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ORIGINAL ARTICLE

Follicular fluid PlGF/sFlt-1 ratio and soluble receptor for advanced glycation end–products correlate with ovarian sensitivity index in women undergoing A.R.T.

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Abstract
Purpose Considering potential roles of soluble receptor for advanced glycation end products (sRAGE) and placental growth factor (PlGF) in ovarian function and embryo implantation, in the present study we have evaluated the association of these factors and also PlGF/sFlt-1 ratio with the ovarian response and implantation rate by dividing patients according to the OSI.
Methods In a cross-sectional study, 90 infertile women who were undergoing ICSI cycle using long protocol were recruited. The patients were divided according to ovarian sensitivity index (OSI). ICSI cycle outcomes were evaluated for each patient and PlGF, sFlt-1 and sRAGE levels of follicular fluid were assayed using commercial ELISA kits.
Results Follicular fluid (FF) sRAGE levels and PlGF/sFlt-1 ratio were statistically greater in high-responder women than other responders ($p < 0.05$). Positive correlations were obtained between sRAGE level with the number of oocytes, follicles and OSI level. sRAGE levels with cutoff value of 4.83 (ng/ml) for evaluating the pregnancy outcome showed 81.8 % sensitivity and 60.7 % specificity. Furthermore, there were positive associations between PlGF/sFlt-1 ratio with the number of oocytes, embryos and OSI level.
Conclusion In conclusion, the results of current study supported that good ovarian response is independent of pregnancy outcome. Our results showed that FF levels of sRAGE and PlGF/sFlt-1 ratio could be used as markers for determining the high-responder women. Also, FF sRAGE levels could be a good predictor for ART outcome.

Keywords Ovarian response · Soluble receptor for advanced glycation end products · Placental growth factor · Embryo implantation · ICSI cycle

Abbreviations

OSI	Ovarian sensitivity index
PlGF	Placental growth factor
AMH	Anti-mullerian hormone
sFlt-1	Soluble fms-like tyrosine kinase-1
AGEs	Advanced glycation end products
RAGE	Receptor for advanced glycation end products
sRAGE	Soluble receptor for advanced glycation end-products

Introduction

In in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles, optimal ovarian responsiveness to gonadotropin leads to obtaining the sufficient number of oocytes and embryos and thus increases the chance of successful pregnancy [1]. On the other hand, excessive ovarian response accompanies the risk of ovarian hyperstimulation syndrome (OHSS). It has been demonstrated that OHSS patients have lower pregnancy rates compared to other cases [2]. Thus, appropriate ovarian response could be a

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vital factor for decreasing the risk of OHSS and enhancing IVF/ICSI outcomes by increasing the number of oocytes and embryos [2].

Ovarian sensitivity index (OSI) is an index which is calculated by dividing the number of retrieved oocytes on the total dose of exogenous FSH. It has been shown that OSI is a better measure for evaluating ovarian responsiveness to gonadotropin compared with the number of retrieved oocytes. Also, it can be useful index in studies with various ovarian stimulation doses [3]. Recently, Huber et al. [4] have presented cutoff for definition of poor, normal and high-responders and made it possible to study various factors among the groups.

Placental growth factor (PlGF) is the second member of vascular endothelial growth factor (VEGF) family which binds to flt-1 (VEGFR-1) and sFlt-1 (sVEGFR-1). It has been demonstrated that the PlGF knockout mice are healthy, fertile with no evidence of vascular impairment, although they indicate decreased ovarian angiogenesis [5]. PlGF levels in follicular fluid (FF) are higher than serum [6] and a positive correlation between follicular fluid PlGF levels and follicle size has been reported [7]. These evidences confirm the important roles of the PlGF in follicular angiogenesis and development. Santi et al. [8] have reported that the expression of PlGF in good endometrium with subsequent pregnancy was significantly higher than bad endometrium without success to pregnancy. PlGF level is correlated with anti-mullerian hormone (AMH) level which is known as an ovarian responsiveness marker [6].

