Altered circulating levels of matrix metalloproteinases 2 and 9 and their inhibitors and effect of progesterone supplementation in women with endometriosis undergoing in vitro fertilization

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Objective: To investigate differences in the activity of matrix metalloproteinases (MMPs) 2 and 9 and their respective tissue inhibitors (TIMPs) in follicular fluid of women with endometriosis, to correlate the findings with IVF outcome, and to examine the therapeutic potential of progesterone supplementation in restoring the fine balance between MMPs and TIMPs.
Design: Prospective case-control clinical study.
Setting: Infertility clinic and reproductive health research unit.
Patient(s): A total of 340 infertile women undergoing IVF.
Intervention(s): Natural micronized progesterone capsules were administered for luteal support.
Main Outcome Measure(s): Association of MMPs 2 and 9 and TIMP-1 with oocyte maturity and embryo development.
Result(s): An abnormal expression of MMP-2, MMP-9, and TIMP-1 with extensive MMP-9/TIMP-1 imbalance in women with endometriosis undergoing IVF was observed. Transforming growth factor β1 plays an important role in these women with possible involvement of Smad-2 and -3 proteins. Progesterone supplementation improves the imbalance in MMP-9/TIMP-1 ratio significantly in women with endometriosis who conceive after IVF.
Conclusion(s): Increase in MMP-2 and -9 and decrease in TIMP-1 expression was associated with poor oocyte and embryo development in women with endometriosis undergoing IVF. MMP-9/TIMP-1 balance was highly affected in these women, and progesterone supplementation appeared to restore this imbalance to a considerable degree. (Fertil Steril® 2013;100:127–34. ©2013 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, matrix metalloproteinases, TGF-β1, progesterone supplementation, IVF outcome

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Development of ovarian follicles and breakdown of the follicular walls to release multiple oocytes at the time of ovulation during in vitro fertilization (IVF) involves extensive tissue remodeling. This remodeling of extracellular matrix (ECM) is determined greatly by the activity of the enzymes matrix metalloproteinases (MMPs) and their inhibitors, the tissue

inhibitors of metalloproteinases (TIMPs). Though several studies have discussed the behavior of MMPs and their inhibitors in IVF, inconsistent results persist. Marked differences in MMPs and their inhibitors in women undergoing IVF and normally ovulating women are reported (1). It is reported that MMP-2 and -9 and their inhibitors are significantly higher in follicular fluid of women with polycystic ovary syndrome (PCOS) undergoing IVF (2). An intense ECM remodeling due to multiple follicular development and cyst formation is suggested. In a similar study, 1.7-fold and 1.6-fold increases in the activities of MMP-9 and -2, respectively, were documented (3). In contrast, it has been shown that activities of MMP-2 and -9 remain unaltered, whereas TIMP-1 decreases significantly in women with PCOS undergoing IVF (4).

One of the persistent and frustrating problems in women with endometriosis is infertility and poor IVF success rate. It is estimated that >150 million women suffer from endometriosis worldwide, of which 30%–40% are infertile (5). It is suggested that endometriosis leads to altered oocyte growth and maturation resulting in poor embryo quality (6). Moreover, decreased potential of embryo to implant is thought to be one of the critical causes for low IVF success rate in these women (7). Involvement of MMPs in the development of endometriosis is being increasingly confirmed by several research groups, with studies indicating that the pattern of MMP expression in endometrium of these women significantly differs from that of healthy women (8–10).

MMP-2 and MMP-9 have the ability to hydrolyze gelatins, collagens, fibronectin, laminin, aggrecans, and elastin present in ECM (11). In its inactive form, MMP-9 is complexed to TIMP-1, whereas pro MMP-2/MMP-2 is complexed to TIMP-2, and their balance determines the degree of matrix degradation. Disturbed balance of MMPs and TIMPs is found in various pathologic conditions, such as cancer, rheumatoid arthritis, and others (12). The role of MMP-2 and MMP-9 in the development of endometriosis holds a special interest in view of the fact that these two gelatinases are known to actively participate in tumor invasion and progression (13). Transforming growth factor (TGF) β1, a member of the peptide growth factor family, induces the production of MMP-2 and -9 and is one of the potent regulators of these molecules (14). Smad-2 and -3 proteins are essential intracellular signaling components for TGF-β1 and have been implicated in the development of endometriosis (15).

Progesterone (P) is known to restrain endometrial breakdown by inhibiting the MMPs (16). Therefore, it is hypothesized that P may restore balance between MMPs and TIMPs. Most IVF patients are associated with a defective luteal phase, and P supplementation during IVF cycles improves clinical pregnancy outcome significantly.

The aim of the present study was to investigate possible differences, if any, in the activity of MMP-2 and -9 and their respective inhibitors in follicular fluid of women with endometriosis and to correlate the findings with IVF outcome. The therapeutic potential of P supplementation in restoring the fine balance between MMPs and TIMPs was also examined.

MATERIALS AND METHODS

This prospective case-controlled study was conducted at a tertiary care hospital, the Institute of Reproductive Medicine, Kolkata, India. Approval was obtained from the Institutional Research Ethics Board. Written informed consent was taken from each couple participating in this study. A part of the study was done at the Reproductive Health Research Unit, School of Medical Science and Technology, Indian Institute of Technology, Kharagpur, India.

Subject Selection

A total of 340 infertile women (26–40 years old) with body mass index (BMI) < 25 kg/m^2 and duration of infertility >24 months undergoing IVF were recruited from February 2010 to December 2011. Out of these 340 women, 200 women with endometriosis (stage III and IV) confirmed by laparoscopy and biopsy were included as the study group (group A) and 140 normal ovulating women with tubal-factor infertility (without endometriosis) were included as control subjects (group B). Baseline FSH, LH, and E_2 levels and various IVF outcome parameters were assessed in both groups.

Inclusion and Exclusion Criteria

In this study, tubal-factor infertility refers to women who had fallopian tube(s) removed for tubal pregnancy and to proximal tubal obstruction because of low-grade infection or fimbrial occlusion with or without mild peritubal adhesion. Tubal infertility associated with gross hydrosalpingeal changes, dense pelvic adhesions because of endometriosis, and pelvic inflammatory diseases were excluded. Women with adenomyosis, severe pelvic adhesions, thyroid disorders, hypoprolactemia, and diseases such as diabetes mellitus and cardiovascular diseases were excluded. Only those women were considered whose partners/donors were normozoospermic with semen parameters normal according to World Health Organization guidelines (17) and having no identifiable symptom of infertility. It was also ensured that women included had not received any medication other than the standard IVF protocol for the preceding 3 months.

Ovarian Stimulation Protocol

Ovarian stimulation was performed with the conventional down-regulation protocol. The patients were down-regulated with a GnRH agonist (Lupride; Sun Pharmaceuticals) midluteal phase onward and when optimally down-regulated, were stimulated with recombinant FSH (Gonal-F; Serono). Follicular size was monitored regularly by ultrasound and serum E_2 assays. Subcutaneous hCG (Pregnyl; Organon) was administered when average diameter of the leading follicles reached $\geq$18 mm. The oocytes were retrieved under transvaginal ultrasound guidance.

Assessment of Oocyte Maturity, Fertilization, and Embryo Culture

Maturity of oocytes and quality of embryos were assessed by the clinical embryologists based on the criteria