Effects of EG-VEGF, VEGF and TGF- β 1 on pregnancy outcome in patients undergoing IVF-ET treatment

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Abstract

Purpose To investigate the correlation of endocrine gland-derived vascular endothelial growth factor (EG-VEGF), vascular endothelial growth factor (VEGF) and transforming growth factor beta 1 (TGF- β 1) with the corresponding reproductive outcome in patients who received in vitro fertilization-embryo transfer (IVF-ET).

Methods Sixty-seven women undergoing IVF-ET at a university tertiary hospital were recruited for a prospective study. Concentrations of EG-VEGF, VEGF and TGF- β 1 were measured by enzyme-linked immunosorbent assay (ELISA) in follicular fluid (FF) collected during oocyte retrieval (OR) and in serum collected 2 days after OR.

Results In FF, concentrations of both EG-VEGF and VEGF were negatively correlated with peak E2 and the number of MII

oocytes retrieved, and positively correlated with each other. In serum, concentrations of all the three growth factors were positively correlated with the rate of good quality embryo, and with one another. Patients in the pregnancy group had lower peak E2 concentrations and higher serum EG-VEGF concentrations than those in the non-pregnancy group, but such tendency was not observed in the case of VEGF and TGF- β 1. **Conclusions** Both concentrations of EG-VEGF and VEGF in FF were negatively correlated with ovarian response and oocyte maturation. Concentrations of all the three growth factors in serum were positively correlated with embryo quality, but only serum concentrations of EG-VEGF were associated with the pregnancy outcome.

Keywords IVF-ET · EG-VEGF · VEGF · TGF- β 1 · Pregnancy

Capsule Prospective study found that concentrations of EG-VEGF, VEGF and TGF- β 1 in FF and serum were correlated with oocyte maturation and embryo quality, but only serum concentrations of EG-VEGF were associated with the pregnancy outcome in IVF women.

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Introduction

The growth and maturation of the ovarian follicle requires the coordinate action of somatic cells and oocytes. Several intra-ovarian growth factors involved in the bidirectional signals between somatic and germ cells have been identified as serving an important role for local autocrine and/or paracrine effects triggering oocyte maturation and apoptosis [1]. Human follicular fluid (FF) contains protein and peptides originating from plasma or the metabolism of granulosa cells, and constitute the microenvironment for the oocyte [2]. Studies have indicated the cytokine network within FF may be related to steroidogenesis, oocyte maturation and subsequent implantation potential [3]. It is intriguing to presume that the concentrations of some growth factors in FF at the time of ovulation might be used as indicators for the development of the follicle, and thus for the quality of the oocyte. However, there is limited information regarding the presence of growth factors or their unique roles in human ovaries.

In routine in vitro fertilization and embryo transfer (IVF-ET), controlled ovarian hyperstimulation (COH) is adopted to stimulate multi-follicles to develop. However, the supraphysiologic concentrations of estradiol (E_2) produced during COH may be detrimental for endometrial receptivity and may affect pregnancy outcome. Studies have reported that the expression and secretion of some growth factors under COH may be altered [4]. Endocrine gland-derived vascular endothelial growth factor (EG-VEGF), also termed as prokineticin (PK1), is the first protein identified as a selective angiogenic mitogen acting on the endothelial cells of steroidogenic endocrine glands [5]. Ovary is the organ with highest production of EG-VEGF, which is predominantly expressed by the granular and thecal cells in follicles. In premenopausal ovaries, EG-VEGF mRNA, with its corresponding protein detected in the follicle and endometrium, is regulated periodically by sexual hormones during menstruation [6]. However, no effect of EG-VEGF on the IVF-ET outcome has been reported. We recently found that EG-VEGF was associated with ovarian response and ovarian hyperstimulation syndrome (OHSS) [7]. But it remains to be determined whether EG-VEGF expression affects oocyte quality and pregnancy outcome.