The advanced glycation end products (AGEs) are produced from non-enzymatic modification of proteins and can alter structure and function of the proteins and thereby affect cellular actions. Receptor for advanced glycation end products (RAGE) as the receptor of these products, has transmembrane, cytosolic and extracellular domains and expresses in various tissues such as the ovary [9]. Soluble receptor for advanced glycation end-products (sRAGE) which has not cytosolic and the transmembrane domains is presented in the circulation. Since sRAGE could bind to AGEs and prevent harmful inflammatory pathways of the RAGE, it is considered as a good receptor [10]. High sRAGE concentration in FF compared to serum (more than fourfold), indicates importance of this factor in ovary function [11]. It has been documented that FF sRAGE positively correlates to the number of retrieved oocytes and AMH levels [12]. Also, high concentration of sRAGE has been reported in pregnant subjects in compared with non-pregnant women [11].

Considering potential roles of FF sRAGE and PlGF in ovarian function and embryo implantation, in the present study, we have evaluated the association of these factors and also PlGF/sFlt-1 ratio with the ovarian response and implantation rate by dividing patients according to the OSI. Also, we have introduced sRAGE levels of FF as a marker for ART outcome.

Materials and methods

Subjects

In a cross-sectional study, 90 women were selected among individuals who were referred to Al-Zahra Hospital of Tabriz, Iran. The agreement of the Ethical Committee of Tabriz University of Medical Sciences was obtained, and all recruited subjects gave written informed consent. The non-smoker 20–40 years-old women with fallopian tube obstruction, idiopathic infertility and male factor infertility (varicocele, oligospermia) enrolled in this study. The exclusion criteria were having history of PCOS, endometriosis, immune system and inflammatory diseases, endocrine disorders and also male infertility with severe oligospermia (concentrations <5 million sperm/mL) and azoospermia.

ICSI cycle outcome

All patients received the long GnRH agonist–recombinant FSH (rFSH) protocol with administration of exogenous gonadotropin (Gonal-F, Serono) and when at least 2–3 follicles with a diameter of 18 revealed, 10,000 IU intramuscular human chorionic gonadotropin (Choriomon, Lugano, Switzerland) was administered. Puncture and aspiration of follicles was carried out 36 h after hCG injection. Single follicular fluid aspiration without blood was done and after separation of oocytes, the remaining fluid was centrifuged and the supernatant was frozen at −80 °C until the assays. The number of follicles and oocytes were evaluated on the same day. Ovarian sensitivity index (OSI) that is defined as the number of retrieved oocytes × 1000 per the total dose of FSH, was calculated for all patients and according to the index enrolled in one of the poor, normal and high-responder groups. Patients with OSI value of <1.697, between 1.697 and 10.07 and >10.07, were considered as poor-responders, normo-responders and high-responders, respectively [4]. All patients underwent ICSI treatment and the number of fertilized oocytes counted 84 h after performing ICSI. Clinical pregnancy was assessed by presence of the intrauterine gestational sac via transvaginal ultrasound. Implantation rate was calculated by dividing the visible sacs number to number of transferred embryos. Furthermore, fertilization rates were obtained from the ratio of the number of fertilized oocytes per number of mature oocytes.

Follicular fluid parameters assay

FF PlGF, sFlt-1 and sRAGE levels were determined by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer instructions. The sensitivities for sRAGE, PlGF and sFlt-1 were 4.12, 7 and 3.5 pg/ml, respectively. Moreover, intraassay and interassay coefficients of variation (CV) were respectively 3.6–5.6 and 11 % for PlGF, 6.6 and 6.1 % for sRAGE, and also 2.6–3.8 and 7–9.8 % for sFlt-1.

Statistical analysis

Data are presented as mean ± SD. The normality of data was tested by Kolmogorov–Smirnov test. We used parametric tests for data with normal distribution and non-parametric tests for data with abnormal distribution. Comparisons between two pregnant and non-pregnant groups were performed using Mann–Whitney *U* or *T* tests, depending on the data distribution. Kruskal–Wallis or One way ANOVA with Tukey's post-hoc test were used to compare the data among OSI groups. In addition, correlations between study variables were investigated by Pearson and Spearman's correlation tests depending on the data distribution. Receiver operated characteristics (ROC) area under the curve (AUC) was done to assessment sensitivity and specificity. *p*-value <0.05 assumed statistically significant and all analysis run on SPSS 19.0 software.

