Endometriosis and infertility: biomarkers affecting implantation rate

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Endometriosis is an inflammatory disease defined by the presence of ectopic endometrial tissue outside the uterine cavity and is associated with infertility. An aberrant molecular phenotype of the eutopic endometrium in women with endometriosis is thought to affect endometrial receptivity. For successful maternal-embryonic dialog, an appropriate expression of growth factors, cytokines and hormones and their regulation of cell adhesion molecules is necessary. Many articles have found that certain biomarkers such as leukemia inhibiting factor (LIF) from the IL-6 family, glycodelin A (GdA) and αvβ3 integrin are abnormally downregulated in women with endometriosis. The altered expression of progesterone receptor expression also decreased expression of HOX genes in the eutopic endometrium, leading to an abnormal activation or repression of the downstream genes regulated by the HOX genes that produce biomarkers of endometrial receptivity. Understanding the molecular and cellular mechanisms of endometriosis-related reduction in uterine receptivity will guide future research for fertility treatment in women with endometriosis.

KEYWORDS: biomarkers  endometriosis  endometrium  endometrial receptivity  HOX10  implantation window  infertility  in vitro fertilization  progesterone receptor

Endometriosis is an inflammatory disease defined by the presence of ectopic endometrial tissue outside the uterine cavity and is associated with pelvic pain and infertility [1,2]. The prevalence of endometriosis in women of reproductive age is 10–15%, but is found to be 35–50% in infertile women [3]. Pathogenic mechanisms in endometriosis-associated fertility include a distorted adnexal anatomy secondary to scarring and chronic inflammation, which in turn leads to abnormal folliculogenesis, poor oocyte quality, abnormal embryogenesis and decreased implantation rate [4].

A previous meta-analysis found that in both natural and assisted reproductive technology (ART) cycles, implantation rates were lower in patients with endometriosis, even in those with minimal disease [5]. The eutopic endometrium in women with endometriosis appears to be genetically, biochemically and functionally different from the endometrium of women without the disease [6]. This aberrant molecular phenotype of the endometrium in women with endometriosis is thought to affect endometrial receptivity [7] and contribute to implantation failure.

Over a decade of research and advancing technology have resulted in the discovery of several implantation biomarkers expressed abnormally in infertile women with endometriosis. Using the keywords “implantation”, “endometrial receptivity”, “endometriosis”, “infertility” and “biomarkers”, this review summarizes the current biomarkers thought to affect endometrial receptivity in women with endometriosis, which have been divided into four groups: cytokines and growth factors, adhesion molecules, signaling molecules and the consequences of progesterone resistance.

Implantation: the blastocyst-endometrial dialog
Embryo implantation is an intricate biological phenomenon that is still not well understood. It requires a functional embryo to appose, adhere to and penetrate a receptive endometrium during a specific period of the menstrual cycle known as “the window of implantation” [89]. This putative time frame of uterine receptivity occurs between days 5 and 10 following the surge of luteal hormone (LH). A primed
endometrium facilitates the establishment of a bi-directional maternal-embryonic dialog with the appropriate expression of growth factors, cytokines and hormones and their regulation of cell adhesion molecules [10].

**Implantation biomarkers in women with endometriosis**

**Cytokines & growth factors**

A delicate balance between pro-inflammatory and anti-inflammatory cells will allow the uterine microenvironment to accept a semi-allogeneic embryo [11]. The leukemia inhibiting factor (LIF) is an important cytokine belonging to the IL-6 family and is among the many factors expressed in the uterine luminal endometrium during the window of implantation [12]. In 2006, Dimitriadis et al. found that the levels of LIF expressed in the luminal epithelium during the mid-to late secretory phase of the menstrual cycle were lower in infertile women with endometriosis when compared with fertile controls [13]. Studies on LIF-knockout mice have demonstrated the functions of LIF in the endometrium, which include controlling the number of immune cells during the time of implantation, mediating the interactions between the decidual leukocytes and the invading embryo and altering the expression of glycans on the cell surface [11,14]. Anti-LIF monoclonal antibody administration to the uterus in rhesus monkeys resulted in reduced pregnancy rates [15]. All these findings were described in animals. *Note with caution:* There maybe difference between animal and human findings.

