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Editorial Article

Improving the chance of successful implantation – part I – embryo attachment to the endometrium and adequate trophoblast invasion

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Summary

The first in a series on improving embryo implantation is presented with emphasis on embryo attachment and trophoblast invasion. *Purpose:* To present knowledge of events needed for embryo attachment to the endometrium and subsequent trophoblast invasion and uterine remodeling leading to successful pregnancy. *Materials and Methods:* Based on normal events, some practical suggestions are proposed as to possible means of improving pregnancy rates by enhancing possible embryo attachment and trophoblast invasion. *Results:* Potential benefits of achieving adequate serum estradiol levels at peak follicular maturation, and the benefits of progesterone in the luteal phase are discussed. Also the potential benefits of purposeful endometrial injury is considered. *Conclusions:* Knowledge of the events leading to embryo attachment and trophoblast invasion could lead to novel research ideas helping to improve pregnancy rates in addition to proper hormone supplementation and endometrial biopsy.

Key words: Spiral arteries; Uterine natural killer cells; Uterine macrophages; Chemokines; Trophoblast invasion.

Introduction

Successful pregnancy requires adequate sperm concentration, with survival in cervical mucus with sperm, traversing patent non-diseased fallopian tubes, meeting an oocyte that was released from a dominant follicle that was picked up by normal fimbria, and transported to the upper third of the fallopian tube. Furthermore, for successful pregnancy the oocyte and sperm have to be chromosomally normal resulting in fertilization and subsequent embryo cleavage to the blastocyst stage.

The present editorial (and subsequent series to follow) will discuss potential reasons why a chromosomally normal embryo may fail to implant and possible methods to improve implantation failure. The present editorial will deal with embryo attachment and adequate trophoblast invasion.

Normal events enabling implantation

The first step in implantation leading to a successful pregnancy involves the blastocyst. After about six days after fertilization, the blastocyst must invade the maternal decidua. The decidua is a cellular substratum that allows the placenta to co-opt maternal blood flow. Interactions between the stromal and vascular components of the placenta and uterus are mostly regulated by maternal immune cells that populate the decidua.

The outer layer of the blastocyst generates the placenta which is composed of both maternal decidual cells and fetal trophoblast cells. The villous trophoblast cells cover the placental villous tree. The trophoblast cells mediate the transport between the maternal blood in the intervillous space and the fetal circulation. The extravillous trophoblast cells invade deep into the uterine wall. Natural killer (NK) cells destroy and then replace the muscular wall of the uterine arteries. With further development from angiogenic factors secreted by the invading trophoblast, specialized spiral arteries are formed. These spiral arteries ensure adequate blood flow to and from the fetus [1].

White blood cell population at the maternal-fetal interface

The most prominent of the white blood cell population of the maternal-fetal interface are NK cells [1, 2]. These cells are determined by flow cytometry and tissue immunostaining to represent approximately 70% of the cells at the maternal-fetal interface [3, 4]. Machrophages are the next most prominent white blood cell representing about 20% of the population [3, 4]. Most of the remaining cells are T cells with rare dendritic cells, B cells or NK T cells.

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The decidual NK (dNK) cells first appear in the secretory endometrium prior to implantation. These NK cells are recognized by their CD56 bright CD16-cell surface phenotype. Though having a similar surface phenotype to these same phenotypic cells that are present in 10% of NK cells in the peripheral blood, they have a different transcriptional profile leading to the production of a wide variety of chemokines, angiogenic factors, and cytokines [5-8]. Thus the dNK cells differentiate into cells with highly specialized pregnancy specific function [5-8]. Though the majority of peripheral NK cells in the blood have the surface phenotype of CD56dim CD16+, two cytokines, both expressed in the decidua, promote the conversion of CD56dim CD16+ peripheral (P) NK cells to decidua NK like-cells with the CD56 bright CD56- phenotype with the pregnancy specific factors: interleukin (IL-15) and transforming growth factor beta (TGF beta) [9,10].

Maximizing maternal blood flow through the placenta

The dNK cells seem to be the most important of the inflammatory white cells present in the preimplantation time period to promote the change of the high resistance low flow uterine vessels into low resistance (high capacitance) high-flow spiral arterioles that supply the placenta with maternal blood [2]. The NK cells also play a role in replacing the endothelium of the uterine arteries with trophoblasts (termed extravillous trophoblast) that have migrated from the placenta and invade the placenta. Thus, these extravillous trophoblast cells that have migrated into the lining of the uterine arterioles form a pseudoendothelium replacing the previous uterine artery endothelium. The diverted blood flows into the space surrounding the placental villous tree and thus fosters gas and nutrient exchange between mother and conceptus. Thus, if there is insufficient spiral arteriolar transformation, with the consequential failure of trophoblasts to invade into the vessels all the way to the superficial layer of the myometrium, this could result in pathological pregnancies resulting from placental underperfusion, e.g., pre-eclampsia and intrauterine growth restriction [11, 12]. One could easily envision a more serious problem with inadequate trophoblast invasion leading to such defective spiral arteriole formation leading to demise of the early conceptus before a positive pregnancy test is obtained or a miscarriage from a clinical pregnancy.