Results

General characteristics of patients according to the OSI (Poor, normal and high-responders) are presented in Table 1. Our results demonstrated that the mean age of high-responders was significantly lower than normal and poor-responder groups. In addition, total dose of FSH was lower for this group when compared with other ones ($p < 0.05$). Although the numbers of oocytes, follicles and also embryos were higher in high-responders according to our results ($p < 0.05$), but we did not observe any significant differences in pregnancy and embryo implantation rates among individuals with various ovarian responsiveness ($p > 0.05$). Investigation of the clinical characteristics of pregnant and non-pregnant women indicated no significant differences between these groups (Table 2).

As shown in Table 3, OSI levels were significantly higher in women with age <35 years compared to those with age >35 years. Furthermore, other follicular fluid parameters showed no significant difference between the two age groups ($p > 0.05$).

As shown in Table 4, FF sRAGE levels were statistically greater in high-responder women than other responders ($p < 0.05$) but PlGF and sFlt-1 values did not show any significant differences between groups. We calculated PlGF bioavailability as a ratio of PlGF to sFlt-1 (PlGF/sFlt-1) because sFlt-1 reduces its free levels and prevents its cellular signaling. However, the PlGF/sFlt-1 ratio was significantly higher in high-responders compared to poor-responders ($p < 0.05$).

Table 1 Clinical characteristics of patients according to the ovarian sensitivity index (OSI)

Parameters	Poor responders ($n = 30$) OSI < 1.697	Normo responders ($n = 40$) OSI = 1.698–10.07	High responders ($n = 20$) OSI > 10.07
Age (years)	33.44 ± 7.36	31.62 ± 4.26	26.92 ± 3.37[b,c]
BMI (kg/m^2)	27.73 ± 4.12	27.34 ± 3.84	26.81 ± 2.67
Number of follicles	10.23 ± 13.07	11.72 ± 5.03	32.71 ± 22.42[b,c]
Number of oocytes	2.59 ± 2.033	10.17 ± 4.19[a]	19.15 ± 6.29[b,c]
Total dose of FSH	2372.82 ± 874.23	2149.76 ± 478.37	1503.92 ± 536.148[b,c]
OSI	0.952 ± 0.648	4.79 ± 1.78	13.04 ± 2.72[a,b,c]
Implantation rate	0.026 ± 0.082	0.052 ± 0.134	0.0762 ± 0.144
Fertilization rate	0.688 ± 0.421	0.756 ± 0.152	0.685 ± 0.135
Number of embryos	1.86 ± 1.35	6.9 ± 3.34[a]	10.42 ± 4.18[b,c]
Pregnancy rate	2 (6.66 %)	7 (17.5 %)	4 (20 %)
Causes of infertility			
Male factor ($n = 36$)	15 (50 %)	16 (40 %)	5 (25 %)
Female factor ($n = 41$)	11 (36.67 %)	19 (47.5 %)	11 (55 %)
Male and female factor ($n = 13$)	4 (13.33 %)	5 (12.5 %)	4 (20 %)

Significant differences: [a] Normo-responders vs Poor-responders, [b] High-responders vs Poor-responders, [c] High-responders vs Normo-responders

Table 2 Clinical characteristics of patients according to the pregnancy

Parameters	Pregnant ($n = 13$)	Non-pregnant ($n = 77$)	p value
Age (years)	30.58 ± 4.2	31.31 ± 5.42	0.66
BMI (kg/m^2)	27.1 ± 3.7	27.6 ± 4.1	0.657
Number of follicles	10.86 ± 5.04	14.23 ± 13.04	0.716
Number of oocytes	9.00 ± 4.81	10.04 ± 6.8	0.59
Total dose of FSH	1922.92 ± 762.58	2107.31 ± 602.59	0.348
OSI	6.055 ± 4.63	5.19 ± 3.99	0.735
Implantation rate	0.33 ± 0.117	0	<0.001
Fertilization rate	0.742 ± 0.17	0.727 ± 0.235	0.831
Number of embryos	5.08 ± 2.46	6.67 ± 4.35	0.208
Causes of infertility			0.485
Male factor ($n = 36$)	7 (54 %)	29 (37.67 %)	
Female factor ($n = 41$)	4 (30.6 %)	37 (48.05 %)	
Male and female factor ($n = 13$)	2 (15.4 %)	11 (14.28 %)	