Vascular endothelial growth factor (VEGF) is a potent vasoactive protein produced in many different tissues. In the ovary, VEGF is produced by granulosa cells and theca cells and increases in response to gonadotropin (Gn) and hypoxia [8]. Besides, with its function in angiogenesis and increase of vascular permeability (VP), largely production of VEGF is considered to be associated with OHSS [9]. Furthermore, VEGF is not only pivotal for the conversion of relatively avascular pre-ovulatory follicle into vascular corpus luteum (CL), but also an important angiogenesis factor related to implantation and early pregnancy [10]. The cellular localization and spatiotemporal expression of VEGF in ovary tissue are complementary to EG-VEGF [11]. In vitro studies also showed that hCG could stimulate expression of both VEGF and EG-VEGF mRNA [6]. These phenomena suggest that both VEGF and EG-VEGF may participate in ovarian angiogenesis synergistically. So we included VEGF in this study together with EG-VEGF.

Transforming growth factor beta 1 (TGF- β 1) has multiple functions in promoting cell proliferation, differentiation and angiogenesis, and is essential for oocyte and early embryo development [12]. Both granulosa cells and thecal cells in the follicle can produce TGF- β 1 [13]. TGF- β 1 was also detected in the endometrium during the window of implantation (WOI), and was shown to modulate maternal immunotolerance during implantation, as well as to regulate several implantation-related molecules such as VEGF and leukaemia inhibitory factor (LIF) [14]. Previous studies have shown that FF TGF- β 1 might have predictive value in monitoring ovarian stimulation treatment for IVF [15], but further confirmation is necessary.

Despite reports on the important roles of these growth factors in follicular development, direct clinical data supporting their correlation with pregnancy outcome following ET still lacks. In this study, we measured concentrations of EG-VEGF, VEGF and TGF- β 1 in FF during oocyte retrieval (OR) and in peripheral blood during the early luteal phase, aiming to confirm correlations among them, and to determine whether there is a relationship between these cytokines and reproductive outcome in women undergoing IVF-ET.

Materials and methods

Patients

We conducted a prospective study utilizing clinical pregnancy rates as endpoints. A total of 67 consecutive infertile women received first or repeated in vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) treatment for tubal and/or male factors in the department of reproductive medicine at Renji Hospital (School of Medicine, Shanghai Jiaotong University, China) were recruited. All women had normal body mass index (BMI, 18.5–23.9 kg/m²) and regular menstrual cycles with basal follicle stimulating hormone (FSH) <10 IU/L. No history of ovarian operation. Normal ovulation and the presence of a normal uterine cavity were verified by ultrasound examination. None had accepted hormone therapy during the 3 months just before entering the study. Patients undergoing COH with low ovarian response (≤ 5 follicles with diameter ≥ 16 mm and $E_2 \leq 1,000$ pg/ml on the day of hCG injection) were excluded. The study was approved by the institutional ethics committee of Renji Hospital and all subjects provided written informed consent.

IVF-ET process

All subjects underwent the long COH protocol with GnRHa (Decapetyl, Ferring GmbH, Kiel, Germany)/Gn (Gonal-F, Merck Serono, Geneva, Switzerland)/hCG (Livzon Biochemical Pharmaceutical Co., Zhuhai, China), gamete manipulation, embryo culture and ET, as regularly performed at our centre. Doppler ultrasound (SSD-1400, Aloka, Tokyo, Japan) examination was carried out to monitor the follicle diameter, as well as thickness and morphology of the endometrium during COH. Serum E_2 was determined via chemiluminescence (Immulife 1,000, Simens, Erlangen, Germany). Injection of hCG (5,000 IU) was applied when a dominant follicle reached 18 mm or two follicles reached 17 mm and serum E_2 concentrations increased to 300 to 400 pg/ml per follicle above 14 mm in diameter. Oocyte retrieval under vaginal ultrasound guide

was carried out 34 to 36 h later. A good embryo was defined as being of 4 to 5-cells on day 2, 7-cells or more on day 3, equally-sized blastomeres and less than 20 % fragmentation and no multinucleation [16]. ET was performed 2 or 3 days after OR with prior consideration of good quality embryo, and 1 to 3 embryos were transferred (up to 2 embryos for women younger than 35 years old and up to 3 embryos for those older than 35 years). There were 33 IVF and 34 ICSI in this cycle, and all subjects had transferable embryos.