The dNK cell is under the influence of the killer cell immunoglobulin-like receptor (KIR) family which encodes NK cells surface receptors. Also there are three HLA1 molecules and it is HLAC which is the dominant KIR ligand and the only one expressed by the extravillous trophoblasts. For further detailed discussion of these interactions, the authors would suggest reading the manuscripts by Parham *et al.* and by Hiby *et al.* [13-15].

The trophoblasts have relatively low levels of classical MHC 1 expression which should normally lead to immune rejection [16]. The dNK cells, similar to pNK cells, have granules containing cytotoxic molecules, e.g., perforin and granzymes [17]. There are many theories as to how the trophoblast escapes immune surveillance, but the one favored by the authors based on their research is that it predominantly involves the intracellular expression of a 34-36 kDa molecule known as the progesterone induced blocking factor (PIBF) which stabilizes perforin and granzymes [18]. This topic will be discussed in detail in the next editorial on "Improving the chance of successful implantation – part II – the importance of immune suppression against the fetal semi-allograft.

Macrophages

The bulk of the rest of decidual white blood cells are macrophages. The d-macrophages are well known to produce the interleukin IL10 [19]. Their main function may be to inhibit decidual infection but their possible role in promoting or inhibiting implantation is not known for sure [19]. There have been erudite hypotheses expounded and for further details, the authors recommend the reader to the studies of Renaud *et al.* and Nagamatsu *et al.* [20, 21].

Dendritic cells

As mentioned these cells are rare at the maternal fetal interface. Dendritic cells are normally very important cells in the adaptive immune response. When dendritic cells (DC) are exposed to pathogens or inflammation they migrate to draining lymph nodes by lymphatic vessels. At these draining lymph nodes, the dendritic cells present antigens to naïve T cells. This fosters T cell proliferation and polarization. Tagliani and Erlebacher have speculated that part of the process of successful implantation of the conceptus, which is a semi-allograft, is to inhibit DC's to reduce the tissue's ability to initiate adaptive T cell responses in the draining lymph notes [22].

T-cells

The presence of T cells in the first trimester human decidua represents about 10% of the white blood cell population. About 30-45% of these T cells are CD3+ TCR2B+ T cells which can be divided into CD4+ T cells (30-45%) and CD8+ T cells (45-75%) (23, 24). About 5% of the CD4+ T cells are regulatory T Cells (T reg) with the surface phenotype of CD25 bright FOXP3+ (23, 24).

At present the function of decidual T cells is generally unknown. The population remains stable in cases of miscarriage. Their role may become more clear with future research.

Attachment of the blastocyst to the endometrium

In order for the early embryo to produce a live baby, the first step is apposition of the blastocyst to the uterine endometrium. The second step is attachment to the endometrial surface endometrium. In humans, the uterus becomes receptive to attachment five to nine days after fertilization,

which has been referred to as the window of implantation (WOI).

It is during the WOI that one of the two distinct cellular components of the uterine endometrium, the stromal cells, transform into larger and rounded decidual cells (decidualization). The epithelial cells, probably under regulation by corpus luteum progesterone and estradiol secretion, produce cytokines, chemokines, growth factors, and adhesion molecules. Part II of this series will present evidence for the role of progesterone secretion in suppression of immunosurveillance especially by NK cells of the fetal semiallograft. The possibility exists that inadequate early luteal phase secretion of estradiol or progesterone could impair trophoblast attachment to the endometrium. Thus the possibility exists that some cases of infertility may be related to poor embryo attachment which could be corrected by early supplementation of additional progesterone and/or estrogen.

Before a woman can achieve blastocyst invasion and placentation, it must be preceded by attachment of the trophectoderm to the endometrial epithelium. The uterine surface, however, is covered by various molecules, especially Mucin I (MUCI) carbohydrate that prevents the attachment of the highly adhesive blastocyst to an improper site. Mucin I is upregulated during the implantation period [25]. Thus the possibility exists that the human endometrial surface epithelium prevents blastocyst adhesion, except for the precise spot where the embryo attaches. Dekel *et al.* have hypothesized that one role of the minority DC's present in the uterine stroma is to produce cytokines to induce local degradation of MUC I, and chemokines to attract the blastocyst to that spot [26]. Thus, failure to establish a successful pregnancy may be related not only to inadequate decidualization or immune rejection, but to deficient specialized chemokine or cytokine production to allow blastocyst recruitment and attachment.