Table 3 Clinical characteristics of patients according to the age

Parameters	Age < 35 years ($n = 59$)	Age > 35 years ($n = 31$)	p value
Number of follicles	14.63 ± 14.46	12.22 ± 7.06	0.958
Number of oocytes	10.95 ± 6.88	8.04 ± 5.1	0.68
Total dose of FSH	1972.55 ± 603.71	2413.04 ± 662.63	0.005
OSI	6.1 ± 4.45	3.41 ± 2.19	0.01
BMI (kg/m^2)	27.12 ± 2.97	27.41 ± 4.32	0.745
Implantation rate	0.058 ± 0.13	0.03 ± 0.12	0.477
Fertilization rate	0.685 ± 0.239	0.842 ± 0.147	0.006
Number of embryos	6.98 ± 4.4	5.55 ± 3.31	0.195
Pregnancy rate	11 (18.64 %)	2 (6.45 %)	0.303
Causes of infertility			
Male factor ($n = 36$)	25 (42.38 %)	11 (35.49 %)	0.493
Female factor ($n = 41$)	24 (40.68 %)	17 (54.84 %)	
Male and female factor ($n = 13$)	10 (16.94 %)	3 (9.67 %)	

Table 4 Comparison of PIGF, sFlt-1, sRAGE levels in follicular fluid and PIGF/sFlt-1 ratio among poor-, normo-and high-responders according to the ovarian sensitivity index (OSI)

Parameters	Poor responders ($n = 30$) OSI < 1.697	Normo responders ($n = 40$) OSI = 1.698–10.07	High responders ($n = 20$) OSI > 10.07
PlGF (pg/ml)	374.44 ± 284.76	448.2 ± 319.171	739.22 ± 519.87
sFlt-1 (ng/ml)	499.79 ± 276.45	384.56 ± 224.85	365.94 ± 292.42
sRAGE (ng/ml)	4.29 ± 1.05	5.12 ± 2.13	6.46 ± 2.23[b,c]
PlGF/sFlt-1	0.80 ± 0.45	1.47 ± 1.95	1.96 ± 1.66[b]

PIGF placental growth factor, *sFlt-1* soluble fms-like tyrosine kinase-1, *sRAGE* soluble receptor for advanced glycation end products

Significant differences: [a] Normo-responders vs Poor-responders, [b] High-responders vs Poor-responders, [c] High-responders vs Normo-responders

We found higher level of FF sRAGE in pregnant women in comparison with non-pregnant group ($p = 0.011$), but other evaluated parameters had not significant differences (Table 5).

For determination of the predictive value of PlGF/sFlt-1 and sRAGE in high responsiveness and pregnancy assessment we used receiver operating characteristic (ROC) analyses. Using cutoff value of 0.825, the sensitivity and specificity of PlGF/sFlt-1 ratio as a candidate biomarker of high-responder women were 77.8 % (CI, 98–57.6 %) and 59.6 % (CI, 79.8–39.4 %) respectively (Fig. 1a). Based on the ROC curve, cutoff value for sRAGE as a high-responder

Table 5 Comparison of PIGF, sFlt-1, sRAGE levels in follicular fluid and PIGF/sFlt-1 ratio between pregnant and non-pregnant individuals

Parameters	Clinical pregnant ($n = 13$)	Non-Clinical pregnant ($n = 77$)	p value
PlGF (pg/ml)	697.53 ± 493.45	419.88 ± 305.50	0.153
sFlt-1 (ng/ml)	518.78 ± 336.23	390.07 ± 223.6	0.089
sRAGE (ng/ml)	7.5 ± 3.73	5.1 ± 1.98	0.011
PlGF/sFlt-1	0.97 ± 0.67	1.32 ± 1.50	0.431

