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Concentration of stromal cell-derived factor-1 (SDF-1/CXCL12) in the follicular fluid is associated with blastocyst development

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Abstract

Purpose: To study the association between stromal cell-derived factor-1 (SDF-1/CXCL12) and vascular endothelial growth factor (VEGF) concentrations in individual human ovarian follicles and IVF outcomes.

Methods: Concentrations of SDF-1 and VEGF in 261 follicular fluid samples were measured with enzyme-linked immunosorbent assay. IVF outcome parameters were included in fertilization rate, cleavage rate, embryo morphology on day 3, and blastocyst morphology on day 5.

Results: The follicular concentration of SDF-1 and VEGF was not significantly associated with fertilization and cleavage outcome, and embryo morphology. The rates of full blastocysts and good-quality blastocysts were significantly higher in follicles with an SDF-1 concentration of 275-350 pg/mL than in the follicles with SDF-1 concentrations of <200 and ≥350 pg/mL ($P < 0.05$). The follicular concentration of VEGF was not associated with the blastocyst morphology.

Conclusion: Our findings showed that follicular concentration of SDF-1, and not VEGF, may be a valuable biochemical marker of blastocyst development.

KEYWORDS

blastocyst development, embryo quality, follicular fluid, SDF-1/CXCL12, vascular endothelial growth factor

1 | INTRODUCTION

Oocyte quality is the most important factor determining the outcome of embryo development. The quality of an oocyte is influenced by the characteristics of follicular microenvironment such as the intrafollicular oxygen level and the degree of follicular vascularity.[1] Vascularization is considered to play an important role during folliculogenesis, because the oocytes retrieved from highly vascularized follicles have higher fertilization rates.[2,3] The ovary is a site of active angiogenesis, and the development of follicular microvasculature is regulated by the angiogenic factors present in the ovary.[4] Recently, we showed that the concentrations of stromal cell-derived factor-1 (SDF-1/CXCL12) and vascular endothelial growth factor (VEGF) in the follicular fluid (FF) increased with an enlargement in the follicular diameter and volume, thus suggesting that these angiogenic factors may play an important role in folliculogenesis.[5]

Vascular endothelial growth factor is a key regulator of angiogenesis. VEGF plays a potential role in the development of the perifollicular capillary network and may be a potential marker of the quality of follicular microenvironment. VEGF, which is produced by follicular granulosa and theca cells, and also known as vascular permeability factor, is a potent angiogenic factor and a mitogen for vascular

endothelium. The regulatory role of VEGF can be observed at almost all stages of follicular angiogenesis, from the development of the antral follicle to the formation and maintenance of the corpus luteum.[6,7]

The SDF-1 as well as VEGF plays important roles in angiogenesis. SDF-1, a CXC chemokine, is a potent chemoattractant for endothelial cells, leukocytes, and hematopoietic progenitor cells.[8] Previous studies have shown that SDF-1 is produced by the luteinizing granulosa cells present in the follicular aspirates of patients undergoing in IVF-ET.[9] VEGF and SDF-1 interact synergistically to promote the functions of vascular endothelial cells, such as cell migration, gene expression, and cell survival.[8,10,11] In a previous study, we found that a positive correlation exists between the concentrations of SDF-1 and VEGF in FF.[5]

In view of the pleiotropic functions of SDF-1 and VEGF, we hypothesized that the levels of these angiogenic factors are associated with the parameters of oocyte quality, such as fertilizability and developmental competence. In the present study, we investigated the association between the concentrations of SDF-1 and VEGF in individual follicles and the fertilization outcome and embryo development.

2 | MATERIALS AND METHODS

2.1 | Patients, stimulation protocol, and retrieval

This study was approved by the Institutional Review Board of Kansai Medical University. A total of 31 Intracytoplasmic Sperm Injection (ICSI) patients considered for blastocyst (BL) culture until day 5 were included in this study. FF samples were obtained at the time of oocyte retrieval following ovarian stimulation from women fulfilling the following inclusion criteria. We analyzed FF from 38 year or less women (range 25-38; mean ± SEM, 34.00 ± 0.60 years), because it has been reported that an elevated level of VEGF in FF correlated positively with the chronological age of the patient.[12] They had undergone between 0 and 2 previous IVF cycles (0.60 ± 0.13 cycles). All patients had both ovaries and regular menstrual cycles with normal ovulatory function as shown by cycle day 3 FSH concentration <10 mIU/mL (6.60 ± 0.73 mIU/mL), and ultrasonographic scanning indicative of ovulatory cycles. All of them had normal body mass index (20.26 ± 0.31 kg/m^2) and multiple follicular development and successful oocyte retrieval, the peak serum estradiol (E_2) concentration attained being 3552.46 ± 278.58 pg/mL and the number of oocyte obtained being 11.68 ± 0.60. The exclusion criteria were presence of very severe male infertility with the sperm concentrations <1 × 10^6/mL, because seminal characteristics may affect the embryo development.[13] The protocol for ovarian stimulation was described previously.[5]