Collection of FF and serum

FF from follicles (with a diameter of 16–20 mm) containing oocytes were collected in separate tubes during OR. FF without blood contamination obtained from 3 to 5 follicles of the same patient was pooled. Embryo quality evaluation and ET are generally performed on 2 or 3 days after OR in clinic, therefore corresponding serum samples were collected at 2 days after OR. All samples were centrifuged (Labofuge 400R, Thermo Heraeus, Wiesbaden, Germany) for 10 min at 2,000 g within 1 h after collecting. Supernatant fluid was put in Eppendorf tubes and stored at -80°C in an ultra-low temperature freezer (MDF-382E (N), Sanyo, Japan) immediately after collecting. Except serum E_2 concentrations examined on the collection day, all other factors were examined within 6 months after the sample collecting.

ELISA assay

Concentrations of EG-VEGF, VEGF and TGF- β 1 were evaluated using commercially available ELISA kits (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). Validation test were performed for both serum and FF, and standard curve was obtained every time of detection. All samples were examined in duplicate. The sensitivity of the ELISA kits to EG-VEGF, VEGF and TGF- β 1 were 16, 25 and 15 pg/ml, respectively. The intra- and inter-assay coefficients of variation (CVs) for EG-VEGF were both lower than 10 %. The intra- and inter-assay CVs for VEGF were lower than 10 % and 15 %, respectively. The intra- and inter-assay CVs for TGF- β 1 were both lower than 8.9 %.

Statistical analysis

All data were analysed by SPSS 13.0 software (SPSS Inc., Chicago, IL). The values of measurement data are expressed as mean $\pm$ S.D. when applicable. Pearson's correlation coefficient was calculated. Differences between the groups were tested for significance using independent-samples *t*-test and ANOVA analysis for parametric variables. Bonferroni correction was adopted for multiple comparisons. Statistical significance was defined as $P<0.05$.

Results

The demographic data and IVF-ET cycle characteristics for the 67 women included in this study are summarized in Table 1.

Among all subjects, the mean concentrations of EG-VEGF, VEGF and TGF- β 1 in FF were 815.77 ± 367.58 , 1373.52 ± 484.81 and 1293.02 ± 694.01 pg/ml, respectively. The mean concentrations of EG-VEGF, VEGF and TGF- β 1 in serum were 42.08 ± 21.79 , 164.36 ± 100.65 and 2554.84 ± 715.67 pg/ml, respectively. The average FF/serum ratios for EG-VEGF, VEGF and TGF- β 1 were 22.00 ± 9.23 , 11.63 ± 8.32 and 0.55 ± 0.32 , respectively. These ratios were significantly different from one another ($P<0.01$).

In FF, concentrations of all the three growth factors EG-VEGF, VEGF and TGF- β 1 were not correlated with age. Concentrations of both EG-VEGF and VEGF were positively correlated with Gn dosage ($r=0.291$, $P<0.05$; $r=0.252$, $P<0.05$, respectively), and negatively correlated with peak E_2 ($r=-0.639$, $P<0.01$; $r=-0.490$, $P<0.01$, respectively) and the number of MII oocytes retrieved ($r=-0.456$, $P<0.01$; $r=-0.325$, $P<0.01$, respectively). None were correlated with the rate of fertilization and the rate of good quality embryos. Besides, concentrations of EG-VEGF were positively correlated with that of VEGF in FF ($r=0.382$, $P<0.01$) (Table 2).