PIGF placental growth factor, *sFlt-1* soluble fms-like tyrosine kinase-1, *sRAGE* soluble receptor for advanced glycation end products

predictor was 4.83 (ng/ml) with sensitivity and specificity of 77.8 % (CI, 94–61.6 %) and 61.7 % (CI, 77.9–45.5 %) respectively (Fig. 1a). Also, sRAGE levels with cutoff value of 4.83 (ng/ml) for evaluating the pregnancy outcome showed 81.8 % (CI, 99–64.6 %) sensitivity and 60.7 % (CI, 77.9–43.5 %) specificity (Fig. 1b).

The data of correlation assessment between follicular fluid factors and ICSI cycle parameters are shown in Table 6. The result demonstrated inverse correlation between PlGF and PlGF/sFlt-1 ratio with women's age ($r = -0.255$, $p = 0.027$ and $r = -0.252$, $p = 0.048$ respectively). Positive correlations were obtained between sRAGE level with the number of oocytes, follicles and OSI level. Furthermore, there were positive associations between PlGF/sFlt-1 ratio with the number of oocytes, embryos and OSI level. In addition, our results indicated that there was an inverse relation between PlGF/sFlt-1 ratio and fertilization rate ($r = -0.251$, $p = 0.043$).

We also evaluated possible associations among PlGF, sFlt-1, sRAGE and PlGF/sFlt-1 levels in follicular fluid of patients, but there were no significant correlations among these parameters and only a positive and strong correlation was found between sRAGE and PlGF levels ($r = 0.642$, $p < 0.001$).

Discussion

Excessive ovarian response may accompany the cycle cancellations, painful follicle aspirations and risk of OHSS. On the other hand, poor ovarian response can be an indicator of diminished ovarian reserve. Such situations could decrease chance of pregnancy, thus it is important to manage ovarian responsiveness for enhance safety, success rate and decrease the costs of assisted reproduction technology (ART) program [13]. Also, it should be noted that ovarian