2.2 | Samples collection

Follicular fluid was aspirated separately from each follicle during oocyte retrieval. The collection tube was flushed to prevent contamination from sample to sample. Any follicle aspirates that were no clear or contaminated with blood were discarded. After removal of the oocytes, the clear FF samples were immediately centrifuged for 10 minutes at 1000 *g* and were stored at −80°C for further analysis.

2.3 | Fertilization and culture

Immediately after retrieval, all oocytes were cultured in HFF media (Fuso Pharmaceutical Industries, Ltd. Osaka, Japan) containing 10% LGPR (LifeGlobal, Guelph, ON, Canada) at 37°C in an atmosphere of 5% CO_2, 5% O_2 in air. After a period of 4 hours in culture, the collected oocytes were briefly exposed to 80 IU/mL hyaluronidase (SynVitro® Hyadase; Medicult, Jyllinge, Denmark) to facilitate mechanical removal of the surrounding cumulus cells. Oocyte was checked for nuclear maturity, and ICSI was performed for MII oocyte. Oocyte was cultured individually after injection. The fertilization assessment was performed 16-18 hours after ICSI and the presence of two pronuclei (2PN) as well as two polar bodies characterized normal fertilization. Zygotes and embryo were cultured individually in global media (LifeGlobal) containing 10% LGPR. Embryo grading was based on the criteria described by Veeck.[14] BL was evaluated according to a previously published scoring system.[15] We analyzed the proportion of full BLs and good-quality BLs on day 5 as described previously.[16] We calculated the number of BL forming three or more per total number of cleavage embryo as the rate of full BL and the number of BL forming three or more (AA, AB, BA, or BB) per total number of cleavage embryo as the rate of good-quality BL.

2.4 | Biochemical assay

Concentrations of SDF-1 and VEGF in FF were determined with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Duoset® ELISA human CXCL12/SDF-1 and human VEGF; R&D Systems, Minneapolis, MN). Intra- and inter-assay coefficients of variation (CVs) in FF were 3.3% and 7.2% for SDF-1, 2.2% and 8.9% for VEGF, respectively. All the procedures were performed according to the manufactures instructions.

2.5 | Statistical analysis

Comparative evaluation of fertilization rate, cleavage rate, full BL rate, and good- quality BL rate between groups was done using χ^2 analysis or Fisher's exact test. Differences in the measured parameters across the different groups were statistically assessed using ANOVA with repeated measurements, followed by Fisher's protected least significant difference, multiple range test. Data are expressed as mean ± SEM. Results were analyzed with a statistical software package, Stat View II version 4.0 (Abacus Concepts, Berkeley, CA). A level of $P < 0.05$ was considered statistically significant.

3 | RESULTS

We analyzed 261 FF samples with oocytes at the MII stage from 31 women undergoing ICSI. Of the 261 oocytes at the MII stage,

200 were successfully fertilized; all these 200 oocytes had 2PN and developed into growing embryos. Of the 61 residual oocytes, 18 had three or one PN, 17 failed to fertilize, and 26 degenerated after ICSI. We measured the concentrations of SDF-1 and VEGF in the FF samples. The mean ± SEM concentrations of SDF-1 and VEGF in FF between different fertilization outcomes and different embryo development outcomes were not significantly difference ($P > 0.1$). These results are suggested that more detailed data need to investigate the association between the concentrations of these angiogenic factors and the fertilization outcome and embryo development. Therefore, a possible relation between the concentrations of these factor and IVF outcomes was evaluated by dividing SDF-1 and VEGF concentrations into five intervals creating five approximately similar sized groups as described previously.[17]

3.1 | Fertilization and embryo morphology

The fertilization rates, cleavage rates, and the embryo morphology on day 3 of the SDF-1 and VEGF concentration groups are shown in Tables 1 and 2, respectively. The FF concentrations of SDF-1 and VEGF were not significantly associated with fertilization and cleavage outcomes, blastomere number, and the embryo grade.

3.2 | Blastocyst morphology

The numbers of full BLs and good-quality BLs on day 5 in the SDF-1 and VEGF concentration groups are shown in Figures 1 and 2, respectively. The rates of full BLs and good-quality BLs were significantly higher in the follicles with an SDF-1 concentration of 275-350 pg/mL than in the follicles with SDF-1 concentrations of <200 and ≥350 pg/mL ($P < 0.05$). The FF concentrations of VEGF were not significantly associated with the BL morphology.

