Journal of Reproductive Immunology 103 (2014) 53–58

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

What is the impact of Th1/Th2 ratio, SOCS3, IL17, and IL35 levels in unexplained infertility?

Zehra Sema Ozkan[a,*], Derya Deveci[c], Banu Kumbak[b], Mehmet Simsek[a], Fulya Ilhan[d], Samet Sekercioglu[d], Ekrem Sapmaz[a]

[a] Firat University Hospital, In Vitro Fertilization Unit, Elazig, Turkey
[b] Medipol University Hospital, In Vitro Fertilization Unit, Istanbul, Turkey
[c] Firat University School of Medical Sciences, Elazig, Turkey
[d] Firat University Hospital, Department of Immunology, Elazig, Turkey

ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form
10 November 2013
Accepted 14 November 2013

Keywords:
Infertility
Implantation
IL17
IL35
SOCS3
T helper

ABSTRACT

Implantation necessitates complex interactions among the developing embryo, decidualizing endometrium, and developing maternal immune tolerance and/or alterations in cellular and humoral immune responses. Overstimulation of T helper 1 (Th1) or Th2 cytokines in systemic and local environments, alterations of the prevalence of IL17 and regulatory T cell (Treg) cytokines have also been suggested to contribute to the pathogenesis of implantation failure. We aimed to investigate the plasma levels of IL4, IL6, IL10, TNFα, IFNγ, TGFβ, IL17, IL35, and SOCS3 in infertile and fertile women. This case–control study was conducted with 80 women suffering from unexplained infertility and 40 fertile women. Peripheral venous blood samples were drawn on day 21 of the menstrual cycle. The extracted plasma samples were assayed by an enzyme linked immunosorbent assay. Statistical analysis was performed using SPSS version 16.0. Our main findings were as follows: despite the significantly high IL17 and IL35 plasma levels of infertile women, IL35/IL17 ratio was significantly lower in the infertile group compared with that in the fertile group; SOCS3 plasma levels showed an inverse relation with plasma levels of all cytokines except IL35; increased plasma IL17 levels (>3.42 pg/mL) have a negative impact on fertility; TNFα/IL10, IFNγ/IL10, IFNγ/IL6, and IFNγ/IL4 ratios were significantly higher in infertile group compared with those in the fertile group. It is not possible to show the major immunological factor(s) of unexplained infertility, but our findings point out that the decreased suppressor activity of the immune system may play a role in implantation failure.

1. Introduction

In practice, less than 5% of oocytes were collected and only 20–25% of embryos transferred lead to a live birth (Patrizio and Sakkas, 2009). Implantation of the embryo, which is crucial for successful reproduction, takes place in a receptive endometrium. In humans, the endometrium becomes receptive during the mid-secretory phase, between days 19 and 23 of the menstrual cycle (Dunn et al., 2003). Implantation necessitates complex interactions among the developing embryo, decidualizing endometrium, and developing maternal immune tolerance and/or alterations in cellular and humoral immune responses (Guzeloglu-Kayisli et al., 2009; Mor et al., 2011). This immune response is characterized by increased production of endometrial cytokines, prostaglandins, and leukocytes (Kelly et al., 2001).

During implantation, trophoblast cells break through the epithelial and stromal cells. The endometrial tissue is

* Corresponding author at: Firat University Hospital, In Vitro Fertilization Unit, 23119 Elazig, Turkey. Tel.: +90 5053983219.
E-mail address: zehrasema@yahoo.com (Z.S. Ozkan).

0165-0378/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jri.2013.11.002

then repaired and reorganized by the growing placenta. This local 'wound healing-like' process is characterized by a strong T helper1 (Th1), pro-inflammatory response in which high levels of pro-inflammatory cytokines such as interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα) are involved (Kelly et al., 2001; Dominguez et al., 2005; van Mourik et al., 2009). Among their other functions, these cytokines assemble immune cells to the decidua. Infiltration of large populations of decidual leukocytes to the implantation site has been observed. Of these cells, 65–70% are uterine-specific natural killer (uNK) cells and 10–20% are antigen-presenting cells (APC) such as macrophages and dendritic cells (Dcs) (Kämmerer, 2005; Hanna et al., 2006). Macrophages and DCs secrete anti-inflammatory cytokines (IL4, IL10) that are involved in tissue remodeling and angiogenesis (David Dong et al., 2009). DCs in the decidua could regulate the Th1/Th2 balance to maintain a Th2-dominant (anti-inflammatory) environment, leading to maintenance of pregnancy (Miyazaki et al., 2003; Lee et al., 2011).