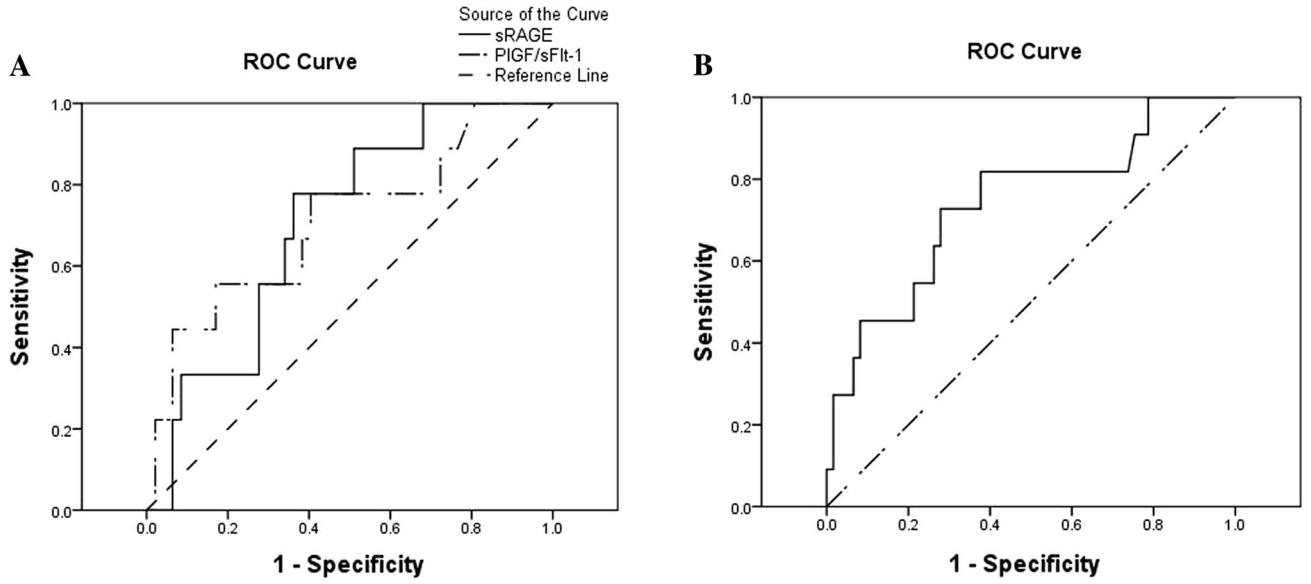

Fig. 1 **a** ROC curves for follicular fluid PlGF/sFlt-1 ratio and sRAGE levels were obtained for high responders women compared to other responder groups and area under the curve (AUC) were 0.707 ($p < 0.05$, cut-off = 0.825) for PlGF/sFlt-1 ratio and 0.704 ($p < 0.05$, cut-off = 4.83 ng/ml) for sRAGE. **b** ROC curve for follicular fluid sRAGE levels were obtained for pregnant women compared to non-pregnant ones and area under the curve (AUC) was 0.704 ($p < 0.05$, cut-off = 4.83 ng/ml) for sRAGE

Table 6 Correlation of PIGF, sFlt-1, sRAGE levels in follicular fluid with ICSI cycle parameters in studied population

	PlGF (pg/ml)		sFlt-1 (ng/ml)		sRAGE (ng/ml)		PlGF/sFlt-1	
	r	*p*	r	*p*	*r*	*p*	*r*	*p*
Age	−0.255	**0.027**	0.170	0.154	−0.175	0.166	−0.252	**0.048**
BMI (kg/m^2)	−0.277	0.234	0.571	0.456	−0.372	0.132	−0.189	0.349
N of follicles	0.02	0.884	0.018	0.899	0.307	**0.038**	0.117	0.422
N of oocytes	0.064	0.568	−0.176	0.123	0.3	**0.014**	0.3	**0.013**
Total dose of FSH	−0.298	**0.007**	−0.073	0.531	−0.239	**0.05**	−0.86	0.490
OSI	0.084	0.458	−0.087	0.452	0.251	**0.039**	0.243	**0.047**
Implantation rate	0.094	0.412	0.144	0.216	0.283	**0.018**	−0.16	0.2
Fertilization rate	−0.056	0.630	0.107	0.361	−0.08	0.530	−0.251	**0.043**
N. of embryos	0.04	0.737	−0.174	0.144	0.155	0.230	0.257	**0.042**

Bold values are statistically significant at $P < 0.05$

stimulation could has effect on ovarian responsiveness [14]. Various markers have been suggested for prediction of ovarian responsiveness [13, 15, 16]. However, predictive value of these markers is poor [17] and nowadays multi-marker models are used for evaluation of ovarian response [18]. AMH and AFC are two ovarian response markers which both of them have good predictive value for poor [15] and high responsiveness [13]. It has been reported that OSI has positive correlation with AMH and AFC [18] and has higher efficiency for evaluation of ovarian responsiveness compared with the number of oocytes [3]. Since OSI consists of FSH total dose in addition to the number of oocytes, it may have utility when different doses of FSH are used in ICSI cycle [3]. In the current study, we have assessed relationship of PlGF, sFlt-1 and sRAGE levels in follicular fluid with ICSI cycle outcome and ovarian responsiveness according to the OSI.

In the current study, we observed that mean age of high responders is lower than other responder groups and also OSI values were significantly higher in younger women (age < 35) than the older ones (age > 35). Since natural fertility starts to decrease after the age of 30 and this reductive manner accelerates in the mid −30 s and leads to decline ovarian follicle pool, therefore observed poor ovarian responsivness in women with age of >35 years was expected [17]. In addition, inverse correlation between OSI and age has been reported by previous studies [3, 18].