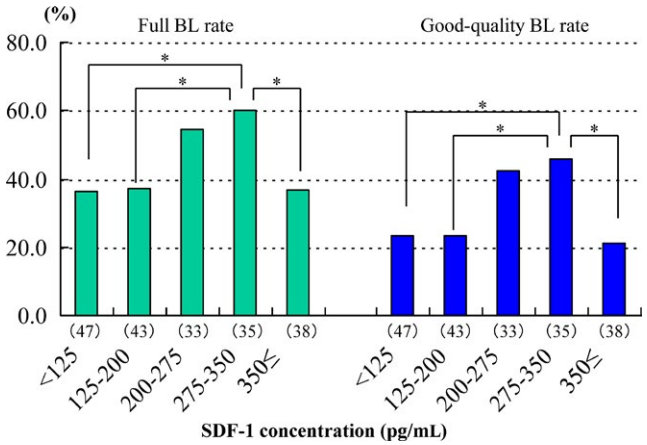

FIGURE 1 Association with SDF-1 concentration in follicular fluid and blastocyst (BL) morphology at 120 h. Numbers in parentheses indicate the number of individual follicles. Statistically significant differences are indicated by brackets: *$P < 0.05$

4 | DISCUSSION

In the present study, we determined the concentrations of SDF-1 and VEGF in the preovulatory follicles of women who were undergoing

TABLE 1 Association with SDF-1 concentration in follicular fluid and clinical data

SDF-1 (pg/mL)	<125 (n = 61)	125-200 (n = 60)	200-275 (n = 42)	275-350 (n = 44)	350≤ (n = 54)
Day 1					
Fertilization rate (%)	78.7	71.7	81.0	81.8	72.2
Day 3					
Cleavage rate (%)	97.9	100.0	97.1	97.2	97.4
Number of blastomeres	8.4 ± 0.4	8.0 ± 0.4	8.5 ± 0.4	7.8 ± 0.3	8.0 ± 0.4
Embryo grade	1.9 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.1 ± 0.1

Values are means ± SEM. Differences were not statistically significant.

TABLE 2 Association with vascular endothelial growth factor (VEGF) concentration in follicular fluid and clinical data

VEGF (pg/mL)	<180 (n = 51)	180-270 (n = 48)	270-360 (n = 67)	360-450 (n = 38)	450≤ (n = 57)
Day 1					
Fertilization rate (%)	74.5	77.1	77.6	73.7	78.9
Day 3					
Cleavage rate (%)	97.4	100.0	98.1	96.4	97.8
Number of blastomeres	8.3 ± 0.4	8.1 ± 0.3	8.1 ± 0.3	8.7 ± 0.5	7.7 ± 0.3
Embryo grade	2.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	2.2 ± 0.1

Values are means ± SEM. Differences were not statistically significant.

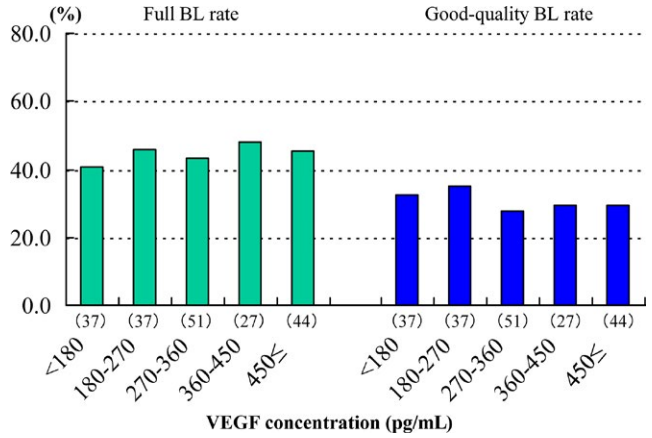

FIGURE 2 Association with vascular endothelial growth factor (VEGF) concentration in follicular fluid and blastocyst (BL) morphology at 120 h. Numbers in parentheses indicate the number of individual follicles. Differences were not statistically significant

ICSI. We also evaluated the relationship between the FF concentrations of these angiogenic factors and embryo development. This is the first study to show that the FF concentration of SDF-1, and not VEGF, is closely related to the BL morphology on day 5. The rates of full BLs and good-quality BLs increased with an enlargement in the FF concentration of SDF-1, reaching a maximum in the follicles with an SDF-1 concentration of 275-350 pg/mL. However, the follicular concentrations of SDF-1 and VEGF were not significantly associated with fertilization and cleavage outcomes, blastomere number, and the embryo grade on day 3.

