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The relationship of seminal transforming growth factor-β1 and interleukin-18 with reproductive success in women exposed to seminal plasma during IVF/ICSI treatment

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ABSTRACT

It has been proposed that the transforming growth factor (TGF)-β1 present in seminal plasma (SP) triggers a female immune response favorable for implantation. We hypothesize that seminal interleukin (IL)-18, a cytokine that can potentially cause implantation failure, interferes with the beneficial effect of TGF-β1. This study aims to determine whether the levels of seminal TGF-β1 and IL-18 are associated with reproductive outcomes in patients exposed to SP during in vitro fertilization (IVF) or IVF with intracytoplasmic sperm injection (ICSI). A prospective study, which included 71 couples undergoing IVF/ICSI was carried out. Female patients were exposed to their partners' SP via timed intercourse before the day of ovum pick-up (OPU) and also subjected to intravaginal SP application just after OPU. Quantitative measurements of total TGF-β1 (active plus latent) as well as IL-18 were determined by FlowCytomix™ technology in the SP to be used for intravaginal applications. Comparison of SP cytokine profiles between pregnant and non-pregnant groups revealed that pregnancy was correlated with a lower concentration of IL-18 ($P = 0.018$) and lower content per ejaculate for both of IL-18 ($P = 0.0003$) and TGF-β1 ($P = 0.047$). The ratio of TGF-β1-to-IL-18 concentration was significantly higher in the pregnant than in the non-pregnant group ($P = 0.026$). This study supports the notion that two key cytokines TGF-β1 and IL-18, both present in SP are associated with reproductive outcomes in female patients exposed to SP during IVF/ICSI treatment.

1. Introduction

It has become increasingly evident that the physiological changes at implantation are associated with interaction between the female reproductive tract and seminal plasma (SP). Previous studies have shown that SP deposition in the female reproductive tract induces active inflammatory response required to accommodate pregnancy in several animal species (Robertson et al., 1996; O'Leary et al., 2004) and humans (Sharkey et al., 2012a). Seminal plasma is a key factor involved in the generation of maternal-fetal immune tolerance, which is essential for a successful pregnancy (reviewed by Schjenken and Robertson, 2015).

Thus, it is reasonable to consider that the pronounced immunomodulatory potential of SP can be exploited to improve the efficiency of IVF/ICSI. Seminal plasma has been previously used in clinical practice in an effort to increase the success rate of assisted reproductive techniques (reviewed by Crawford et al., 2015). However, studies examining SP exposure during IVF/ICSI procedures have generated conflicting results. Some authors have reported beneficial effects of SP application (Chicea et al., 2013); others acknowledge a small trend towards a higher pregnancy rate (von Wolff et al., 2009; Friedler et al., 2013), or found no significant effect (von Wolff et al., 2013).

It should be noted, that along with a favorable effect, an adverse effect of SP on fertility and pregnancy may occur if SP were to provide an insufficient, excessive or altered signal to the female reproductive system (Anderson and Politch, 2015). It is possible that seminal soluble factors including pro-inflammatory and anti-

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inflammatory cytokines could be related to either success or failure of IVF/ICSI treatment.

In mice seminal TGF-β1 plays a key role in female reproductive physiology initiating a cascade of molecular and cellular events that precede implantation (Tremellen et al., 1998). Additionally several studies have demonstrated that seminal TGF-β1 promotes maternal tolerance to paternal antigens (Dekker, 2002; Robertson et al., 2002, 2003). Using the mouse model of recurrent spontaneous resorption CBAxDBA/2, in vivo experiments have shown that exogenous TGF-β delivered vaginally at mating can reduce abortion rates (Clark et al., 2008).

In human male reproductive tract, TGF-β1 is produced by seminal vesicles and the prostate (Lee et al., 1999; Pannek et al., 1999). Interestingly, the concentration of TGF-β in the SP is ~400 ng/mL; this value is several-fold higher than in the blood serum (Sharkey et al., 2012b). The TGF-β family includes three polypeptides, TGF-β1, TGF-β2 and TGF-β3, all present in human SP (Sharkey et al., 2012b). The TGF-β1 is the most abundant TGF-β isoform detected in SP (Nocera and Chu, 1995; Sharkey et al., 2012b).

The human SP also contains the pro-inflammatory cytokine interleukin (IL)-18 (Matalliotakis et al., 2006; Qian et al., 2014). IL-18 is a member of the IL-1 cytokine family that was originally identified as interferon-γ (IFN-γ) inducing factor (Okamura et al., 1995). IL-18 plays a major role in the production of IFN-γ from T-cells and natural killer cells (Dinarello et al., 2013). The presence of IL-18 has been previously demonstrated in the human endometrium (Yoshino et al., 2001) and at the fetomaternal interface during human and murine pregnancy (Tokmadzić et al., 2002; Chaouat et al., 2002; Ostojic et al., 2003). It seems that the tight regulation of IL-18 expression is important for normal implantation and decidual remodeling events in early pregnancy (reviewed by Laskarin et al., 2005). However, the excess level of IL-18 in the uterine lumen at the Day-OPU has been associated with a poor implantation rate (Lédée-Bataille et al., 2004, 2005).