In serum, concentrations of both EG-VEGF and TGF- β 1 were negatively correlated with serum E_2 at 2 days after OR ($r=-0.350$, $P<0.01$; $r=-0.286$, $P<0.05$, respectively).

Table 1 Baseline and IVF/ICSI cycle characteristics of enrolled patients ($n=67$)

Variables	Mean	SD	Min–Max
Age (years)	30.00	3.93	22–39
Infertility duration (years)	4.61	2.94	1–12
Cycle number	1.94	1.60	1–7
Gn duration (days)	9.00	1.19	7–12
Gn dosage (IU)	1616.04	492.24	825–3,300
Peak E_2 (pg/ml)	4174.25	1637.18	1,486–9,446
Serum E_2 on 2 days after OR (pg/ml)	1376.07	623.97	204–2,650
Number of oocytes retrieved	15.19	5.97	3–30
Number of mature oocytes	12.57	4.97	2–26
Number of oocytes fertilized	10.07	4.47	1–21
Number of good quality embryos	6.06	3.53	1–17
Number of embryos transferred	2.21	0.48	1–3
Pregnancy rate (%)	52.24	NA	NA

Gn, gonadotropin; E_2 , estradiol; OR, oocyte retrieval; NA, not available; SD, standard deviation

Table 2 Correlations among the clinical characteristics of the studied IVF/ICSI cycles and the concentrations of growth factors in FF

Variable/ <i>r</i>	FF EG-VEGF	FF VEGF	FF TGF-β1
Age	0.156	0.181	−0.017
Gn dosage	0.291 ^a	0.252 ^a	−0.014
Peak E ₂	−0.639 ^b	−0.490 ^b	−0.048
Mature oocytes	−0.456 ^b	−0.325 ^b	0.215
Rate of fertilization	−0.099	0.048	0.171
Rate of good quality embryo	0.227	0.133	−0.128
FF EG-VEGF		0.382 ^b	−0.153
FF VEGF			0.116
FF TGF-β1			

Gn, gonadotropin; E₂, estradiol; FF, follicular fluid; EG-VEGF, endocrine gland-derived vascular endothelial growth factor; VEGF, vascular endothelial growth factor; TGF-β1, transforming growth factor beta 1

^a $P < 0.05$; ^b $P < 0.01$

Concentrations of all the three growth factors were positively correlated with the ratio of good quality embryo ($r = 0.290$, $P < 0.05$; $r = 0.249$, $P < 0.05$; $r = 0.311$, $P < 0.05$, respectively). Furthermore, concentrations of serum EG-VEGF, VEGF and TGF-β1 were positively correlated with one another (EG-VEGF and VEGF, $r = 0.394$, $P < 0.01$; EG-VEGF and TGF-β1, $r = 0.391$, $P < 0.01$; VEGF and TGF-β1, $r = 0.241$, $P < 0.05$, respectively) (Table 3).

Among the 67 patients received ET, 35 patients got pregnant (52.24 %). There were no differences between pregnancy group and non-pregnancy group in average age, number of MII oocytes retrieved, number of embryos transferred and number of good quality embryos transferred. Pregnant patients had lower peak serum E₂ concentrations as compared to non-pregnant recipients ($P < 0.05$). Average concentrations of EG-VEGF in serum but FF were significantly higher in the

Table 3 Correlations among the clinical characteristics of the studied IVF/ICSI cycles and the concentrations of growth factors in serum

Variable/ <i>r</i>	Serum EG-VEGF	Serum VEGF	Serum TGF-β1
Serum E ₂ on 2 days after OR	−0.350 ^b	−0.072	−0.286 ^a
Mature oocytes	−0.235	−0.291 ^a	−0.155
Rate of fertilization	0.030	−0.113	0.050
Rate of good quality embryo	0.290 ^a	0.249 ^a	0.311 ^a
Serum EG-VEGF		0.394 ^b	0.391 ^b
Serum VEGF			0.241 ^a
Serum TGF-β1			

