Original Article
The assesment of follicular fluid presepsin levels in poor ovarian responder womenandits relationship with the reproductive outcomes

Ali Ovayolu[1], Özkan Özdamar[3], İsmet Gün[1], Cansev Y Arslanbuğa[2], Tayfun Kutlu[1], Gülden Tunalı[1], Ramazan Uluhan[2]

Departments of [1]Obstetrics and Gynecology, [2]Clinical Microbiology, Zeynep Kamil Training and Educational Hospital, Istanbul, Turkey; [3]Department of Obstetrics and Gynecology, Gölcük Military Hospital, Kocaeli, Turkey

Abstract: A considerable proportion of all women undergoing IVFrespond poorly to gonadotropin stimulation. These women are reported to be associated with increased cancellation rates and lower pregnancy rates. It has been hypothesized that poor response to ovarian stimulation is a first sign of ovarian ageing or premature ovarian failure, which might be related to altered inflammatory response in the body. We aimed to compare follicular fluid presepsin levels between poor- and normo-responder patients to ovarian stimulation, to assess its relationship with reproductive outcomes. This study included infertility patients who underwent ovulation induction with either long GnRH agonist or GnRH antagonist protocols and who subsequently underwent IVF/ICSI. Included patients were assigned to two groups according to the Bologna criteria for poor ovarian response. Group 1 and 2 consisted of normo- and poor-responder patients, respectively.The 2 groups were compared in terms of FF presepsin levels. Also, any relationship between the FF presepsin levels and fertility outcomes was assessed within the groups. The groups were compared by using student's t-test, Mann-Whitney U test and X^2 test, where appropriate. Pregnancy rates were not significantly different between the groups (22.6% and 17.6%; P=0.650, respectively). FF presepsin levels were higher in Group 1, however, the difference was not statistically significant (298.0±797.4 and 149.2±422.3; P=0.190, respectively). FF presepsin levels did not significantly differ between pregnancy positive and the pregnancy negative patients in both Group 1 (243.6±531.1 and 314.3±866.5; P=0.055, respectively) and Group 2 (112.2±79.8 and 157.1±464.3; P=0.394, respectively). Consequently, FF presepsin seems not to be a reliable marker in predicting pregnancy in both normo-responder and poor-responder infertility groups.

Keywords: Presepsin, infertility, poor responder, follicular fluid

Introduction

Since the first successful live birth conceived by in vitro fertilization (IVF) in 1978, significant advances have been achieved in the assisted reproduction techniques (ART), aimed to improve clinical outcomes from IVF. However, success rates are still modest, with clinical pregnancy rates still at around 33% [1]. A considerable proportion of women undergoing ART, respond poorly to the usual gonadotropin stimulation, who are also known as poor ovarian responders, and these patients constitute 9-24% of all women undergoing ovarian stimulation for IVF [2]. Low ovarian response was first described in patients with peak estradiol (E2) levels <300 pg/ml and decreased follicular

response, expressed as fewer retrieved and fertilized oocytes and hereby fewer transferred embryos [3]. Now that there has been no universally accepted consensus to define patients who respond poorly to ovarian stimulation, the European Society of Human Reproduction and Embryology (ESHRE) working group on Poor Ovarian Response Definition recently developed new criteria for the definition of poor ovarian response, the so-called 'Bologna criteria' [4]. These criteria incorporate age, ovarian reserve tests such as anti-müllerian hormone (AMH) levels or antral follicle count (AFC) and ovarian response in previous IVF/ICSI cycles. On the other hand, it has been hypothesized that poor response to ovarian stimulation is a first sign of ovarian ageing or premature ovarian

failure [5, 6], which might be related to altered inflammatory response in the body.