During follicular development, an oocyte is exposed to the intrafollicular environment containing various elements like steroid hormones, cytokines, and growth factors. These factors, which are secreted or transported into the FF, play an important role in the development of a mature and competent oocyte.[18,19] Therefore, it is important to study these factors in detail at the time of oocyte recovery in order to study their role in folliculogenesis. Recently, we showed that locally produced angiogenic factors such as VEGF and SDF-1 participate in follicular growth and development via angiogenesis. A low oocyte recovery rate has been observed in the follicles with SDF-1 or VEGF concentrations of <100 pg/mL; the recovery rates increased with the follicular concentrations of SDF-1 and VEGF.[5] Some studies have hinted at the possibility of a significant correlation between VEGF concentrations and the grade of perifollicular vascularity.[3] Higher VEGF concentrations may indicate increased ability of the follicle to create its own vascular network.

Some reports have discussed the relationship between VEGF concentration in the FF and embryo development.[22,23] To the best of our knowledge, our study is the first to evaluate the relationship between VEGF concentration in the FF and BL morphology. Our study was designed to include only women aged <38 years, because a positive correlation has been reported between the elevated levels of VEGF in the FF and chronological age of patients.[12] All the patients had undergone identical procedure for controlled ovarian stimulation and had developed adequate response. Nevertheless, our study failed to show any significant association between VEGF concentration in the FF and fertilization rate and embryo development. Our findings are consistent with those of some previous studies that reported an insignificant association between the follicular VEGF concentration, and fertilization outcome and embryo quality.[18,22] Like our work, these studies adopted criteria such as chronological age.[22] However, Barroso et al[24] showed that VEGF concentration in the FF negatively correlated with the embryo morphology on day 3 in unselected patients. Some previous studies, which support the results of Barroso et al,[24] have shown that elevated VEGF concentration in the FF is a potential marker of diminished pregnancy and that it negatively correlates with embryo quality.[25]

In the present study, the SDF-1 concentration in the FF was found to be associated with the numbers of full BLs and good-quality BLs on day 5, but not with embryo morphology on day 3. This may be attributed to the differences between the morphological evaluations conducted on days 3 and 5, because BL culture permits the selection of more viable embryos. It is suggested that the morphological criteria which govern embryo selection on day 3 are highly subjective and do not reflect the quality of embryos as accurately as the criteria used on day 5 do.[26,27] Embryo quality is an important predictor for IVF treatment, because a high-quality embryo increases the pregnancy rate.[16,29] Therefore, SDF-1 concentration in the FF might be an early predictor of BL formation.

The disparity between the numbers of full BLs and good-quality BLs in the SDF-1 and VEGF concentration groups indicates the existence of distinct control mechanisms of these factors during folliculogenesis. VEGF is locally secreted from granulosa and theca cells of the human ovary, whereas SDF-1 is produced only by granulosa cell.[6,9] VEGF plays a central role in the regulation of ovarian angiogenesis and is critical for the growth of ovarian follicles. On the other hand, SDF-1 may not only play an important role in ovarian angiogenesis but also contribute to T-lymphocyte recruitment and coordinates with local lymphocytes to enhance granulosa cell survival and embryo quality.[9] Elevated SDF-1 level indicates an increased ability of the follicle to create its own vascular and immune networks, and therefore, guarantees an improved follicular microenvironment for the developing oocyte.

SDF-1 serves as the ligand to a single receptor-the seven transmembrane G-protein-coupled receptor CXCR4.[30] SDF-1 and CXCR4 mRNA and protein have been reported to present highly similar expression patterns in oocytes and granulosa cells.[31] In addition, SDF-1 directly promotes key periovulatory genes in cumulus-oocyte complexes. Holt et al[32] demonstrated that incubation with recombinant SDF-1 results in a dose-dependent increase in follicle densities and inhibition of CXCR4 reduced follicle densities back to control levels. Zhang et al[31] also showed that inhibition of CXCR4 suppressed oocyte nuclear maturation while an addition of recombination SDF-1 significantly increased percent of oocyte undergone metaphase 1 phase. However, the mechanisms by which the actions of SDF-1 during folliculogenesis are related to the late stages of embryo development are not still well understood.

In conclusion, we showed that FF concentration of SDF-1, and not VEGF, may be a valuable biochemical marker of blastocyst development after ICSI. This knowledge will provide new insights into our understanding of the aspects of ovarian physiology, such as follicular development and oocyte competence.

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DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human and animal rights*: All the procedures were followed in accordance with the ethical standards of the institutional ethical committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all the patients who underwent IVF treatment in the study. This study was approved by the Institutional Review Board at Kansai Medical University. This article does not contain any study that was performed by any of the authors that included animal participants.

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