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Oxidative stress biomarkers in endometrial secretions: A comparison between successful and unsuccessful in vitro fertilization cycles

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ABSTRACT

A potential role of oxidative stress has been implicated in the outcome of various steps of assisted reproductive technology (ART). In a prospective cohort study, a total of 100 patients undergoing IVF/ICSI procedure due to male factor infertility were recruited based on the inclusion criteria. In all patients, 1–2 ml of endometrial secretions was aspirated prior to embryo transfer. The oxidative stress markers in endometrial secretions, including superoxide dismutase (SOD), catalase (CAT) activities, lipid peroxidation (LPO), total thiol groups (TTG), and total antioxidant power (TAP) were investigated and compared among study groups including term pregnancy, failed IVF cycle, and miscarriage. $P < 0.05$ was considered statistically different. Of the 100 patients, 28 cases (28%) resulted in ongoing pregnancy (biochemical pregnancy followed by clinical pregnancy), 11 cases (11%) resulted in miscarriage, and 61 cases (61%), resulted in failed IVF cycle. SOD, LPO, CAT, and TAP levels in the endometrial secretions of the three groups were statistically different (P-value <0.01, <0.001, <0.001, and <0.001, respectively). TTG levels in endometrial secretion of three groups were not statistically different (P-value = 0.837). Our results indicated that higher levels of antioxidants such as SOD, CAT, or TAP, and lower levels of oxidative stress markers such as LPO in the endometrial secretions were associated with successful IVF outcome.

1. Introduction

Despite significant advances in assisted reproductive technologies (ARTs), the success rate of these methods is still remarkably low. Many factors appear to influence the ART outcome. Oxidative stress has been considered one of the most important factors that affect the various steps of ART procedure and inevitably its outcome (Agarwal et al., 2006).

Oxidative stress is defined as the imbalance between reactive oxygen species (ROS) and antioxidant defense (Agarwal et al., 2003). The antioxidant enzymes including copper–zinc containing superoxide dismutase (Cu, Zn-SOD), manganese containing SOD (Mn-SOD), glutathione peroxidases (GPXs), and catalase are the body's defense system against oxygen species oxidizing actions (Jezek and Hlavata 2005; Halliwell 2009).

ROS increase as a consequence of some diseases and environmental conditions (e.g. smoking, obesity, and nutrition) and play a causative role in several pathologic processes such as cancer, periodontitis, neurodegeneration, cardiovascular diseases, diabetes, and kidney diseases (Montuschi et al., 2007; Pisoschi and Pop 2015; Rahiminejad et al., 2015b). Previous studies have shown that menstrual discharge and endometrial secretions are novel samples for identification of oxidative stress biomarkers (Ametzazurra et al., 2009; Dikareva et al., 2013).

Implantation and early post-implantation developmental periods are critical steps in ART, and the outcome of the entire ART process is largely dependent

on their success. Previous studies suggest that at this stage, developing organisms are very sensitive to ROS-induced oxidative damage. Any possible damage can have a negative impact on the establishment of pregnancy (Peter Stein et al., 2008). The adverse effects of oxidative stress on pregnancy include sponta-

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neous abortion and idiopathic recurrent pregnancy loss (Gupta et al., 2007). Successful implantation requires an appropriate interaction between the embryo and the endometrium. Previous studies have shown that, antioxidant enzymatic systems are implicated both in the development of embryo and its receptive uterine endometrium before implantation (Guerin et al., 2001; Orsi and Leese 2001; Blomberg et al., 2005). There are several approaches to evaluate the endometrial maturity and receptivity (Diedrich et al., 2007). A possible method is to analyze endometrial secretions during the implantation window. Recent studies have shown that endometrial secretions can represent the interactions between the intrauterine environment and embryo (Boomsma et al., 2009a; Berlanga et al., 2011; Rahiminejad et al., 2015a). Moreover, it has been demonstrated that endometrial secretion aspiration prior to embryo transfer can be done without affecting the rate of implantation (Van Der Gaast et al., 2003). Boomsma et al. (2009b) investigated the role of cytokines such as IL-1β or TNF-α in predicting in vitro fertilization (IVF) outcomes. To our knowledge, analysis of endometrial secretions to determine the markers of oxidative stress has not previously been evaluated. In this study, we aim to investigate whether markers of oxidative stress can impact IVF success.

2. Methods and materials

2.1. Study design

This prospective cohort study was conducted from October 2012 to December 2014 at the Infertility and Reproductive Research Center of Fatemieh Women's Hospital, Hamadan, Iran. The Institutional Review Board of Hamadan University of Medical Sciences, Hamadan, Iran, approved the study protocol. A total of 100 candidates undergoing IVF/intra-cytoplasmic sperm injection (ICSI) were recruited using the following inclusion criteria: (1) nulliparous women less than 35 years old with history of male factor infertility for less than 5 years; (2) normal menstrual cycles lasting between 25 and 35 days; (3) less than 3 prior failed ART attempts; (4) body mass index (BMI) less than 30 kg/m^2; (5) sperm obtained through ejaculation; (6) normal responders with more than 6 antral follicles on ultrasound and FSH less than 10 mIU/ml.