The role of chemokines and cytokines in implantation and placental development

Uterine NK cells are present in low levels pre-ovulation. However, they peak in the late luteal phase [27]. The chemokine CCL4 increases from early secretory phase and may be the main chemokine responsible for the increase in UNK cells and the recruitment of other immune cells, e.g., which are needed to allow implantation, decidualization, and fetal tolerance [28]. The exception may be T reg cells which seem to peak by the rise in estradiol at the peak sexually receptive time [29]. Actually T reg cells decrease with the secretion of progesterone. As mentioned, inadequate progesterone secretion in the luteal phase may lead to increased T reg cells during the luteal phase and these cells may be involved in immune rejection. It is not clear what the role of T regs are at peak follicular maturation, but assuming it plays some important role in establishing a successful pregnancy, it could explain why progesterone supplementation is so successful in correcting infertility and preventing miscarriage when the follicle is making adequate estradiol, but where the combination of a follicle maturing drug plus progesterone supplementation in the luteal phase provides the best success when ovulation occurs with lower peak serum estradiol levels [30].

Trophoblast invasion is directed by uterine NK cells. The UNK cells express the chemokines IL-8 and IP-10 which bind the receptors CXCR1 and CXCR3 on the extra villous trophoblast cells [31]. The trophoblast invasion and vascular remodeling are influenced by the release of the angiogenic factors VEGF and placental growth factor [31]. Other cytokines play a role. Transforming growth factor beta (TGF-B) may both downregulate inflammatory activity and provide differentiation of UNK cells [31].

The EVT cells express the non-classical class I molecules HLA-E which bind CD94/NKG2A [32]. Another such immunomodulatory molecule HLA-G, binds to KIR2DL4 [33]. Furthermore, trophoblast cells express the highly polymorphic classical HLA-C1 and HLA-C2 [34, 35]. HLA-C-KIR interaction is crucial for promoting placental vascularization. Certain combinations of KIR haplotypes and HLA-C groups have a detrimental effect and may increase the risk of pre-eclampsia [36].

Can certain treatments improve blastocyst attachment and trophoblast invasion?

Are there other possible treatments that can correct blastocyst attachment and subsequent trophoblast invasion besides providing adequate estrogen in the follicular phase and by supplementing progesterone and possibly estradiol in the luteal phase?

There is the possibility that local injury to the endometrium can result in increased chance of a successful pregnancy. As early as 1907, in the German literature, Loeb reported that scratching the guinea pig uterus during the progestational phase of the estrous cycle provoked a rapid growth of decidual cells [37]. Subsequently, it was found that decidua formation occurred in pseudopregnant rodents by other forms of local injury, e.g., suturing the uterine horn or intrauterine injection of oil [38, 39].

In humans, Barash *et al.* inadvertently found that performing endometrial biopsies in the preceding cycle to measure levels of connexin 43 protein led to a doubling of the pregnancy rates in the subsequent in vitro fertilization-embryo transfer (IVF-ET) cycle [40]. A subsequent study by Raziel *et al.* found that performing an endometrial biopsy in the preceding luteal phase, and thus performing local injury, could improve subsequent success rates in IVF-ET cycles in women with high-order implantation failure [41]. Zhou *et al.* showed that this local injury could be performed even during the follicular phase of an IVF-ET cycle and produce improved outcome [42].

A Cochrane meta-analysis reviewed 14 clinical trials (2,128 women) evaluating the effect of endometrial injury

in women undergoing IVF-ET [43]. Thirteen of the trials had the endometrial biopsy performed in the preceding luteal phase and one study performed the biopsy on the day of oocyte retrieval. Their conclusions were that if 26% of women achieve live birth without endometrial injury, between 28% and 48% will achieve live birth with this intervention when the biopsy is performed in the luteal phase [43]. However, a lower live delivered pregnancy rate occurs if the biopsy occurs on the day of oocyte retrieval [43].

There have been no studies to date on the effect of endometrial local injury on pregnancy rates in non-IVF cycles. A study on the effect of endometrial injury on non-IVF cycles is presently being conducted by our new fellow in reproductive endocrinology and infertility, Dr. Eric Chang, who is also one of the co-authors of this editorial.

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