Numerous physiological reproductive phenomena including folliculogenesis, ovulation and implantation of the embryo and a set of processes employed during the ART, including controlled ovarian hyperstimulation (COH), oocyte pick-up (OPU) and embryo transfer (ET), are known to involve complex inflammatory processes. However, the point beyond which physiological inflammation convert to pathological and where the reproductive outcomes are compromised still remains unclear. Recent reports suggest that uncontrolled inflammation affects both IVF results and the ovarian reserve adversely [7]. Hence, researchers have studied on the inflammation markers to establish a realistic and reasonable relationship between these markers and IVF success. C-reactive protein (CRP), procalcitonin (PCT) and lipopolysaccharid-binding protein (LBP) are substantial markers, biological fluid levels of which provide useful information about the inflammatory response. Presepsin is a novel, 13 kDa molecular weight biomarker for diagnosing systemic inflammation and sepsis. CD14 (cluster of differentiation), the receptor for lipopolysaccharide/lipopolysaccharide binding protein (LPS/LBP) complexes, are expressed on the cell membranes of macrophage, monocyte and granulocyte cells [8]. The LPS/LBP-CD14 complex is released into circulation by shedding of CD14 from the cell membrane, yielding soluble-CD14 (s-CD14), which is activated by the plasma proteases, generating sCD14-subtype, Presepsin [9, 10]. Collected data provide evidence that presepin may serve as an acute phase reactant, analogous to CRP [11]. While the biological role of presepsin has not been completely elucidated, it is considered to be a regulatory factor capable of modulating cellular and humoral immune responses by interacting directly with T and B cells [12]. Pathfast assay method appears to be an adequate technique for the determination of presepsin levels in the biological fluids and its sensitivity has been demonstrated to be 100% in the presence of infection [13]. Even though the clinical significance of acute phase reactant, CRP, in the infertility population has been evaluated by some previous published reports [14-18], there is no publication assessing the significance of presepsinlevels in women with infertility, available in the literature. Moreover, in the current

ART practice, an accurate test to predict ovarian response, which would have both increased the efficacy and reduced the costs of ART, is still lacking and there is a need for data on the predictability of ovarian response in COS.

In the present study, we aimed to compare follicular fluid presepsin levels between poor- and normo-responder patients to ovarian stimulation, to assess its relationship with reproductive outcomes and hereby to identify whether presepsin can be used as a predictor of ovarian response.

Material and methods

The present is a prospective study conducted with the patients who admitted to the Assisted Reproduction Department of Zeynep Kamil Training and Educational Hospital due to the desire of having a child between June 2013 and December 2013. Approval of the ethics committee was obtained prior to the initiation of the study.

This study included infertility patients who underwent ovulation induction with either standard long GnRHagonist or fixed GnRH antagonist protocols and who subsequently underwent IVF/ICSI. Eligibility criteria were as follows; body mass index (BMI) 18-28 kg/m^2, no uterine (fibroids, adenomyosis, mullerian malformations), ovarian (endometrioma, polycystic ovaries), or adnexal (hydrosalpinx) abnormalities detected by transvaginal ultrasonograghy and/or hysteroscopy and/or laparoscopy and normal semen analyses according to the World Health Organization criteria for normality [19].

Patients were assigned to two groups according to the Bologna criteria for poor ovarian response [4]. Group 1 consisted of normo-responder and Group 2 consisted of poor-responder patients. Two out of three of the following criteria were essential in order to classify a patient as poor ovarian responder: (i) advanced maternal age (≥40 years) or any other risk factor for poor ovarian response; (ii) a previous poor ovarian response (≤3 oocytes with a conventional stimulation protocol); or (iii) an abnormal ovarian reserve test (AFC <5-7follicles or AMH <0.5-1.1 ng/ml).

