Dysregulated leukemia inhibitory factor and its receptor regulated signal transducers and activators of transcription 3 pathway: a possible cause for repeated implantation failure in women with dormant genital tuberculosis?

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Objective: To investigate the influence of dormant *Mycobacterium tuberculosis* on the expression of various endometrial receptivity markers and leukemia inhibitory factor (LIF)-signal transducers and activators of transcription 3 (STAT3) signaling pathway. Expression of endometrial receptivity markers and LIF-STAT3 signaling in in vitro decidualized human endometrial stromal cells (hESC) treated with 65 kDa mycobacterial heat shock protein (HSP65) is also explored.
Design: A prospective study.
Setting: Tertiary care hospital and reproductive health research unit.
Patient(s): Endometrial tissue samples were collected from 38 women who tested positive for *Mycobacterium tuberculosis* and 30 normal women with proven fertility undergoing sterilization. In vitro decidualization of hESC was performed.
Intervention(s): Endometrial biopsies collected from all women during implantation window and treatment of hESC with HSP65.
Main Outcome Measure(s): Measurement of various endometrial receptivity markers including $\alpha v\beta 3$ integrin, E-cadherin, MECA-79, mucin-1, and pinopodes and LIF/LIFR-STAT3 signaling molecules expressed in the endometrium of women with dormant genital tuberculosis (GTB) during implantation window and measured also in HSP65-treated hESC.
Result(s): Significantly reduced levels of endometrial receptivity markers LIF, LIFR, and pSTAT3 were observed in endometrium of women with dormant GTB as compared with controls. A similar trend was observed under in vitro conditions with decreased level of phosphorylated STAT3 in HSP65-treated hESC. However, no change in the expression of endometrial receptivity markers under in vitro conditions was observed.

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Conclusion(s): Our findings suggest that endometrium of women with dormant GTB is associated with poor receptivity, as evidenced by reduced receptivity markers and aberrant LIF-STAT3 signaling. In vitro treatment of hESC with HSP65 also confirms compromised endometrial decidualization. (Fertil Steril® 2016;105:1076–84. ©2016 by American Society for Reproductive Medicine.)
Key Words: Endometrial receptivity, infertility, decidualization, dormant genital tuberculosis, HSP65

Genital tuberculosis (GTB) is a major pelvic factor causing infertility in Indian women (1, 2), and IVF has emerged to be the technological solution for such infertile couples. The global incidence of genital tuberculosis is estimated to be 1% of infertile women ages 20–40 years in developed countries, whereas in India it is reported to be 18% (3, 4). The major difficulty in treating GTB is that it often exists in a latent form without any clinical symptoms.

A receptive endometrium together with a good-quality embryo and an efficient maternal embryo signaling process is necessary for successful implantation to occur (5, 6). Extensive investigations have associated endometrial receptivity with the expression of various molecular mediators (7, 8). The adhesion molecules $\alpha v\beta 3$ integrin (ITGAVB3; $\alpha v\beta 3$), E-cadherin (CDH1), and MECA79 are known to play an important role in attachment of the blastocyst to the endometrial surface (7). Reduced expression of ITGAVB3, a transmembrane protein, has been associated with an unreceptive endometrium (9). CDH1 represents the most studied subclass of glycoproteins responsible for the calcium-dependent cell-to-cell adhesion mechanism. MECA-79, the ligand of L-selectin, binds with L-selectin molecules and helps in the adhesion of the blastocyst to the endometrium (10, 11). Mucin 1 (MUC1) is an anti-adhesion molecule and inhibits implantation of the blastocyst to an improper endometrial region (9). In addition, P-dependent morphological markers (pinopodes) appearing as apical cellular protrusions over the endometrium during implantation window are believed to play a critical role in the early steps of human embryo-endometrial interaction (7, 9).

Leukemia inhibitory factor (LIF), an IL-6-family cytokine, is regarded as an important local mediator in the endometrium for successful implantation to occur (12, 13). Involvement of LIF in complex sequences of signaling events is necessary for the establishment of pregnancy (14, 15). LIF binds with LIF receptor (LIFR) and signals primarily through the signal transducers and activators of transcription 3 (STAT3) signaling pathway (16, 17). There is increasing evidence that LIF-mediated STAT3 activation regulates uterine receptivity (15, 16, 18). The role of vascular endothelial growth factor (VEGF), an indispensable angiogeneic factor in endometrial growth and differentiation during implantation, is well established (19, 20). Numerous studies have shown that STAT3 activates transcription of the VEGF gene (21) and blocking of STAT3 reduces angiogenesis (22). However, these reports are mostly limited to cancer (23), retinopathy (24), and lymphatic endothelial cell migration (25, 26).

Numerous studies exist related to the expression of endometrial receptivity markers in recurrent spontaneous miscarriage (27), polycystic ovary syndrome (PCOS) (28), endometriosis (7), and unexplained infertility (29, 30). An extensive literature search shows no reports related to the influence of mycobacterial infection on endometrial receptivity. There is only one study documented so far where we have examined the role of latent tuberculosis in repeated IVF failure of unexplained infertility in women (2). Impairment in subendometrial blood flow and reduced endometrial thickness motivate us to assess the receptive status of the endometrium in the presence of dormant mycobacterial bacilli during the window of implantation.

The objective of the present study is to assess the expression of various cell adhesion molecules including ITGAVB3, MECA79, CDH1, and MUC1 and pinopodes in the endometrium of women with dormant GTB. In addition, the LIF-STAT3 signaling pathway, proven to be essential for implantation and angiogenesis, is examined in these women. We have also validated the expression of endometrial receptivity markers and signaling pathway in decidualized human endometrial stromal cells (hESC) treated with 65 kDa mycobacterial heat shock protein (HSP65) under in vitro conditions.

MATERIALS AND METHODS

Subject Inclusion and Sample Collection

This study was approved by the Research Ethics Board of the Institute of Reproductive Medicine, Salt Lake City, Kolkata, India. Written informed consent was obtained from all couples participating in the study. Couples reporting to the institute between October 2011 and March 2013 for infertility treatment were screened. Of these, couples with no identifiable cause of infertility (n = 484) were included in this study for selecting the dormant GTB cases following stringent exclusion criteria. Dormant GTB refers to the group of asymptomatic infertile women testing positive for *Mycobacterium tuberculosis* (*M. tuberculosis*) without any clinical manifestation of tuberculosis or evidence of tubal or endometrial damage. The patient selection flow chart is provided as Supplemental Figure 1.

Hysterosalpingography and hysteroscopy were performed to evaluate the tubal and endometrial abnormalities. Single-tube nested polymerase chain reaction (PCR) was