Even though a relevant role for a seminal TGF-β1 and IL-18 in the human reproduction has been suggested, no data documenting their effects on female reproductive status are available. We hypothesize, that seminal IL-18 potentially causing implantation failure may interfere with a beneficial effect of TGF-β1 on the early stages of pregnancy. This study aims to determine whether the levels of TGF-β1 and IL-18 in SP are associated with IVF/ICSI outcome in patients exposed to SP during IVF/ICSI treatment.

2. Materials and methods

2.1. Study subjects

Seventy one female patients diagnosed with tubal factor infertility and undergoing IVF/ICSI treatment were recruited into this study. The study also included the sexual partner for each patient. Ethical approval was obtained from the Research Center for Obstetrics, Gynecology and Perinatology Ethics Committee. Informed consent was given by all patients at the time of recruitment. The inclusion criteria for female patient enrolment into the study were as follow: age ≤41 years; tubal factor infertility; 0–2 previous IVF attempts; normal ovarian reserves testing by evaluation of serum levels of anti-Müllerian hormone and follicle stimulating hormone (FSH) and antral follicular count with transvaginal sonography; normal anatomy of the uterus, estimated by transvaginal sonography combined with saline contrast sonohysterography, and absence of pelvic inflammatory disorders or autoimmune diseases. No detectable pelvic pathology based on history of any previous surgery and history of symptoms such as pelvic pain, severe dysmenorrhea, dyspareunia were also considered as criteria for patient selection. The exclusion criteria were also TORCH (TOxoplasmosis, Rubella, Cytomegalovirus and Herpes) infections and sexually transmitted diseases with ureaplasma urealyticum, mycoplasma, chlamydia, gonococcus, fungi, trichomonas vaginalis, human immunodeficiency virus, treponema pallidum, hepatitis B. Male patient with clinical signs of genital tract inflammation, varicocele, leukocytospermia, or autoimmune diseases were also excluded in the study.

2.2. Stimulation protocol

Female patients included in the study were treated with a controlled ovarian hyperstimulation protocol for IVF with a GnRH antagonist. All patients received recombinant FSH or highly purified hMG. When at least 3 follicles reached pre-ovulatory size (17–18 mm), 10,000 IU of hCG was administered. Transvaginal oocyte retrieval was performed ~36 h later. Just after oocyte pick-up, 0.5 mL fresh undiluted SP was injected into the vaginal vault of each patient. All embryos were allowed to cleave and the best two embryos were transferred into the uterus on the third or fifth day after oocyte collection. Micronized progesterone was used for luteal support. Patients had unprotected sexual relations until the 3–5th day prior the Day-OPU. Just after oocyte pick-up, 0.5 mL fresh undiluted SP was injected into the vaginal vault of each patient. The couples were then advised not to have intercourse until the pregnancy results were known. Clinical pregnancy was defined as a visible intrauterine pregnancy sac, as estimated by ultrasound at 3 weeks gestational age. Live birth was defined as any deliveries in which at least one baby was born alive and survived for more than 1 month.

2.3. Specimen collection and preparation

Semen samples were collected at the day of ovum pick-up by masturbation following a 3–4 day period of sexual abstinence. Each ejaculate was collected into sterile containers for conventional semen analysis, according to the World Health Organization (WHO) protocol (WHO, 2010). DNA flow cytometry was used to distinguish leukocytes from immature sperm present in the ejaculate (Hacker-Klom et al., 1999). Patients with leukocytospermia, defined according to the WHO as the presence of $\geq 1 \times 10^6$ white blood cells/mL (WHO, 2010), were excluded from the study. The direct mixed agglutination reaction (MAR) test was performed as described previously (Comhaire et al., 1988) using a SpermMar Test (FertiPro, Beernem, Belgium). Only specimens with a MAR percentage ≤10% were used. After centrifugation, 0.5 mL SP was used for the intravaginal application. The remaining SP was aliquoted and stored at −80 °C.

2.4. Measurements of TGF-β1 and IL-18 in seminal plasma

Quantifications of total TGF-β1 (active plus latent) as well as IL-18 were performed using a FlowCytomix™ Human TGF-beta-1 and IL-18 Simplex Kits (Bender MedSystems, Vienna, Austria). Acidic activation of the samples at the time of TGF-β1 testing was performed according to the manufacturers' instructions. Seminal plasma was tested at 1:120 and 1:4 dilutions for TGF-β1 and IL-18 analysis, respectively. All samples were analyzed by FACSCalibur cytometer (Becton Dickinson, San Jose, CA, USA). The concentrations of TGF-β1 (the sum of free TGF-β1 and TGF-β1 released from latent complexes at acidification, ng/mL) and IL-18 (pg/mL) were calculated using FlowCytomix Pro 3.0 Software (Bender MedSystems). The ratio of TGF-β1-to-IL-18 concentration (TGF-β1/IL-18), the content of TGF-β1 and IL-18 in the intravaginally applied SP (calculated as the concentration of TGF-β1 or IL-18 × 0.5 mL), and the TGF-β1 and IL-18 content per ejaculate (calculated as the con-