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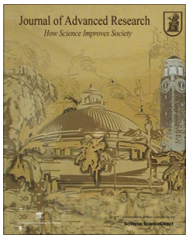

ORIGINAL ARTICLE

Pilot study on molecular quantitation and sequencing of endometrial cytokines gene expression and their effect on the outcome of *in vitro* fertilization (IVF) cycle

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ABSTRACT

Human trophoblast invasion and differentiation are essential for successful pregnancy outcome. The molecular mechanisms, however, are poorly understood. Interleukin (IL)-11, a cytokine, regulates endometrial epithelial cell adhesion. Leukemia inhibitory factor (LIF) is one of the key cytokines in the embryo implantation regulation. The present study aimed to assess the levels of LIF, IL-11, and IL-11 α receptor gene expression in the endometrium of women undergoing IVF and correlate their levels with the IVF pregnancy outcome. Also, the study aimed to detect any mutation in these three genes among IVF pregnant and non-pregnant women versus control menstrual blood of fertile women. Endometrial tissue biopsies were taken from 15 women undergoing IVF on the day of oocyte retrieval. The quantitative expression of IL-11, IL-11Rα, and LIF genes was assessed by real-time PCR and PCR products were sequenced. Menstrual blood from 10 fertile women was used as control to compare the DNA sequence versus DNA sequence of the studied genes in endometrial biopsies. LH, FSH, and E2 were assessed for enrolled patients by ELISA. Endometrial thickness was also assessed by pelvic ultrasonography. No significant difference was detected between quantitative expression of the three studied genes and pregnancy IVF outcome. Although DNA sequence changes were found in IL-11 and LIF genes of women with negative pregnancy IVF outcome compared to women with positive pregnancy IVF outcome, no DNA sequence changes were detected for IL-11Rα. Other studied

Abbreviations: IL-11, interleukin 11; IL-11Rα, interleukin receptor α; LIF, leukemia inhibitory factor; IVF, ***in vitro*** fertilization; FSH, follicular stimulating hormone; LH, Luteinizing hormone; E2, Estradiol 2.

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parameters (e.g., age, LH, FSH, E2, and endometrial thickness) showed no significant differences or correlation of quantitative expression of the three studied involved genes. Data suggested that there were no significant differences between quantitative expression of IL-11, IL-11Rα, and LIF genes and the IVF pregnancy outcome. The present study may reveal that changes in IL-11 and LIF genes sequence may contribute in pregnancy IVF outcome.

Introduction

Embryo implantation is a complex process requiring synchronized endometrial receptivity and blastocyst competence [1]. The initial apposition, attachment, and adhesion of the blastocyst to an adequately prepared or receptive maternal endometrium occur via a coordinated dialog of locally produced molecules, including cytokines, adhesion, and extracellular matrix (ECM) molecules [2].

A class of cytokines, which play an important role in embryonic implantation, is the interleukin-(IL) 6 superfamily. That family consists of numerous cytokines, including leukemia inhibitory factor (LIF), IL-6, interleukin-11 (IL-11), neurotrophic factor, oncostatin-M, and cardiotrophin-1. An important characteristic of that class of cytokines is their sharing of intracellular signaling through gp130 [3]. IL-11 and LIF signal via a hetero-dimeric receptor complex comprising either the specific IL-11 receptor α chain or the low-affinity LIF receptor, associated with the common signaling component gp130. Binding of IL-11 or LIF to their receptors forms a complex that signals via activation of Janus kinases (JAKs) that subsequently phosphorylate tyrosine residues in the cytoplasmic domain of the gp130 subunit. This in turn triggers signaling cascades involving mitogen activated protein kinases (MAPKs) and signal transducer and activator of transcription (STAT) family, in particular STAT3 and STAT1 proteins, resulting in the activation of transcription of specific genes [4,5].

During the secretory phase of the menstrual cycle, human endometrial stromal cells spontaneously differentiate into decidualized stromal cells which are morphologically and biochemically distinct. If pregnancy ensues, decidualization proceeds further and provides the maternally derived component of the placenta. The molecular interactions that regulate the formation, maintenance, and remodeling of decidua are poorly understood although many factors are known to be involved [6]. IL-11 is absolutely required for decidualization of endometrial stromal cells and blastocyst implantation in mice [7]. In humans, IL-11 mRNA and protein are expressed in the endometrium throughout the menstrual cycle, while its expression in the stroma was reported to be restricted to the predecidualized stromal cells in the late secretory phase to help the blastocyst implantation. The expression of IL-11 and its receptor (IL-11Rα) was found to be maximal during decidualization, suggesting that their interactions in the decidua are important in that process [3,6].

Leukemia inhibitory factor (LIF) derived its name from its ability to induce the terminal differentiation of myeloid leukemia cells, thus preventing their continued growth. One of the main properties attributed to LIF is the regulation of embryo implantation. LIF had been shown to facilitate implantation in the mouse model and possibly in humans [8]. LIF is expressed in the luminal epithelium during the mid-late secretory phase (days 18–28) of the menstrual cycle, supporting a role in implantation [9]. It has been suggested that recombinant human LIF might help to improve the implantation rate in women with unexplained infertility [10]. Many *in vitro* fertilization (IVF) studies using gene-matrix technology had revealed some differences in the expression of many molecules, cytokines, and other factors in endometrium of infertile women compared with fertile women [11,12].

The aim of the present study was to assess the levels of LIF, IL-11, and IL-11 α receptor gene expression in the endometrium of women undergoing IVF and correlate their levels with the IVF pregnancy outcome. Also, the study aimed to detect any sequence mutation in these three genes among IVF pregnant and non-pregnant women versus control menstrual blood of fertile women.

Methodology

Patients and tissues

Fifteen women were enrolled in the current study; they were under IVF long protocol in The IVF Centre, Kasr El Aini Hospital, Cairo University, Egypt. Patients fulfilled the inclusion criteria that included the following: age between 23 and 35 years, FSH < 10 mIU/ml, no endometriosis, no previous uterine operations, no history of poor response in previous IVF cycles, no diabetes mellitus, and no antral follicle count (AFC) > 5. All patients gave their written informed consent to participate in the study.

Endometrial tissue samples were taken on the day of oocyte retrieval using soft suction plastic catheter. The original plane of this study was to take the endometrial biopsy twice on day of pick up and on day of transfer (day 5 post-LH surge), but we observed the occurrence of endometrial bleeding, so we stopped the procedure and assess results by chemical pregnancy rate. The pregnancy rate was 50% among the done cases, but the IVF board reconsiders the biopsy at day of pick up only. Standard long protocol was used. Down regulation started on day 21 of the previous cycle using decapeptyl 0.1 mg sc daily till withdrawal occurs, serum E 2 done on day 2 of cycle when less than 50 and endometrial thickness less than 5, stimulation with 150–300 IU of HMG was started. Folliculometry started 7 days then continued every other day till more than 4 follicles of 18 mm size are seen, and HCG 5000–10,000 iu GIVEN IM 36 hs before ovum pick up all embryos were day 3 6–8 cell embryos. The menstrual blood of 10 women with regular menstrual cycles and with no apparent endometrial dysfunction was taken as control samples. *The study protocol and informed consents were approved by the Human Ethics Committee of Cairo University*.