E₂, estradiol; OR, oocyte retrieval; EG-VEGF, endocrine gland-derived vascular endothelial growth factor; VEGF, vascular endothelial growth factor; TGF-β1, transforming growth factor beta 1

^a $P < 0.05$; ^b $P < 0.01$

pregnancy group than in non-pregnancy group (48.72 ± 24.69 pg/ml vs 34.81 ± 15.44 pg/ml, $P < 0.01$). There were no significant differences of VEGF or TGF-β1 concentrations between pregnancy group and non-pregnancy group in FF and serum ($P > 0.05$) (Table 4).

Discussion

The growth factors contained or generated within the ovary may act alone or in concert to regulate the growth of follicles. These factors are ultimately controlled by endocrine, paracrine and autocrine regulation, and may affect oocyte quality and fertility potential if aberrantly expressed. Further elucidation of specific physiological role(s) of factors involved in the pre-ovulatory follicle may be useful in judging the ovarian function and in treating infertility.

In this study, we select FF from mature follicles during OR to measure concentrations of the three growth factors. Meanwhile, as ET is usually performed on day 2 or 3 after OR in our center, so we chose the second day after OR for the measurement of serum concentrations of the three growth factors. Our results showed that the concentrations of EG-VEGF and VEGF were much higher in FF than in serum, whereas concentrations of TGF-β1 were lower in FF than in serum. This result is in line with their origin and demonstrates the contribution of both passive transfer and local synthesis in the granulosa cells. Moreover, we also found that the FF/serum ratio of EG-VEGF was significantly higher than that of VEGF. This may be due to the fact that EG-VEGF is generated specifically by the ovary while VEGF is non-specific to ovarian tissues [17]. However, as serum E₂ concentrations decreased quickly 2 days after OR, serum concentrations of the three growth factors might change subsequently, making the FF/serum ratio to vary during the 2 days interval. Up to now, there has been no study reported on the periodic variation of serum concentrations of EG-VEGF and TGF-β1 during IVF cycle, only serum concentrations of VEGF were reported remained unchanged during the normal menstrual cycle but elevated in the luteal phase during IVF cycle [18], therefore further studies with multiple time-points measuring are needed to clarify their time-relevant changes during IVF cycle.

An analysis on the relationship of FF concentrations of the three growth factors with clinical characteristics of IVF cycles showed that they all did not correlate with age, which seems illogical with the decreased follicular development in the elderly. This may be due to the patient characteristics of the study, as all subjects included were younger than 40 and had normal ovarian reserve, and low ovarian responders were excluded. Concentrations of both EG-VEGF and VEGF were positively correlated with Gn dosage, but negatively correlated with peak serum E₂ concentrations and the number of MII oocytes retrieved. In general, E₂

Table 4 Comparison between pregnancy and non-pregnancy groups for serum E_2 levels and EG-VEGF, VEGF and TGF- β 1 levels in FF and serum

Variable/means $\pm$ SD	Pregnancy group (<i>n</i> =35)	Non-pregnancy group (<i>n</i> =32)	<i>P</i> value
Age (years)	30.03 $\pm$ 3.93	29.97 $\pm$ 3.99	0.951
Number of MII oocytes retrieved	11.57 $\pm$ 4.74	13.66 $\pm$ 5.06	0.087
Number of embryos transferred	2.20 $\pm$ 0.47	2.22 $\pm$ 0.49	0.874
Number of good quality embryos transferred	1.89 $\pm$ 0.58	1.97 $\pm$ 0.65	0.582
Peak E_2 (pg/ml)	3766.17 $\pm$ 1491.71	4620.59 $\pm$ 1694.81	0.032 ^a
Serum E_2 on 2 days after OR (pg/ml)	1279.80 $\pm$ 672.57	1481.38 $\pm$ 557.56	0.189
FF EG-VEGF (pg/ml)	877.46 $\pm$ 413.97	748.29 $\pm$ 301.20	0.152
FF VEGF (pg/ml)	1412.44 $\pm$ 450.82	1330.95 $\pm$ 523.38	0.496
FF TGF- β 1 (pg/ml)	1243.00 $\pm$ 699.38	1347.72 $\pm$ 695.04	0.541
Serum EG-VEGF (pg/ml)	48.72 $\pm$ 24.69	34.81 $\pm$ 15.44	0.007 ^a
Serum VEGF (pg/ml)	177.42 $\pm$ 109.24	150.08 $\pm$ 89.87	0.270
Serum TGF- β 1 (pg/ml)	2633.33 $\pm$ 746.73	2469.00 $\pm$ 681.41	0.352

