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Serum and follicular fluid levels of soluble Fas and soluble Fas ligand in IVF cycles

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Abstract

Objective: To determine follicular fluid (FF) and serum levels of soluble Fas (sFas) and soluble Fas ligand (sFasL) in patients undergoing IVF cycles.
Study Design: A prospective comparative study among patients with endometriosis ($n = 12$), infertility due to male factor ($n = 12$) and poor responders ($n = 32$) undergoing IVF cycles in Centrum IVF Clinic. Individual FF and serum samples were collected from patients during transvaginal ultrasonography-guided follicle aspiration. Patients were classified as poor responder patients undergoing IVF cycles with GnRHa, triptorelin and GnRH antagonist, cetrotide, patients with endometriosis and patients with infertility due to male factor. sFas, sFasL levels in both FF and serum samples and their correlations with clinical outcomes of IVF were measured in each study group.
Results: Serum and FF levels of sFas, sFasL were similar in the poor responder and male factor groups. There were no differences between the serum and FF levels of both sFas and sFasL among poor responder patients receiving either GnRH agonist or antagonist therapies. Serum levels of sFas were significantly lower in the endometriosis group compared to the male factor group. Serum and FF levels of sFas, sFasL were similar among patients with or without clinical pregnancy.
Conclusion: sFas and sFasL are detected in both serum and follicular fluid samples from IVF cycles, their levels are similar between poor responder and male factor groups as well as between GnRH agonist and antagonist treatment groups. These soluble apoptotic factors may not be predictive for the outcomes of IVF. Decreased serum levels of sFas, suggests increased apoptosis in endometriosis.

Keywords: sFas; sFasL; Follicular fluid; Serum; IVF; Poor responder

1. Introduction

Programmed cell death or apoptosis is the co-ordinated collapse of cell with protein degradation, DNA fragmentation, followed by rapid engulfment through neighboring cells. It is an essential component of the human reproductive system including endometrial, ovarian function and development [1,2]. Apoptosis is also suggested as a regulator of maternal immune-tolerance for the implanting of the semi-allograft embryo during pregnancy and similarly, as part of the immune-tolerance predisposing the survival of ectopic implants in endometriosis [3–5].

In normal physiological cellular turnover, cytokines or death factors, such as Fas ligand (FasL), initiate the apoptotic stimuli. Fas and FasL are proteins that exist in both transmembrane and soluble forms. Transmembrane Fas triggers apoptosis when bound by FasL, whereas soluble Fas (sFas) inhibits Fas-mediated apoptosis by preventing death signal transduction. Hence, Fas-mediated apoptosis is a

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result of receptor–ligand interactions whereas sFas acts as a functional antagonist of FasL-mediated apoptosis [6].

Apoptotic and proliferative factors recently detected in both follicular fluid (FF) and serum samples of women during in vitro fertilization (IVF) cycles suggest that the rate of apoptosis might be one of the regulatory mechanisms for oocyte maturation and survival [7]. sFas concentrations in FF from individuals undergoing IVF cycles are higher in follicles containing mature oocytes indicating a lower apoptotic rate. Similarly, follicles with immature oocytes contain higher levels of sFas than follicles with atretic oocytes [7].

Most of the current protocols of controlled ovarian hyperstimulation for IVF combine gonadotropin-releasing hormone agonist (GnRHa) and follicle stimulating hormone (FSH) regimens. These hormones are suggested to regulate granulosa cell apoptosis in opposite directions with GnRHa as an inducer and FSH as an inhibitor of apoptosis [8–10]. Recently, GnRH antagonists have been employed for selected patients in current protocols of IVF as an alternative to GnRH agonists [11,12]. They are effective in the prevention of premature LH surges with significantly shortened duration of treatment compared to those with GnRH agonists [13–15].