The same study by Dimitriadis et al. also showed that IL-11 and IL-11 receptor-α were absent in the endometrium of infertile with endometriosis [13]. IL-11 is an anti-inflammatory cytokine produced by stromal and epithelial cells, with its maximal production occurring during decidualization [16]. IL-11 receptor-α knockout mice were infertile, possibly due to abnormal decidualization [17]. Although the precise roles of LIF and IL-11 in affecting fertility in humans are not well understood, the dysregulation of these cytokines may be one of the pathogenic mechanisms behind decreased implantation rate in women with endometriosis.

Insulin-like growth factor binding protein-1 (IGFBP-1) is produced by the stromal cells during decidualization, under the influence of progesterone [18]. It is hypothesized that IGFBP-1 may limit the invasiveness of the trophoblast by inhibiting the action of IGF-2 [19]. The IGF/IGFBP system demonstrates the importance of controlled aggression, where any disruption on either side will result in failure of implantation. Klemmt et al. in 2006 showed that the levels of IGFBP-1 in stromal cells derived from the ectopic and eutopic endometrial stromal cells were lower in women with endometriosis than in women without the disease [20]. The reduced expression of IGFBP-1 is now thought to be secondary to the overactivation of the PI3K/AKT signaling pathway resulting in reduced nuclear FOXO1 levels [21].

Glycodelin is another cytokine that is upregulated during the implantation window and is thought to play a role in establishing blastocyst-endometrial communication. Like IGFBP-1, its production is induced by progesterone. Using a qualitative immunohistochemistry test, Wei et al. found that glycodelin A (GdA) levels were decreased in women with endometriosis during the late secretory phase [22]. GdA appears to have a suppressive effect on the maternal immune response and allows invasion of the blastocyst during implantation; however, the results about glycodelin in the literature are very controversial. Vigne et al. in 2001 found an increased GdA levels in endometriosis over ‘healthy’ women. He described these fusing quantitative methods on serum/plasma and/or peritoneal fluid [23,24].

Osteopontin (OPN) is a multifunctional, acidic glycoprotein belonging to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of extracellular matrix proteins and cytokines. TNF-β, TNF-α, IL-1, IFN-γ, estrogen and progesterone are among the many stimulators of OPN production [25]. Endometrial glands and decidual cells produce OPN during the time surrounding the implantation window, supporting its role in implantation [25]. As a cytokine, it has both pro- and anti-inflammatory effects. OPN is also important for adhesion and cell-cell/cell-extracellular matrix communication at the uterine–placental interface and facilitates the attachment of integrins such as αvβ3. Lessey in 2002 reported that in women with endometriosis, there is a decreased level of αvβ3 integrin but no change in the level of OPN [26]. Wei et al. in 2009 found no difference OPN expression in the eutopic endometrium of women with endometriosis during the mid-secretory phase [22]. Interestingly, in the same year, Cho et al. found significantly increased OPN mRNA levels in eutopic endometrium of women with endometriosis [27].

A recent immunohistochemical analysis of OPN and its ligand αvβ3 integrin by Casals et al. did not show a difference in expression of these two markers in women with endometriosis and those without [28]. The authors try to explain the possible reasons for the discrepancies between the different studies, which included the heterogeneity of endometriosis, the lack of appropriate controls and the inclusion of endometrial samples taken at different times of the menstrual cycle [28]. Stoikos et al. in 2010 hypothesized that the cytokine activin A may contribute to successful adhesion of the blastocyst to the endometrium. Dysregulated activin A levels in endometriosis during the secretory phase of the menstrual cycle could cause decreased production of cell adhesion molecules in trophoblast cells and affect endometrial receptivity [10].

**Adhesion molecules**

L-selectin ligands expressed on the luminal uterine epithelium support early stages of blastocyst attachment [11]. Apposition to the endometrium is impaired when L-selectin is blocked with specific antibodies [29,30]. Studies showed that women with higher levels of L-selectin ligands have higher pregnancy and implantation rates [31,32]. In 2005, Lai et al. demonstrated that L-selectin ligands were highly expressed in the glandular epithelium during the mid-secretory phase of the menstrual cycle [33].
Margarit et al. in 2009 found that there was a predominance of low affinity ligands over high affinity (sulfated) ligands in women with endometriosis \[34\]. Abnormal expression of L-selectin ligands may result in defects in endometrial receptivity.