We observed higher number of oocytes, follicles and embryos in high responders, but there were no significant differences in embryo implantation and pregnancy rates among various responder groups. The possible association of ovarian responsiveness with occurrence of pregnancy has been evaluated by different studies but they have taken various strategies to divide responder groups. In previous studies in which dividing was according to the number and quality of follicles, controversial results have been obtained. Some of them have reported significant differences in pregnancy rate among various responder groups [19, 20] while others have not observed such differences [21, 22]. Saldeen et al. [19] have shown that pregnancy rate of poor-responder women was significantly lower than other responder groups. On the other hand in a study in which patients were divided based on OSI, higher pregnancy rate was observed in high-responders than normal and poor-responder women [4]. However, in this study, we found no significant difference in pregnancy rate when compared among various responder groups based on OSI. Although there were no methodological differences in grouping and also stimulation protocol between our study with study of Huber et al. [4], but different results were obtained regarding the relation of ovarian responsiveness and pregnancy rate. This kind of contradictory results might be explained by differences in sample size and inclusion criteria of the patients, as unlike Huber et al. we did not enroll PCOS patients who often have high responsiveness. Positive correlation of OSI with ovarian response and also lack of association with chance of pregnancy probably indicates that better IVF/ICSI cycles outcome including the high number of follicles, oocytes and embryos could not warrant achieving successful pregnancy, and other factors such as sperm/egg genetics integrity, embryo quality, stimulation protocol, transfer technique, and endometrial receptivity may have crucial roles. Thus the results of current study supported that good ovarian response is independent of pregnancy outcome. Nonetheless, higher number of the oocytes, follicles and embryos provides an opportunity to transfer higher number of embryos and increases chance of pregnancy.

It has been reported that FF PlGF has positive correlation with follicle size [7], AMH levels and number of oocytes [6], and knockout of PlGF gene decreases ovarian angiogenesis [5]. Therefore, it could be speculated that PlGF plays dominant role in follicular development. In addition, it has been reported that the expression of PlGF is higher in the endometrium with good quality based on sakumoto-masamoto grading in compared with bad endometrium [8].

Furthermore, the expression of PlGF in healthy implantation sites is higher than arrested implantation sites [23]. In the current study, FF PlGF levels were not statistically different among various responder groups and also between pregnant and non-pregnant women. Moreover, we did not observe significant association between FF PlGF levels and the number of oocytes. In contrast to our findings, Tal et al. [6] have reported a positive correlation between FF PlGF and the number of oocytes in women with PCOS. A possible reason for divergence between these two studies might be related to difference in study population, as some effective factors on PlGF levels such as ovarian reserve, FSH and AMH levels are totally different in women with PCOS in compare with healthy females [24, 25]. In accordance with Tal et al. [6] study, we obtained no correlation between PlGF and fertilization rate.

sFlt-1 is a soluble receptor for two angiogenic factors, PlGF and VEGF-A and it could inhibit PlGF and VEGF-A angiogenic effects by binding them [26]. We did not find any significant differences in FF sFlt-1 levels among responder groups and also there was no correlation between sFlt-1 levels with the number of oocytes and total dose of FSH. Savchev et al. [27] have also obtained similar results. Only in study conducted by Neulen et al. [28] an inverse relation between sFlt-1 levels and the number of oocytes has been observed. Since PlGF and VEGF have determinant role in FF sFlt-1 levels by binding to it, and Neulen et al. have obtained totally different levels of VEGF, this reason could cause the controversial results. In our study, there were no significant differences in levels of FF sFlt-1 between pregnant and non-pregnant groups. The same results have been achieved in previous study [27]; although in a study on porcine, high expression of sFlt-1 in endometrium of pregnant animals have been reported [29].

PlGF/sFlt-1 ratio in our study was statistically higher in high-responders than poor-responder patients. High level of PlGF/sFlt-1 ratio in high-responders possibly leads to an increase in availability of PlGF and thus this factor exerts through VEGFR-1 (Flt-1). Occupation of VEGFR-1 by PlGF causes VEGF-A acts through VEGFR-2 and not VEGFR-1 [5]. In this condition, activation of both VEGF receptors (1 and 2) may reinforce the angiogenesis in the ovaries and increase oogenesis. In this study, there were no differences in PlGF/sFlt-1 ratio between pregnant and non-pregnant women while we found positive correlations between the ratio with number of oocytes, embryos and OSI levels and also a negative correlation with fertilization rate.

