Proinflammatory cytokines induced altered expression of cyclooxygenase-2 gene results in unreceptive endometrium in women with idiopathic recurrent spontaneous miscarriage

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Objective: To investigate the expression pattern of proinflammatory, anti-inflammatory, and angiogenic cytokines and their effect on various mediators of endometrial receptivity in women with idiopathic recurrent spontaneous miscarriage (IRSM).
Design: A prospective study.
Setting: Tertiary care hospital and reproductive health research unit.
Patient(s): Thirty-six women with IRSM (<35 years) and 30 fertile women as controls matched by age and body mass index undergoing sterilization.
Intervention(s): Endometrial biopsies in all women corresponding to the window of implantation.
Main Outcome Measure(s): Assessment of endometrial expression of proinflammatory, anti-inflammatory, and angiogenic cytokines, mediators of matrix turnover and angiogenesis, markers of receptivity.
Result(s): A statistical significantly higher level of proinflammatory cytokines, mediators of matrix turnover and angiogenesis, and a reduced expression of anti-inflammatory and angiogenic cytokines were observed in women with IRSM. Additionally, the markers of endometrial receptivity were poorly expressed in women with IRSM.
Conclusion(s): Aberrant expression of proinflammatory, anti-inflammatory, and angiogenic cytokines during implantation window in women with IRSM is one of the key factors that adversely affect endometrial development, as evidenced by the inadequate expression of various endometrial receptivity markers. (Fertil Steril® 2013;99:179–87. ©2013 by American Society for Reproductive Medicine.)
Key Words: Cyclooxygenase-2, cytokines, endometrial receptivity, MMP, VEGF

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Recurrent spontaneous miscarriage is defined as three or more consecutive pregnancy losses within 20 weeks of gestation (1). In approximately 50% of these cases, the cause remains unsolved. An unsupportive endometrium, leading to abnormal implantation, is considered to be one of the key factors contributing to idiopathic recurrent spontaneous miscarriage (IRSM).

Structural and functional modifications of the endometrial matrix and vasculature during the periimplantation period to acquire a receptive state remain an active area of research (2–4). Cytokines play an

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important role as local mediators in the endometrium during this preparative phase of the menstrual cycle (5). The process of implantation shares similarities with the proinflammatory response (6). Cyclooxygenase-2 (COX-2), whose expression is induced by inflammatory stimuli such as interleukin-1β (7), tumor necrosis factor-α (8), and interferon-γ (9), has been suggested as playing an important regulatory role in successful implantation. However, little is known about its role in IRSM. Only one study has reported decreased expression of COX-2 resulting in recurrent pregnancy loss (10). Inflammatory cytokines have been reported to induce transforming growth factor-β (TGF-β), a multifunctional cytokine with a wide range of physiologic and pathologic effects (11). Further, TGF-β up-regulates matrix metalloproteinase-2 (MMP-2) and MMP-9 (12), the main mediators of endometrial extracellular matrix (ECM) turnover during menstruation. An alteration in this turnover before implantation has been reported to occur in IRSM (13).

The expression of a major COX-2 product, prostaglandin E2 (PGE2), is induced by the proinflammatory mediator TGF-β (14). As a vasoactive factor, PGE2 is known to play an important role in embryo implantation (15) and angiogenesis (16). The role of PGE2 during the implantation window in women with IRSM is poorly understood. One study has reported a higher PGE2 level in the cervical ovulatory mucus of women with IRSM compared with healthy fertile controls (17).

Angiogenic cytokines induce the expression of vascular endothelial growth factor (VEGF), a prime angiogenic factor (18–21) that regulates angiogenesis and neovasculogenesis, which are fundamental for endometrial growth and differentiation during implantation (22). Vascular endothelial growth factor messenger RNA (mRNA) and its proteins are reported to be down-regulated in IRSM when compared with normal fertile women (10, 23).