SD, standard deviation; E_2 , estradiol; OR, oocyte retrieval; FF, follicular fluid; EG-VEGF, endocrine gland-derived vascular endothelial growth factor; VEGF, vascular endothelial growth factor; TGF- β 1, transforming growth factor beta 1

^a Statistical difference remained after Bonferroni correction test

concentrations are positively correlated with Gn dosage. However, heterogeneous effect among patients is a common feature of clinical data, for it may be resulted from inter-patient variability including different age, infertility cause, ovarian reserve and Gn start dose etc., so in patients with different ovarian responses, E_2 concentrations were not always Gn dependent [19]. So we think that our data did not show a positive correlation between Gn dosage and E_2 concentrations perhaps reflects the specific situation in our center. As for VEGF, our results were consistent with the reported finding that FF VEGF concentrations were positively correlated to Gn dosage, and negatively correlate to peak E_2 levels and the number of oocytes retrieved [20]. Although the significant association of FF EG-VEGF and VEGF with ovarian response implies that their production may be inhibited by the high E_2 concentrations generated during COH, the underlying mechanism is unknown. Our study also showed that EG-VEGF and VEGF concentrations in FF had positive association with each other, suggesting that they may have synergistic interactions to affect follicular development during COH cycles, which is supported by the previous report that EG-VEGF could stimulate VEGF expression in steroid cells in CL [21]. However, FF concentrations of the three growth factors have no significant correlation with the rate of fertilization and the embryo quality. Therefore, while these factors may be related to early follicular development and oocyte maturation, their concentrations in FF do not contribute to the subsequent embryo development potentiality.

Interestingly, serum concentrations of the three growth factors were all positively correlated with the rate of good quality embryo, and with one another, though only EG-VEGF and TGF- β 1 were negatively related with serum E_2 concentrations of the same day. It is possible that these growth factors are related to oocyte development through serum, but follicular fluid pathway.

A comparison of concentrations of E_2 and growth factors between the pregnancy and non-pregnancy group showed that, peak E_2 concentrations were significantly lower in the pregnancy group than in the non-pregnancy group. Noticeably, on the day of embryo transfer, the serum E_2 concentrations were no longer different between the two groups. As for the three types of growth factors in FF and serum, only serum EG-VEGF concentrations were significantly higher in the pregnancy group than in the non-pregnancy group. In contrast, some studies reported that the high concentrations of VEGF or TGF- β 1 in FF were related to low rate of pregnancy [20, 22]. For high E_2 concentrations were reported to have an adverse influence on the pregnancy outcome of IVF [23], and our study also showed that E_2 concentrations were negatively correlated with serum EG-VEGF concentrations, so it is proposed that high E_2 concentrations in patients with strong ovarian response may inhibit the expression of EG-VEGF, and in turn reduce the opportunity of pregnancy.