The treatment protocol choices and the gonadotropin dose adjustments were individualized according to age, body mass index (BMI), ovari-

Table 1. Demographic, stimulation and treatment outcomes characteristics and FF presepsin values of the groups

Characteristics	Group 1 (n=53)	Group 2 (n=68)	P
Age, y	32.3±7.7	33.4±4.5	0.351[a]
GnRH agonist/antagonist protocol, n	24/29	22/46	0.206[b]
Induction duration, d	8.8±1.7	8.5±1.5	0.317[a]
Average used gonadotropin dose, IU	2427.6±1097.8	3558.8±662.2	<0.0001[a]
FF presepsin values, pg/mL	298.0±797.4	149.2±422.3	0.190[a]
MII oocytes, n	6.2±3.7	3.5±2.3	<0.0001[a]
Pregnancy test positivity ratio, n (%)	12/53 (22.6%)	12/68 (17.6%)	0.650[b]

Data are presented as mean±SD except for GnRH agonist/antagonist protocol and pregnancy test positivity ratios. [a]Student t-test. [b]X^2 test.

an reserve determined by antral follicle count and basal FSH, and experience from previous cycles. Gonadotropin stimulation was achieved by using either rFSH or hMG. All patients were administered acetyl-salicilic acid 100 mg daily and folic acid 400 mcg daily simultaneously with the initiation of the protocol.

Serial ultrasonographic controls and E2 level measurements were made until 3 follicles ≥17 mm and a serum E2 level >500 pg/ml were detected. Choriogonadotropin-alpha 250 µg - (Ovitrelle®; Merck Serono, Turkey) was administered subcutaneously to induce final follicular maturation. Transvaginal ultrasound-guided oocyte retrieval was performed 35-36 hours after hCG administration. All patients were implemented a single dose of cefazolin sodium (Sefazol, Mustafa Nevzat Ilaç San, Turkey) 1 gr, intramuscularly (im.) at the course of OPU procedure and were given doxycycline 100 mg capsule (Tetradox capsule, Fako Ilaç, Turkey) per oral (p.o.) twice daily and methylprednisolone 16 mg capsule (Prednol tablet, Mustafa Nevzat Ilaç San, Turkey) p.o. once daily and continued for 4 days. Luteal support was initiated on the night of oocyte retrieval and continued until the day of pregnancy testing. If the test was positive, progesterone treatment was continued up to 9th gestational weeks. Fertilization was assessed at 16-18 h after ICSI and one or two embryos with the best morphological grade were transferred into the uterine cavity under ultrasound guidance (General Electric, GE, Logiq 200 Alpha Ultrasound, FL, USA). Serum pregnancy test was performed 12 days after the embryo transfer. hCG levels >20 mIU/mL was considered as a positive pregnancy test.

All patients were informed about OPU prior to the procedure and informed consent was obtained. During oocyte retrieval, the FF was collected from the first penetrated mature (>17 mm) follicle at the first entry without contamination. FFs contaminated with blood or that do not contain oocyte were not used. Aspirated FFs were centrifuged for 10 minutes at 2000× g at room temperature and separated supernatant fluids were transferred into the eppendorf tubes to be stored at -80°C until performing the assay. On the assaying day, with in 4 hours after the samples were thawed, presepsin levels were measured by using the PATHFAST Presepsin assay (Mitsubishi Medience, Tokyo, Japan). The 2 groups were compared in terms of demographic and induction characteristics, treatment outcomes and FF presepsin levels. Additionally, any relationship between the FF presepsin levels and fertility outcomes was assessed within the both groups.

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 15.0 software (SPSS, Chicago, IL, USA). Descriptive statistics were given as mean and standard deviation. Parametric comparisons were performed using Student's t-test, and non-parametric comparisons were performed using Mann-Whitney U test. Categorical data were evaluated by using

X^2 test. Statistical significance was defined as P<0.05.