To our knowledge there is limited data in literature about the levels of soluble apoptotic factors in IVF cycles and whether they have any prognostic value on the clinical outcome. We hypothesized that serum and FF levels sFas and also sFasL may differ among different groups of patients undergoing IVF. We compared the serum and FF levels of sFas and sFasL of poor responders and patients with endometriosis with those of the patients with infertility due to male factor. We investigated whether any correlation existed between the levels of these apoptotic factors and the clinical outcomes of IVF patients. We also measured both FF and serum levels of sFas and sFasL in poor responder patients undergoing IVF cycles with GnRHa, triptorelin and GnRH antagonist, cetrotide in order to compare the effect of these two regimens on soluble apoptotic factors and clinical outcomes.

2. Materials and methods

The study was performed with patients attending Centrum IVF Clinic between April 2003 and May 2004. Informed consent in writing was obtained from each patient; consent forms and protocols were approved by the Local Ethic Committee. Individual FF and serum samples were collected from 56 women undergoing IVF treatments. Participants were consecutive patients fitting in the diagnostic categories of poor responders, endometriosis and infertility due to male factor. Thirty-two women were poor responders, described as $\leq$3 recruited follicles or collected oocytes and basal (on day 3 of the menstrual cycle) serum levels of FSH $\leq$ 12 IU/ml [16]. Twelve women had stage 1–2 endometriosis diagnosed by laparoscopy and 12 had infertility due to male factor which was described as $<$500,000 spermatozoa with progressive motility and $>$90% abnormal spermatozoa.

2.1. IVF-ICSI stimulation procedure

All poor responder patients, underwent controlled ovarian hyperstimulation (COH) with either a combination of GnRHa and r-FSH, using a long protocol (n = 16) or with a combination of GnRH antagonist and r-FSH (n = 16) for IVF-ICSI treatment. Long protocol with GnRHa was used for patients with endometriosis and those with infertility due to male factor. During the standard long protocol of pituitary suppression, GnRHa (Decapeptyl 0.1 mg, Ferring, Germany) was administered s.c. from day 21 of the previous cycle followed by recombinant FSH (Gonal F, Serono, Turkey) for ovarian stimulation. The standard initial dose of gonadotropin was 2–4 ampoules of r-FSH (150–300 IU) per day and doses were adjusted based on individual responses. In both groups, with ultrasound examinations and measurement of estradiol (E_2) levels were initiated on day 6 of the stimulation cycle and patients were monitored every 1–2 days. HCG (10,000 IU, Pregnyl, Organon, Turkey) was administered when a minimum of three leading follicles $\geq$18 mm mean diameter and serum levels of $E_2 < 5500$ pg/ml were detected. Oocyte retrieval was performed by vaginal ultrasound-guided follicular puncture, 35–36 h following HCG administration. A maximum number of three embryos were transferred on the 3rd day after a selective assisted hatching procedure with laser. Pregnancy was confirmed when serum HCG concentrations were rising on at least two separate occasions between the 12th and 14th days after the embryo transfer. Clinical pregnancy was diagnosed by ultrasonography during the 7th week of pregnancy.

2.2. Serum and follicular fluid collection and measurement of sFas, sFasL levels

Blood samples were drawn just before follicle aspiration. FF aspirates from all mature follicles ($\geq$14 mm diameter) were collected at the time of transvaginal ultrasound-guided oocyte retrieval and immediately centrifuged at 4 °C. The cell-free supernatants were aliquoted and stored at −80 °C until assay. As a reflection of follicular asynchrony during ovarian stimulation for IVF, wide interfollicular variations in steroid and cytokine concentrations have been reported [17,18]. Therefore, we collected pooled FF aspirations from each patient in an attempt to assess whole ovarian production as previously recommended, rather than evaluate each follicle separately [19–21].

Follicular fluid and serum levels of sFas and sFasL were analyzed by a sandwich ELISA (human sFasL and sFas, Biosource International, California, USA). Sensitivities, intra-assay and interassay coefficients of variation were