Various cell adhesion molecules and pinopodes expressed during the implantation window have been recognized to be potential biomarkers of uterine receptivity (15, 24). There is evidence that reduced expression of $\alpha v\beta_3$ integrin, a transmembrane glycoprotein, is associated with unreceptive endometrium (15). Though E-cadherin represents the most studied subclass of glycoproteins responsible for the calcium-dependent cell-to-cell adhesion mechanism, its expression in IRSM during implantation window has yet to be studied. L-selectin molecules, expressed by the human trophoblast, bind with the ligand MECA-79 aiding the initial steps of blastocyst adhesion to the uterine wall. Absence of MECA-79 may be the reason for repeated implantation failure in patients with multiple implantation failure (25). The expression of L-selectin ligand during implantation widow has not been investigated in IRSM. Without expression of leukemia inhibitory factor (LIF) in the uterus, implantation of a blastocyst cannot occur. Another important anti-adhesion candidate marker is mucin-1 (Muc-1), which blocks the blastocyst from implanting in an improper endometrial region (15). Pinopodes, which appear as apical cellular protrusions over the endometrium during the implantation window, are progesterone-dependent morphologic markers of receptivity and are considered to be the site of the blastocyst's attachment to the endometrium (26).

Because cytokines are physiologically involved in the process of endometrial remodeling, we assessed expression during implantation window in women with IRSM of various proinflammatory cytokines such as interleukin 1β (IL-1β), tumor-necrosis factor α (TNF-α), interferon-γ (IFN-γ), transforming growth factor β1 (TGF-β1), anti-inflammatory cytokines such as IL-4 and IL-10, and angiogenic cytokines such as IL-2, IL-6, and IL-8. Additionally, we also explored the expression of key factors responsible for matrix turnover (MMP-2, MMP-9, and their inhibitors) and angiogenesis (VEGF, prostaglandin E2). Finally, we evaluated endometrial receptivity during implantation period in these women by studying the expression of various biochemical and morphologic markers, including $\alpha_v\beta_3$ integrin, LIF, E-cadherin, MECA-79, Muc-1, and pinopodes.

MATERIALS AND METHODS

Patient Selection

The participants in the study were 36 women with IRSM treated for infertility at the Institute of Reproductive Medicine, Salt Lake, Kolkata. The following tests were performed to confirm that there was no apparent cause of recurrent pregnancy loss: thyroid-stimulating hormone (TSH) and antithyroid antibody tests, antiphospholipid antibodies test (anticardiolipin antibodies and lupus anticoagulants IgG and IgM), TORCH (toxoplasmosis, rubella, cytomegalovirus, and herpes) tests, paternal and maternal chromosomal analysis, hysterosalpingography, and hysteroscopy to rule out uterine defects, abnormal fasting level of homocysteine, exclusion of diabetes mellitus, and estimation of midluteal serum progesterone to exclude luteal phase defect.

Women who have had more than three consecutive miscarriages within first trimester (up to 12 weeks of gestation) of unknown cause (not associated with any other gynecologic disorder), and who had not received any kind of medication since the last 3 months were included. Women with a history of repeated miscarriages of known cause were excluded. Endometrial tissue samples were collected from the participating women (age $<$35 years) during the midsecretory phase of the menstrual cycle. Thirty women of proven fertility undergoing sterilization were enrolled as controls. The study was approved by the institutional ethics committee and written informed consent was obtained from all couples.

Ultrasonography for serial folliculometry was performed every day from day 10 onward in all cases to monitor follicular growth until ovulation occurred. The linear increments of the size of the dominant follicle and endometrial thickness were recorded. After rupture of the dominant follicle (ovulation) and the subsequent crenated appearance of the follicle, the expected period/window of implantation was calculated between day 18 to day 23, corresponding to 5 to 8 days after ovulation. This was supported by the basal body temperature, the persistent elevated temperature of 0.5°F above the preovulatory period. An endometrial biopsy was performed on day 7 after confirmation of ovulation under general anaesthesia by uterine curettage (D&C).

The endometrial biopsy samples were sent for routine pathologic analysis, and endometrial histologic dating was