Th17 cells are directly involved in chronic inflammatory processes by secreting IL17, a pro-inflammatory cytokine (Kolls and Lindén, 2004; Quan et al., 2012). Transforming growth factor beta (TGFβ) has been suggested as a crucial cytokine for Th17 cell development, in conjunction with IL6 and interferon gamma (IFNγ) (O'Garra et al., 2008; Manel et al., 2008; Lee et al., 2013). IL35 is a recently discovered immunoregulatory cytokine that is secreted by regulatory T cells (Tregs) and acts by inhibiting T cell proliferation (Collison et al., 2007). First-trimester human trophoblast cells express and secrete IL35, which may contribute to the suppression of maternal immune cells (Mao et al., 2013). TNFα and IFNγ may stimulate IL35 expression and IL35 may suppress Th17 development (Ye et al., 2013; Long et al., 2013).

Signal transducers and activators of transcription (STATs) are a family of cytoplasmic proteins that act as signal messengers and transcription factors and participate in normal cellular responses to cytokines and growth factors (Abell and Watson, 2005). Suppressor of cytokine signaling 3 (SOCS3) negatively regulates STAT3 activation in response to several cytokines (Carow et al., 2013). The primary function of SOCS3 is to regulate cytokine production.

Overstimulation of Th1 (IL2, IFNγ, TNFα) or Th2 (IL4, IL5, IL6, IL10) cytokines in systemic and local environments, alterations of the prevalence of Th17, and Tregs have also been suggested to contribute to the pathogenesis of implantation failure (Challis et al., 2009). In this study, we aimed to investigate the plasma levels of IL4, IL6, IL10, TNFα, IFNγ, TGFβ, IL17, IL35, and SOCS3 in infertile and fertile women.

2. Materials and methods

2.1. Study subjects

This case–control study was conducted with study subjects and controls at the In Vitro Fertilization (IVF) Unit of Firat University Hospital, Elazig, Turkey during October 2011 and October 2012 after approval of the local ethics committee. The study group comprised 80 infertile women planning to undergo controlled ovarian hyperstimulation–intracytoplasmic sperm injection (COH–ICSI) treatment due to unexplained infertility. The control group comprised 40 fertile women. Inclusion criteria for the study group were as follows: 21–38 years old, primary infertility (no live birth) or secondary infertility (live birth at least 2 years before), regular menstrual cycle (24–35 days), body mass index (BMI) <30 kg/m^2, FSH < 10 mIU/mL, E2 < 50 pg/mL on day 3 of the menstrual cycle (D3). Pituitary down-regulation was carried out with gonadotropin-releasing hormone (GnRH) agonist according to the long luteal protocol.

Inclusion criteria for the control group were as follows: 21–38 years old, 1 live birth less than 2 years before, regular menstrual cycle (24–35 days), BMI < 30 kg/m^2, FSH < 10 mIU/mL, E2 < 50 pg/mL on D3. Women with endometriosis, tubal factor infertility, ovulatory dysfunction, anatomical uterine pathological conditions, male factor infertility, and women with previous implantation failure or recurrent spontaneous abortion history were excluded. Informed and signed consent was obtained at the time of blood sampling.

2.2. Blood sampling

Peripheral venous blood samples were drawn on day 21 (D21) of the menstrual cycle, and the samples were centrifuged at 2500 rpm at 4 °C for 15 min and stored at −20 °C until analysis. In the study group, on the same day GnRH agonist administration was started and COH–ICSI treatment began on D3 of the following menstrual cycle. The plasma samples extracted were assayed by an enzyme linked immunosorbent assay (ELISA) using commercially available kits for IL17, IFNγ, TNFα, TGFβ, IL6, IL4, IL10 (Boster Biological, Fremont, CA, USA) and SOCS3 and IL35 (USCN Wuhan/China) according to the manufacturer's instructions. The samples were analyzed by the same staff under the same laboratory conditions. Within and between assay variations were less than 6% and 8% for all ELISA assays respectively.

2.3. Ovarian hyperstimulation protocol

After pituitary down-regulation with GnRH agonist, ovarian stimulation was started on day 3 with recombinant FSH. Between 300 and 375 IU of gonadotropins were administered from stimulation day 1 until the day of the human chorionic gonadotropin (hCG) trigger. hCG was administered when at least three follicles reached 17 mm in diameter. Estradiol level and endometrial thickness were both measured on the day of the hCG trigger. Retrieval of the oocytes was performed 35–36 h after the hCG trigger. Embryo transfer was performed on day 3 or 5 after retrieval. Twelve days after embryo transfer, pregnancy was tested with blood beta-hCG analysis. If the result was positive, the beta-hCG test was repeated 48 h later. If a regular increment was observed, transvaginal ultrasound examination was carried out for detecting fetal cardiac activity ten days later.