The fact that EG-VEGF concentrations in serum in the early luteal phase but those in FF were related to the IVF pregnancy outcome, possibly suggesting that apart from embryo quality, EG-VEGF could act on the endometrial receptivity, as high serum concentrations of EG-VEGF can be transported into and function in the uterus endometria. Increased VP and angiogenesis around the site of blastocyst invasion is the original sign of successful implantation [24]. EG-VEGF may support pregnancy through angiogenesis and VP regulation [5, 25]. It has been reported that EG-VEGF is expressed in human peri-implantation endometrium, which expression and receptor are elevated in human decidua during early pregnancy, and that EG-VEGF can mediate fetal-maternal dialogue by regulating hCG-mediated endometrial LIF expression [26, 27].

In conclusion, our results indicated that both concentrations of EG-VEGF and VEGF in FF were negatively correlated with ovarian response and oocyte maturation. Concentrations of all the three growth factors in serum were

positively correlated with embryo quality, but only serum concentrations of EG-VEGF were associated with the pregnancy outcome. So EG-VEGF may be applicable as an indicator for IVF pregnancy outcome, but more experimental supports such as EG-VEGF expression in endometrium during WOI phase are needed. Moreover, as serum concentrations of EG-VEGF may vary during the luteal phase, further study with sampling at multiple time-points will assure its role to predict the clinical pregnancy outcome.

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Conflict of interest The authors have no conflicts of interest.