It has been documented that the accumulation of AGEs interferes with folliculogenesis and it has negative impact on oocyte maturation [9]. It was revealed that in PCOS patients, vitamin D inhibits the inflammatory actions of AGEs by increasing serum levels of sRAGE [30]. These findings indicate that sRAGE as a decoy receptor for AGEs, probably inhibits the interaction of AGE-RAGE and thereby protects against cellular damage. In the current study for the first time, we reported that FF sRAGE levels are significantly higher in high-responders than normal and poor-responders. Furthermore, positive correlations were observed between levels of this factor with the number of oocytes and follicles. In agreement with our study, existence of positive correlations between sRAGE levels with the number of oocytes and follicles has been mentioned [9, 11]. However, until now, no study has been evaluated FF levels of sRAGE among various ovarian responder groups. Merhi et al. [9] have shown a positive correlation between sRAGE and AMH levels in FF and have considered sRAGE as a candidate biomarker for ovarian reserve. In support of previous results, we found that increased FF sRAGE levels coincide with the higher ovarian responsiveness. Also, we observed an inverse correlation between FF sRAGE and total dose of gonadotropin which is similar to previous finding [9]. According to the our findings and previous reports, it could be concluded that sRAGE probably improves folliculogenesis and ovulation via suppression of the inflammatory pathways such as Nuclear Factor Kappa B (NF-κB), protection against oxidative stress and prevention the abnormal activation of the ERK-1,2 pathways. On the other hands, the positive correlation between PlGF and sRAGE levels in the follicular fluid and also negative correlation of both of them with the total dose of FSH may indicate that these two factors are partly involved in FSH signaling pathways in the ovaries. Although to be best of our knowledge, this is the first study to present this relation and further studies are required for clarification of the exact mechanism.

In the present study, FF sRAGE levels had a positive correlation with embryo implantation rate. However, in the study of Bonetti et al. [31], lack of such a relation between sRAGE levels and embryo implantation rate has been reported and they only have observed a significant correlation between FF sRAGE and embryo quality. It has been noted that inflammation is a vital process for embryo implantation through release of inflammatory cytokines in the endometrium. However, excessive inflammation may lead to implantation and pregnancy loss [32]. Studies have shown that FF levels of sRAGE as an anti-inflammatory factor are significantly greater and AGEs levels are lower in pregnant women compared with non-pregnant ones [11, 33]. Therefore, it seems that there is a protective role for sRAGE against inflammatory actions of AGEs. Although FF levels of AGEs did not determine in our study, but we found increased levels of sRAGE in pregnant women compared with non-pregnant cases which is in agreement with Malickova et al. [11] study. It is likely that sRAGE as a soluble receptor prevents initiation of AGE-RAGE signaling

pathway and facilitates maintenance of pregnancy by the reduction of inflammation.

Our results showed that FF levels of sRAGE and PlGF/sFlt-1 ratio could be used as markers for determining the high-responder women. However, we evaluated these factors in follicular fluid after finishing ovarian stimulation treatment and it seems that, in order to the clinical utility of these factors, further studies on their serum levels before the stimulation and association of them with ovarian response are needed. We also found that sRAGE levels with sensitivity and specificity of 81.8 and 60.7 %, respectively and with cutoff point at 4.83 (ng/ml) could be a good predictor for ART outcome. However, to find underlying mechanism and utility of its serum levels as a pregnancy predictor, further investigations are needed.

One of the strengths of this study is using OSI as a new index for investigation of ovarian responsiveness, since the application of this index could remove confounding effect of different doses of FSH. The having of appropriate sample size compared to previous studies also is another advantage of the present study. However, since follicular fluid levels of studied factors are many folds higher than the serum; we did not evaluate serum levels of these factors among various responders.

In conclusion, the results showed that good ovarian response is independent of pregnancy outcome. Moreover, our results showed that FF levels of sRAGE and PlGF/sFlt-1 ratio could be used as markers for determining the high-responder women and potentially may permit to identify patients at risk for OHSS only when OHSS is developed yet. Also, FF sRAGE levels could be a good predictor for ART outcome.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval The agreement of the Ethical Committee of Tabriz University of Medical Sciences was obtained.

Informed consent All patients gave written informed consent.

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