Results

Between June and December 2013, 121 couples with infertility were included in the study

among 560 evaluated patients. Group 1 and 2 consisted of 53 and 68 patients, respectively. Demographic and induction characteristics, treatment outcomes and FF presepsin levels are presented in **Table 1**. Age, distribution of stimulation protocols and induction duration did not significantly differ between the groups. Since Group 2 consisted of women who responded poorly to the ovarian hyperstimulation, mean gonadotropin doses were significantly higher in Group 2 than in Group 1 (2427.6±1097.8 vs. 3558.8±662.2; P<0.0001). Although the number of MII oocytes was significantly lower in Group 2 than in Group 1 (6.2±3.7 and 3.5±2.3; P<0.0001, respectively), pregnancy rates were not significantly different between the groups (22.6% and 17.6%; P=0.650, respectively). FF presepsin levels were higher in Group 1 patients, however, the difference was not statistically significant (298.0±797.4 and 149.2±422.3; P=0.190, respectively). Besides, both groups were further divided into two subgroups as, patients who conceived and those who did not. FF presepsin levels did not significantly differ between pregnancy positive and the pregnancy negative patients in both Group 1 (243.6±531.1 and 314.3±866.5; P=0.055, respectively) and Group 2 (112.2±79.8 and 157.1±464.3; P=0.394, respectively).

Discussion

An optimal response to controlled ovarian stimulation is of crucial importance in ART. It has been accepted that both too low and too high ovarian responses are associated with increased cancellation rates and lower pregnancy rates [20]. Although numerous biochemical and sonographic tests have been suggested to predict ovarian response, in the current practice, there is no accurate and highly predictive test to assess ovarian response and no screening test available to detect poor ovarian response. Hence; the diagnosis of poor responder is revealed only during ovulation induction [21]. Antral follicle count (AFC) and basal FSH levels have been proposed to have had best sensitivity and specificity for predicting low ovarian response [22]. Recently, anti-Müllerian hormone (AMH) has been shown to have at least the same level of accuracy and clinical value for the prediction of poor response as AFC [23].

It is well recognized that inflammation is a hallmark of many processes in reproductive physiology, including ovulation, menstruation, and implantation [24]. However, uncontrolled inflammation might negatively affect hormone production, ovulation [25], and fertility [26] by deteriorating ovarian reserve and ovarian response [27, 28]. In many previous studies, the decline in the ovarian reserve was reported to accelerate in the inflammatory states, such as diabetes mellitus [29], Behçet's Disease [30], Takayasu arteritis [31] and myotonic dystrophy [32]. Additionally, endometriosis, a systemic chronic inflammatory condition, is associated with a poor ovarian response to gonadotropin stimulation and with lower pregnancy rates [27, 33]. Even if inflammatory conditions are known to be related to poor IVF outcomes and improving IVF outcome in poor responder population represents a main priority, there is a limited number of studies available in the literature assessing the efficacy of the inflammatory markers in the poor responder patients. Uri-Belapolsky et al. [7] investigated the possible involvement of inflammatory pathways and the role of IL-1 in the ovarian ageing and exhaustion of ovarian reserve in an animal model and concluded that IL-1 was an important participant in the age-related exhaustion of ovarian reserve. Lee et al. [34] demonstrated that elevated levels of FF TNF-α were correlated with poor oocyte quality. Winger et al. [35] concluded that inhibition of TNF-α improves IVF outcomes in infertile women. The soluble receptor of advanced glycation end-products (sRAGE) in the FF has been proposed as a marker of ovarian reserve and diminished ovarian response [36]. In the present study we investigated the predictive value of presepsin, a novel inflammation marker, in women who respond poorly to ovarian hyper-stimulation; however, FF presepsin levels did not significantly differ between normo-responder and poor-responder group. Further, FF presepsin levels were not significantly different between patients who conceived and those who did not, following IVF, in both poor- and normo-responder patients. Nevertheless, more comprehensive studies assessing any possible relationship between low ovarian response and inflammatory markers are needed.

In conclusion, FF presepsin levels were not statistically different between the normo-respond-

er and poor-responder infertility patients. Additionally, achieving pregnancy did not make any significant difference in the both groups. Consequently, FF presepsin seems not to be a reliable marker in predicting pregnancy in both normo-responder and poor-responder infertility groups.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Özkan Özdamar, Gölcük Military Hospital, Department of Obstetrics and Gynecology, 41650, Gölcük, Kocaeli, Turkey.Tel: +90532 4766744; Fax: +90 262 414 11 45; E-mail: ozkan_ozdamar35@hotmail.com

References

[1] Ferraretti AP, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA, Erb K, Korsak V, Nyboe Andersen A; European IVF-Monitoring (EIM) Consortium for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. Hum Reprod 2013; 28: 2318-2331.

