurrent implantation failure discussed here differs from recurrent pregnancy loss (8), which is not the focus of this paper. Furthermore, even when considering recurrent implantation failure, the immune system's role has been poorly understood, and often the testing and treatment for these proposed defects have come before concrete evidence is available (9). In the present paper, we discuss the evidence surrounding the immune system's impact on implantation failure in the setting of euploid embryo transfer.

THE IMMUNE SYSTEM AND EUPLOID IMPLANTATION FAILURE

T-Helper Cells: T_H1 and T_H2 Immunity

Among the potential immune cause of reproductive failure are imbalances in the T-lymphocyte helper cells. These T-helper (T_H) lymphocytes express CD4 and are commonly characterized by the types of cytokines that they secrete and their influence on cell-mediated immunity. There are two broad classes of T-helper lymphocytes–T_H1 and T_H2. T_H1 cytokines are generally considered to be proinflammatory and include interferon-γ,

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tumor necrosis factor (TNF) α, and interleukins (ILs) 1, 2, 12, 15, and 18. The proinflammatory actions of the T_H1 cytokines are counteracted by the T_H2 cytokines, such as ILs 4, 5, 6, 10, and 13 and granulocyte-macrophage colony-stimulating factor. The T_H2 cytokines serve to limit the proinflammatory actions of the T_H1 cytokines and are often characterized as antiinflammatory. Humans see variation in the balance of T_H1 and T_H2 cytokines based on the nature of any immune challenges that are present at any given time (10, 11).

Pregnancy is accompanied by a shift in the ratio of T_H2 to T_H1 cytokines and is considered to be a T_H2-dominant state. In fact, a strong T_H2 response may be necessary to lessen the in utero T_H1 response and to maintain pregnancy. Infants are born with a T_H2-dominant cytokine milieu that shifts rapidly following their initial microbial colonization.

The T_H2 dominance found in early pregnancy is induced by rising levels of progesterone. In addition to increasing the secretion of T_H2 cytokines, progesterone inhibits the secretion of T_H1 cytokines. Specifically, IL-4 and IL-6 increase and IL-2, IL-12 and interferon-γ decrease. Even the embryo directly contributes to T_H2 dominance by secreting IL-10 and transforming growth factor β (12, 13).

Aberrations in these ratios may be induced experimentally in animal models by direct administration of cytokines IL-2, TNF-α, and interferon-γ to produce at T_H1-dominant milieu early in gestation. In that setting, there is an increase in pregnancy wastage. Administration of agents that block the effects of these cytokines (cytokine antagonists) prevent resorption (12, 13).

There are data that demonstrate an association between loss of T_H2 dominance in early gestation and poor clinical outcomes. Anembryonic gestations, even in the presence of sufficient circulating progesterone, lose their T_H2 dominance. Women with recurrent pregnancy loss also have impaired T_H2 responses (reduced IL-4, IL-6, and IL-10) and may even demonstrate T_H1 dominance (12, 13).

Although the changes in T_H1-T_H2 dynamics in early gestation are unequivocal, several important issues remain unknown. It is not known if the aberrations in the transition to T_H2 dominance and its maintenance in early gestation represents the primary defect which leads to failed gestations, or whether they are simple secondary effects that are the result of other factors that are actually responsible for the pregnancy losses. Anembryonic gestations that develop after the implantation of trisomic embryos would seem to be examples of these changes being an effect rather than a cause.

Efforts to improve outcomes by favorably altering the T_H1-T_H2 milieu are complicated by a number of factors. It is very important to recognize that before conception there are no detectable differences in the immune systems of women who have normal T_H2 dominance compared with those who have adverse T_H1 dominance. In fact, it is normal for T_H1 to dominate in the peri-implantation interval. Therefore, no screening tests can be done in advance to predict imbalance in a future gestation.

The lack of effective screening tests and the relatively strong association of abnormal T_H1 dominance have led some investigators to pursue immune therapies to improve outcomes in these settings. Again, sufficient data to suggest that immune imbalance is the primary etiology of the failure is lacking. It is unknown if correction of the imbalance would actually prevent the loss or simply extend the time for the loss to manifest itself clinically. Use of TNF-α blockers, intralipids, intravenous immunoglobulin, etc. may be able to increase T_H2 dominance, but has failed to demonstrate benefit for either recurrent implantation failure or recurrent first-trimester pregnancy loss. Great caution should be exercised before the administration of these agents outside of the setting of Institutional Review Board–approved research.

Natural Killer Cells

Peripheral and uterine natural killer (NK) cells have been the subject of intense research and debate regarding their association with reproductive success and failure (14). The peripheral NK cells make up ~5%–10% of peripheral blood lymphocytes, whereas uterine NK cells constitute ~70%–90% of lymphocytes found in the uterus. The NK cell population is distinguished from other lymphocytes via immunohistochemistry findings; namely, NK cells are CD-56 positive, and peripheral and uterine NK cells represent distinct subsets with the majority of peripheral NK cells being CD-56^{dim} and the majority of uterine NK cells being CD-56^{bright} (15, 16). In addition to the phenotypic differences, the two populations have functional differences. Peripheral NK cells show robust cytotoxic activity and are involved in infection and neoplasia defense; in contrast, uterine NK cells exhibit limited cytotoxic activity (17). Therefore, although much effort has been put forth to correlate the measurement of peripheral NK cells with uterine NK cells, this may not be appropriate and may even lead to the introduction of therapies that are physiologically misguided (9).

The appearance and differentiation of uterine NK cells in the mid-secretory phase of the menstrual cycle does not have a clear mechanism. It is theorized that either peripheral NK cells are recruited and then differentiate into uterine NK cells or that uterine NK cells result from a differentiation of stem cells in the endometrium (18). Another possibility is that mature peripheral NK cells are recruited to the uterus (19–21). The uterine NK cells accumulate in the stroma around glandular tissue and blood vessels, and their numbers, though low in the proliferative phase, rise after ovulation and reach a peak during the middle and late secretory phases of menstrual cycle (22). Importantly, exogenous administration of estrogen and progesterone, as seen with hormone replacement therapy in postmenopausal women, also increases uterine NK cells, so fertilization and presence of an embryo is not required for their presence (23). In contrast, peripheral NK cells do not vary through the menstrual cycle (24).

The study of uterine NK cell fluctuation in the menstrual cycle and their peak during the time of embryonic implantation led to the investigation of uterine NK cells in patients with repeated pregnancy failure. There were a number of reports of abnormal uterine NK cell numbers in these individuals, with investigators finding altered proportions of CD56^{dim} and CD56^{bright} cells in patients with poor outcomes (25, 26). Others found associations between altered