References

- Shimizu T, Abe Y, Wakai T, Hoshino Y, Miyamoto A, Sato E. Recent patents of TGF-beta family and VEGF associated with ovarian follicular development in mammals. *Recent Pat DNA Gene Seq.* 2007;1:195–9.
- Schweigert FJ, Gericke B, Wolfram W, Kaisers U, Dudenhausen JW. Peptide and protein profiles in serum and follicular fluid of women undergoing IVF. *Hum Reprod.* 2006;21:2960–8.
- Hammadeh ME, Fischer-Hammadeh C, Amer AS, Rosenbaum P, Schmidt W. Relationship between cytokine concentration in serum and preovulatory follicular fluid and in vitro fertilization/intracytoplasmic sperm injection outcome. *Chem Immunol Allergy.* 2005;88:80–97.
- Grondahl ML, Borup R, Lee YB, Myrholm V, Meinertz H, Sorensen S. Differences in gene expression of granulosa cells from women undergoing controlled ovarian hyperstimulation with either recombinant follicle-stimulating hormone or highly purified human menopausal gonadotropin. *Fertil Steril.* 2009;91:1820–30.
- LeCouter J, Kowalski J, Foster J, Hass P, Zhang Z, Dillard-Telm L, et al. Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature.* 2001;412:877–84.
- Fraser HM, Bell J, Wilson H, Taylor PD, Morgan K, Anderson RA, et al. Localization and quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum. *J Clin Endocrinol Metab.* 2005;90:427–34.
- Gao M-Z, Zhao X-M, Sun Z-G, Hong Y, Zhao L-W, Zhang H-Q. Endocrine gland-derived vascular endothelial growth factor concentrations in follicular fluid and serum may predict ovarian hyperstimulation syndrome in women undergoing controlled ovarian hyperstimulation. *Fertil Steril.* 2011;95:673–8.
- Guimera M, Morales-Ruiz M, Jimenez W, Balasch J. LH/HCG stimulation of VEGF and adrenomedullin production by follicular fluid macrophages and luteinized granulosa cells. *Reprod Biomed Online.* 2009;18:743–9.
- Ajonuma LC. Is vascular endothelial growth factor (VEGF) the main mediator in ovarian hyperstimulation syndrome (OHSS)? *Med Hypotheses.* 2008;70:1174–8.
- Sugino N, Kashida S, Karube-Harada A, Takiguchi S, Kato H. Expression of Vascular Endothelial Growth Factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy. *Reproduction.* 2002;123:379–87.
- Ferrara N, LeCouter J, Lin R, Peale F. EG-VEGF and Bv8: a novel family of tissue-restricted angiogenic factors. *Biochim Biophys Acta.* 2004;1654:69–78.
- Ingman WV, Robertson SA. The essential roles of TGFβ1 in reproduction. *Cytokine Growth Factor Rev.* 2009;20:233–9.
- Mulheron GW, Bossert NL, Lapp JA, Walmer DK, Schomberg DW. Human granulosa-luteal and cumulus cells express transforming growth factors-beta type 1 and type 2 mRNA. *J Clin Endocrinol Metab.* 1992;74:458–60.
- Dimitriadis E, White CA, Jones RL, Salamonsen LA. Cytokines, chemokines and growth factors in endometrium related to implantation. *Hum Reprod Update.* 2005;11:613–30.
- Fried G, Wramsby H. Increase in transforming growth factor beta1 in ovarian follicular fluid following ovarian stimulation and in-vitro fertilization correlates to pregnancy. *Hum Reprod.* 1998;13:656–9.
- Arce JC, Ziebe S, Lundin K, Janssens R, Helmgård L, Sorensen P. Interobserver agreement and intraobserver reproducibility of embryo quality assessments. *Hum Reprod.* 2006;21:2141–8.
- Maldonado-Perez D, Evans J, Denison F, Millar RP, Jabbour HN. Potential roles of the prokineticins in reproduction. *Trends Endocrinol Metab.* 2007;18:66–72.
- Unkila-Kallio L, Vuorela-Vepsäläinen P, Tiitinen A, Halmesmäki E, Ylikorkala O. No cyclicity in serum vascular endothelial growth factor during normal menstrual cycle but significant luteal phase elevation during an in vitro fertilization program. *Am J Reprod Immunol.* 2000;43:25–30.
- Benadiva CA, Ben-Rafael Z, Strauss 3rd JF, Mastroianni Jr L, Flickinger GL. Ovarian response of individuals to different doses of human menopausal gonadotropin. *Fertil Steril.* 1988;49:997–1001.
- Friedman CI, Seifer DB, Kennard EA, Arbogast L, Alak B, Danforth DR. Elevated level of follicular fluid vascular endothelial growth factor is a marker of diminished pregnancy potential. *Fertil Steril.* 1998;70:836–9.
- Kisliouk T, Levy N, Hurwitz A, Meidan R. Presence and regulation of endocrine gland vascular endothelial growth factor/prokineticin-1 and its receptors in ovarian cells. *J Clin Endocrinol Metab.* 2003;88:3700–7.
- Fujii EY, Nakayama M. The measurements of RAGE, VEGF, and AGEs in the plasma and follicular fluid of reproductive women: the influence of aging. *Fertil Steril.* 2010;94:694–700.
- Joo BS, Park SH, An BM, Kim KS, Moon SE, Moon HS. Serum estradiol concentrations during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner. *Fertil Steril.* 2010;93:442–6.
- Welsh AO, Enders AC. Chorioallantoic placenta formation in the rat: II. Angiogenesis and maternal blood circulation in the mesometrial region of the implantation chamber prior to placenta formation. *Am J Anat.* 1991;192:347–65.
- Lee YL, Chan YL, Chow WN, Ng EH, Lee KF, Yeung WS, et al. Endocrine gland-derived vascular endothelial growth factor stimulates proliferation and tube formation in human uterine microvascular endothelial cell through the mitogen-activated protein kinase but not through the Akt pathway. *Fertil Steril.* 2009;91:2163–71.
- Evans J, Catalano RD, Brown P, Sherwin R, Critchley HO, Fazleabas AT, et al. Prokineticin 1 mediates fetal-maternal dialogue regulating endometrial leukemia inhibitory factor. *FASEB J.* 2009;23:2165–75.
- Haozui D, Mahmoud K, Fourar M, Bendhaou K, Dechaud H, De Vos J, et al. Identification of new biomarkers of human endometrial receptivity in the natural cycle. *Hum Reprod.* 2009;24:198–205.