[2] Ubaldi FM, Rienzi L, Ferrero S, Baroni E, Sapienza F, Cobellis L, Greco E. Management of poor responders in IVF. Reprod Biomed Online 2005; 10: 235-246.

[3] Garcia JE, Jones GS, Acosta AA, Wright G. HMG/hCG follicular maturation for oocytes aspiration: phase II, 1981. Fertil Steril 1983; 39: 174-179.

[4] Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011; 26: 1616-1624.

[5] Beckers NG, Macklon NS, Eijkemans MJ, Fauser BC. Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for in vitro fertilization exhibit follicular phase characteristics suggestive of ovarian aging. Fertil Steril 2002; 78: 291-297.

[6] Nikolaou D, Lavery S, Turner C, Margara R, Trew G. Is there a link between an extremely poor response to ovarian hyperstimulation and early ovarian failure? Hum Reprod 2002; 17: 1106-1111.

[7] Uri-Belapolsky S, Shaish A, Eliyahu E, Grossman H, Levi M, Chuderland D, Ninio-Many L, Hasky N, Shashar D, Almog T, Kandel-Kfir M, Harats D, Shalgi R, Kamari Y. Interleukin-1 deficiencyprolongsovarian lifespan in mice. Proc Natl Acad Sci U S A 2014; 111: 12492-12497.

[8] Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990; 249: 1431-1433.

[9] Mussap M, Noto A, Fravega M, Fanos V. Soluble CD14 subtype presepsin (sCD14-ST) and lipopolysaccharide binding protein (LBP) in neonatal sepsis: New clinical and analytical perspectives for two old biomarkers. J Matern Fetal Neonatal Med 2011; 24: 12-14.

[10] Yaegashi Y, Shirakawa K, Sato N, Suzuki Y, Kojika M, Imai S, Takahashi G, Miyata M, Furusako S, Endo S. Evaluation of a newly identified soluble CD14 subtype as a marker for sepsis. J Infect Chemother 2005; 11: 234-238.

[11] Aalto H, Takala A, Kautiainen H, Siitonen S, Repo H. Monocyte CD14 and soluble CD14 in predicting mortality of patients with severe community acquired infection. Scand J Infect Dis 2007; 39: 596-603.

[12] Rey Nores JE, Bensussan A, Vita N, Stelter F, Arias MA, Jones M, Lefort S, Borysiewicz LK, Ferrara P, Labéta MO. Soluble CD14 acts as a negative regulator of human T cell activation and function. Eur J Immunol 1999; 29: 265-276.

[13] Novelli G, Morabito V, Ferretti G, Pugliese F, Ruberto F, Venuta F, Poli L, Rossi M, Berloco PB. Pathfastpresepsin assay for early diagnosis of bacterial infections in surgical patients: preliminary study. Transplant Proc 2013; 45: 2750-2753.

[14] Levin I, Gamzu R, Mashiach R, Lessing JB, Amit A, Almog B. Higher C-Reactive protein levels during IVF stimulation are associated with ART failure. J Reprod Immunol 2007; 75: 141-144.

[15] Almagor M, Hazav A, Yaffe H. The levels of C-reactive protein in women treated by IVF. Hum Reprod 2004; 19: 104-6.

[16] Orvieto R, Chen R, Ashkenazi J, Ben-Haroush A, Bar J, Fisch B. C-Reactive protein levels in patients undergoing controlled ovarian hyperstimulation for IVF cycle. Hum Reprod 2004; 19: 357-359.

[17] Wunder DM, Kretschmer R, Bersinger NA. Concentrations of leptin and C-reactive protein in serum and follicular fluid during assisted reproductive cycles. Hum Reprod 2005; 20: 1266-1271.