Cycle-dependent integrins are adhesion molecules that play a role in the apposition and adhesion steps of implantation. In 1994, Lessey et al. conducted a prospective double-blind study which found that αvβ3 integrin expression was reduced or absent in patients with endometriosis during the window of implantation \[34-36\]. Women with endometriosis who were treated with gonadotropin-releasing hormone (GnRH) analogs and laser ablation of implants showed an improvement in fertility and return of normal αvβ3 levels \[38,39\].

E-cadherin is a cell adhesion molecule vital for the normal development of the embryo and in establishing a successful dialog between the blastocyst trophoderm and the maternal epithelium \[11\]. It is involved in the formation of a permeability barrier in the primary decidual zone that regulates the various molecules in the microenvironment and also guides the trophoblast in the endometrium for successful implantation \[40\]. Interestingly, a recent case–control study by Matsuzaki et al. showed that there was an impaired downregulation of the E-cadherin and β-catenin expression in infertile women with endometriosis during the mid-secretory phase \[41\]. The authors postulated that a temporal downregulation of these two proteins during the implantation window may be necessary for dissociation of epithelial cells and subsequent invasion of the blastocyst \[41\].

**Progesterone-regulated mucins function**

Mucins expression in human endometrium is downregulated during the window of implantation \[11\]. Margarit et al. in 2010 found that mucin-1 (MUC1) expression was altered in women with endometriosis during the secretory phase of the menstrual cycle. Altered MUC1 at the time of implantation may be one of the causes of diminished endometrial receptivity \[42\].

**Signaling molecules**

Lysophosphatidic acid (LPA) is a cell membrane phospholipid metabolite and its effects are mediated though G-protein coupled receptors \[43\]. LPA signaling is important in many aspects of reproduction including fertilization, embryo implantation and deciduization. In the mouse, it is essential for blastocyst implantation by stimulating cyclooxygenase-2 induction and prostaglandin signaling \[44,45\]. The study by Wei et al. in 2009 was the first to show decreased expression of LPA3 in the mid- and late secretory phase endometrium of women with endometriosis \[22\].

**Progesterone resistance & the HOX genes**

Endometriosis has been well described in literature as an estrogen-dependent disease, but there is shift in paradigm, with newer studies suggesting that it is more a disorder of progesterone resistance \[7,46\]. The human progesterone receptor (PR) has two functionally different isoforms, PR-A and PR-B \[47\].

Progestrone resistance in endometriosis is thought to be the result of the abnormal methylation of the PR-B in endometriosis \[48\]. altered expression of chaperone proteins such as FKBP52 \[49\] and co-regulators like HIC-5/ARA55 \[7,50\]. Abnormalities in progesterone signaling will affect the expression of downstream target genes, leading to a uterine microenvironment unfavorable for implantation \[39\].

The mammalian homeobox (HOX) genes are highly conserved genes that impart developing body segment identities during embryogenesis \[51\]. The progesterone target HOXA10 and HOXA11 genes are cyclically expressed in the endometrium, with maximal expression during the window of implantation \[52,53\]. In women with endometriosis, the altered expression of PR results in decreased expression of HOX genes in the eutopic endometrium \[54\]. This leads to an abnormal activation or repression of the downstream genes regulated by the HOX genes, including markers of endometrial receptivity such as αvβ3 integrin and IGFBP-1 that were discussed earlier \[55\]. Hypermethylation of HOXA10 has recently been implicated as the mechanism involved in HOX gene dysregulation in endometriosis and it results in the permanent silencing of HOXA10 \[56,57\].

FKBP4 (FKBP52) is a co-chaperone protein of the PR. A recent study by Yang et al. assessed the HOXA10 regulation of FKB4 and found that in HOXA10-silenced human endometrial stromal cells, there was a decrease in FKB4 expression leading to impaired decidualization \[58\].

Studying the potential role of P13K/AKT/FOXO1 pathway in patients with endometriosis, Yin et al. described the overactivation of P13K. The authors concluded that the treatment of those overactive pathways may help the patient’s symptoms and may stop the progression of the disease \[21\].

The expression of Empty spiracles homolog 2 (Emx2/EMX2) gene is repressed by HOXA10 during the peri-implantation window \[59\]. In women with endometriosis, the absence of mid-secretory rise of Hoxa10/HOXA10 expression results in high levels of EMX2 \[60,61\]. Mice transfected with EMX2 cDNA in the peri-implantation period had a 40% decrease in litter size \[62\], further suggesting the importance of HOXA10-regulated Emx2 expression for successful implantation.