Exclusion criteria included: (1) presence of any structural abnormality of the reproductive system, any metabolic/endocrine system-associated diseases such as hyperprolactinemia, thyroid dysfunction, or polycystic ovary syndrome (defined by the Rotterdam criteria), as revealed by physical examination, medical history, and routine infertility panel including pelvic ultrasonography, fasting plasma glucose, FSH, LH, prolactin, TSH, free T4, BUN, creatinine, complete blood count with differential, and urine analysis; (2) prior history of any kind of surgery on the reproductive system; (3) smoking; (4) couples with surgically extracted sperm; (5) women with prior diagnosis of endometriosis.

2.2. IVF technique

According to the protocol of gonadotropin releasing hormone (GNRH) antagonist for ovarian stimulation, daily injection of recombinant follicular stimulating hormone (rFSH) (Puregon, Organon, Germany) (100–300 IU/day) was started on day 2 or 3 of a menstrual cycle. To prevent premature luteinization, all patients received co-treatment with GNRH antagonist (Ganirelix acetate, Organon, Netherland) (450 IU once daily). A single injection of human chorionic gonadotrophin (hCG) (Pregnyle, Organon, Germany) (10,000 IU) was given to trigger the final stages of oocyte maturation. Ultrasound-guided oocyte pick-up was performed 34–36 h following hCG injection under appropriate sedation. On the day of oocyte retrieval, sperm obtained from ejaculate is collected into a sterile plastic container. According to the indications and quality of sperm, IVF or ICSI was performed. Embryos were graded according to their morphological appearance 48 h after fertilization. An embryo with stage-specific cell size, <10% of fragmentation, and no multinucleation was considered as grade A. Embryos with stage-specific cell size for the majority of cells, 10–25% of fragmentation, and no evidence of multinucleation considered as grade B, and embryos without stage-specific cell size, severe fragmentation (more than 25%), and evidence of multinucleation counted as grade C (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011). While the majority of embryo transfers were performed on Day 5 and Day 6, in a limited number of cases (n = 8) and in order to avoid transfer cancelation, transfer occurred on Day 4. A maximum of three embryos was transferred using a soft catheter and under ultrasound guidance. A daily injection of intramuscular progesterone (Progestan, Organon, Germany) (100 mg/day) was administered for 72 h before and 5 days after the embryo transfer.in order to support the luteal phase. To detect implantation, serum β-hCG test was measured on day 14 after embryo transfer. In patients with a positive initial hCG measurement (>25 mIU/mL), a two fold increase after 48 h was considered as a biochemical pregnancy. Cases without expected increased β-hCG levels were classified as failed IVF cycles. Visualization of gestational sac in the fifth week of pregnancy was defined as clinical pregnancy. A biochemical pregnancy which was not proven clinically in the fifth week was defined as a failed IVF cycles. Loss of products of conceptions that had been visualized by ultrasound study during the first 20 weeks was considered a miscarriage. Progression of the pregnancy after the 20th week was defined as an ongoing pregnancy.

2.3. Endometrial secretions aspiration

Endometrial secretion aspiration and measurement with regards to its viscosity were performed prior to embryo transfer similarly to the procedures described by Cheong et al. (2013). The patient was placed in the lithotomy position, and after cleaning the cervix, a 2 ml syringe attached to the embryo transfer catheter was gently introduced transcervically into the uterine cavity up to 6 cm. The inner catheter was shielded at this stage in order to avoid contamination of samples by blood and mucus. Aspiration of 1–2 ml of endometrial secretions was performed, and the tip of catheter was removed (Cheong et al., 2013). An equal amount of secretion was poured into microtubes, and the samples were stored in liquid nitrogen at a temperature of −80 °C.

2.4. Oxidative stress biomarkers

2.4.1. Lipid peroxidation (LPO) measurement

The LPO product in secretions was determined by thiobarbituric acid (TBA) reagent expressed as the extent of malondialdehyde (MDA) production during an acid heating reaction. The calibration curve of tetraethoxypropane standard solution was used to determine the concentrations of TBA + MDA adducts in samples (Ohkawa et al., 1979).

2.4.2. Total antioxidant power (TAP) measurement

TAP was measured by the ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma to reduce Fe +3 to Fe +2 in the presence of TPTZ. The reaction of Fe +2 and TPTZ gives a complex with blue color and maximum absorbance in 593 nm (Benzie and Strain, 1996).