[18] Seckin B, Ozaksit G, Batioglu S, Ozel M, Aydoğan M, Senturk B. The relationship between the change in serum high sensitivity C-reactive protein levels and IVF success. Gynecol Endocrinol 2012; 28: 418-421.

[19] World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. Geneva, Switzerland: WHO Press; 2010.

[20] Broekmans FJ, Verweij PJ, Eijkemans MJ, Mannaerts BM, Witjes H. Prognostic models for

high and low ovarian responses in controlled ovarian stimulation using a GnRH antagonist protocol. Hum Reprod 2014; 29: 1688-1697.

[21] Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 2003; 9: 61-76.

[22] Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006; 12: 685-718.

[23] Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril 2009; 91: 705-714.

[24] Jabbour HN. Inflammatory pathways in female reproductive health and disease. Reproduction 2009; 138: 903-919.

[25] Herath S, Williams EJ, Lilly ST, Gilbert RO, Dobson H, Bryant CE, Sheldon IM. Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. Reproduction 2007; 134: 683-693.

[26] Weiss G, Goldsmith LT, Taylor RN, Bellet D, Taylor HS. Inflammation in reproductive disorders. Reprod Sci 2009; 16: 216-229.

[27] Falconer H, Sundqvist J, Gemzell-Danielsson K, von Schoultz B, D'Hooghe TM, Fried G. IVF outcome in women with endometriosis in relation to tumour necrosis factor and anti-Müllerian hormone. Reprod Biomed Online 2009; 18: 582-588.

[28] Fréour T, Miossec C, Bach-Ngohou K, Dejoie T, Flamant M, Maillard O, Denis MG, Barriere P, Bruley des Varannes S, Bourreille A, Masson D. Ovarian reserve in young women of reproductive age with Crohn's disease. Inflamm Bowel Dis 2012; 18: 1515-1522.

[29] Artunc-Ulkumen B, Pala HG, Pala EE, Yavasoglu A, Yigitturk G, Erbas O. Exenatide improves ovarian and endometrial injury and preserves ovarian reserve in streptozocin induced diabetic rats. Gynecol Endocrinol 2014; 4: 1-6.

[30] Mont'Alverne AR, Yamakami LY, Gonçalves CR, Baracat EC, Bonfá E, Silva CA. Diminished ovarian reserve in Behçet's disease patients. Clin Rheumatol 2015; 34: 179-183.

[31] Mont'Alverne AR, Pereira RM, Yamakami LY, Viana VS, Baracat EC, Bonfá E, Silva CA. Reduced ovarian reserve in patients with takayasu arteritis. J Rheumatol 2014; 41: 2055-2059.

[32] Srebnik N, Margalioth EJ, Rabinowitz R, Varshaver I, Altarescu G, Renbaum P, Levi-Lahad E, Weintraub A, Eldar-Geva T. Ovarian reserve and PGD treatment outcome in women with myotonic dystrophy. Reprod Biomed Online 2014; 29: 94-101.

[33] González-Fernández R, Peña Ó, Hernández J, Martín-Vasallo P, Palumbo A, Ávila J. Patients with endometriosis and patients with poor ovarian reserve have abnormal follicle-stimulating hormone receptor signaling pathways. Fertil Steril 2011; 95: 2373-2378.

[34] Lee KS, Joo BS, Na YJ, Yoon MS, Choi OH, Kim WW. Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. J Assist Reprod Genet 2000; 17: 222-228.

[35] Winger EE, Reed JL, Ashoush S, Ahuja S, El-Toukhy T, Taranissi M. Treatment with adalimumab (Humira) and intravenous immunoglobulin improves pregnancy rates in women undergoing IVF. Am J Reprod Immunol 2009; 61: 113-120.

[36] Merhi Z, Irani M, Doswell AD, Ambroggio J. Follicular fluid soluble receptor for advanced glycation end-products (sRAGE): a potential indicator of ovarian reserve. J Clin Endocrinol Metab 2014; 99: E226-233.