**Discussion**

The molecular phenotyping of the eutopic endometrium in women with endometriosis has introduced many potential biomarkers of endometrial receptivity defects. Studies looking at these markers have been criticized for the lack of power and limited validity in their study design \[63\]. Adding complexity to an already enigmatic disease are the multiple discordant results of these studies. Endometriosis is a heterogeneous disease with varying degrees of severity. Not all women with endometriosis are infertile, nor will all have the aberrant expression of biomarkers. A myriad of factors rather than just defective endometrial receptivity can contribute independently or in concert with the pathogenesis of infertility in women with endometriosis.
Hence, the task of finding a single biomarker as a candidate for treatment of endometriosis-related infertility can be a challenge and proper study design with strict inclusion criteria is crucial (Figure 1). The emerging concept of progesterone resistance in endometriosis is important. The shift to an estrogen-enriched milieu via endometrial aromatase and the disturbance of signal transduction and expression of downstream progesterone target genes lead to defective decidualization and ultimately an altered eutopic endometrial phenotype that may be hostile to implantation. Endometriosis is also aptly described as a disease of inflammation, with excessive free radical production and oxidative stress [64–66].

Evidence shows that the peritoneal fluid of women with endometriosis reduces implantation rates in the mouse [67] and the hamster [68]. Oxidative stress was shown to induce genomic and mitochondrial DNA damage, which leads directly to a decrease in fertility. Sperm, oocytes and embryos incubated in the peritoneal fluid of patients with endometriosis exhibited DNA fragmentations indicated by increased apoptosis [4]. An increased level of nitric oxide, as seen in the endometrium of women with endometriosis, is toxic to embryos and inhibits implantation [69]. Defective implantation in patients with endometriosis cannot be attributed to a single biomarker alone but a combination of factors.

There have been many treatments developed at improving implantation rate. Local injury to the proliferative phase endometrium by endometrial biopsy increases implantation and pregnancy rates in subsequent IVF-embryo transfer cycles [70–72]. The wound healing that follows results in massive secretion of different cytokines and growth factors including LIF and IL-11 are beneficial for implantation. The recruitment of stem cells following injury may also form a new endometrium free of epigenetic defects [73]. More studies need to be performed to completely understand the role of stem cells and epigenetic.

The importance of endometrial αvβ3 integrin expression for successful implantation has been discussed earlier. A recent retrospective study showed that in addition to GnRH agonist therapy and laser ablation of endometriotic lesions, letrozole may improve IVF pregnancy outcomes in patients with lack of endometrial αvβ3 integrin expression [74]. Supplementing embryo culture with putative adhesion promoting factors like hyaluronic acid or recombinant heparanase have improved implantation rates in animals but not in humans [75]. Mice transferred with the HOXA10 gene showed an increase in implantation sites. All these could be fertile ground for future research into the treatment of implantation failure in women with endometriosis.

**Expert commentary**

In order to study the implantation window in patients with endometriosis, the perfect animal model would be primates, however few studies have been published using these animals. The papers studying biopsies in humans could also provide key information in this subject.

Many endometrial biomarkers of implantation have been studied during the past decade and their relation to women with endometriosis has been discussed in this review. However, a woman with endometriosis-related infertility might have to go through several obstacles from fertilization to implantation for a successful pregnancy to occur. Researchers must remember that treatment targeted at improving the levels of one biomarker may not necessarily benefit the entire group of women with endometriosis. The advent of proteomics, epigenetics, DNA microarrays will definitely help future research.

**Five-year view**

The authors believe that in 5 years, it will be possible to understand the key pathways involved in implantation window in patients with endometriosis. Knowing the biomarkers using gene therapy will definitely improve assisted reproduction outcomes. They also believe that P13K/AKT/FOXO1 pathway is one of the key pathways in the literature that will help understand endometriosis deeply. Future research is still necessary in order to best treat women suffering from endometriosis.
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Key issues
- This review focuses on biomarkers measure during implantation window in patients with endometriosis.
- Endometriosis is a heterogeneous disease with varying degrees of severity and not all women with endometriosis are infertile and have aberrant endometrium.
- Defective implantation in patients with endometriosis cannot be attributed to a single biomarker alone but a combination of factors.
- The advent of proteomics, epigenetics, DNA microarrays will definitely help future research.

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22 The authors have described the important role of P13K/AKT/FOXO1 pathway in patients with endometriosis.


Knowing that endometrial injury could provide implantation is a very important step to improve assisted reproduction outcomes.

