Follicular fluid growth differentiation factor-9 concentration and oocyte developmental competence

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Objective: To investigate the correlation between concentrations of growth differentiation factor (GDF)-9 in single follicular fluid (FF) and the corresponding oocyte developmental competence.

Methods: Forty-eight infertile women participating in vitro fertilization procedure between March 2007 and July 2009 were recruited when at least one mature oocyte was obtained and the corresponding FF from single follicle was available. Patients were stimulated with recombinant follicle stimulating hormone and the pituitary was suppressed by gonadotropin-releasing hormone agonist long or antagonist protocol. The levels of GDF-9 were estimated by a sandwich enzyme-linked immunosorbent assay using commercial monoclonal antibody.

Results: The median concentration of FF GDF-9 did not differ according to occurrence of fertilization (7.4 vs. 1.9 ng/mL, P=0.665). By using receiver operating characteristic curve analysis, level of FF GDF9 could not predict the occurrence of fertilization or the formation of good-quality embryo. The concentration of FF GDF9 was not correlated with the matched embryo score.

Conclusion: Level of FF GDF9 might not be clinically useful to predict oocyte developmental competence.

Key words: Growth differentiation factor-9; Follicular fluid; Oocyte; Fertilization

Introduction

Growth differentiation factor (GDF)-9 is an oocyte-secreted growth factor and is known to be critical local regulators of ovarian function and preovulatory cumulus expansion.1-3 It belongs to the transforming growth factor (TGF)-β superfamily and is believed to be important for follicular growth beyond the primary stage.

In human, GDF-9 expression is first observed in primary follicles, not in primordial follicles.4 GDF-9 has been shown to promote ovarian follicle growth in vitro and to be mitogenic for granulosa and theca cells in rat as well as in human,5,6 although this effect was not always observed.1 GDF-9 seems to be anti-apoptotic in rat preantral follicles, thus promoting follicular survival and growth during the preantral to early antral transition.7 GDF-9 also induces cumulus expansion in mice,8-10 and supplementation of GDF-9 in oocyte maturation media enhances subsequent embryo development and fetal viability in mice.11 Exogenous administration of GDF-9 can modulate steroidogenic function of granulosa cells. GDF-9 has been shown to stimulate inhibin production in granulosa cells from rat and human.5,12-14 GDF-9 inhibits follicle stimulating hormone (FSH)-induced progesterone production in
granulosa cells from rat, cattle and human.\textsuperscript{15-17} Since GDF-9 suppressed expression of P450arom mRNA in human granulosa cells, it is likely that GDF-9 could affect estradiol secretion.\textsuperscript{17} Inconsistency also exists in the effect of GDF-9 on theca cell function where stimulation\textsuperscript{18} and inhibition\textsuperscript{17} of steroidogenesis has been observed.

GDF-9 is also involved in the interconnections between oocytes and adjacent cumulus cells and regulates amino acid uptake, glycolysis and cholesterol biosynthesis from cumulus cells via gap junctions to the oocyte.\textsuperscript{2}

Although there are accumulating evidences that GDF-9 involves ovarian folliculogenesis, steroid production, and oocyte maturation, studies on the clinical significances of GDF-9 in infertile women are sparse. In situ hybridization study showed that the expression of GDF-9 was lower in oocytes from women with polycystic ovary syndrome (PCOS) compared with that from normal women,\textsuperscript{19} although a subsequent study did not find a significant difference of GDF-9 expression at protein level.\textsuperscript{20} GDF-9 is also detected in follicular fluid (FF) of women with ovarian endometriosis,\textsuperscript{21,22} and its concentration in women with severe endometriosis was lower than in women without endometriosis.\textsuperscript{22}

In the present study, we investigated the direct relationship between the concentrations of GDF-9 in FF and corresponding oocyte developmental competence.

**Materials and Methods**

Our study involved forty-eight infertile women participating in vitro fertilization (IVF) procedure between March 2007 and July 2009. The cycles were included when at least one mature oocyte was obtained and the corresponding FF from single follicle was available. All women were 40 years old or less; the averaged age was 34.6±3.4 year (range, 27 to 40 year). Thirty-nine women were nulliparous including 26 nulligravida. Twenty-seven women had no previous experience of IVF. The indications for IVF were identified as unexplained (n=19), tubal (n=18), male (n=6), polycystic ovary syndrome (n=3), and diminished ovarian reserve (n=2). Informed consent was obtained from all study participants. The study was approved by the Institutional Review Board of Seoul National University Hospital.

Patients were stimulated with recombinant FSH (Gonal -F; Merck-Serono, Geneva, Switzerland). The pituitary was suppressed by gonadotropin-releasing hormone (GnRH) agonist long protocol (n=3) or antagonist protocol (n=45). For GnRH agonist long protocol, daily injection of 0.1 mg trip-torelin acetate (Decapeptyl; Ferring, Saint-Prex, Switzerland) was started in the mid-luteal phase of the previous cycle. After confirmation of pituitary down-regulation, gonadotropin injections were initiated on cycle day 2 or 3. The initial gonadotropin dose was fixed for the first 4 or 5 days, followed by adjustment on the basis of individual follicular growth and estradiol (E2) levels until triggering. For the GnRH antagonist protocol, gonadotropin was started on cycle day 2 or 3. When the leading follicle reached a diameter of 14 mm, cetrorelix 0.25 mg (Cetrotide; Merck-Serono) was added daily until triggering.

When at least three follicles reached 17 mm or two follicles reached 18 mm in diameter, exogenous human chorionic gonadotropin (Ovidrel; Merck-Serono) was administered and oocytes were retrieved via transvaginal route 36 hours later. At the time of oocyte retrieval, single dominant follicle (>17 mm in diameter) was firstly punctured. If one intact mature oocyte was present and the FF was not contaminated by visible blood, the FF was isolated. To prevent contamination from other follicles, FF was collected from only one follicle for each ovary, and different needle was used for each side of ovary. All FF samples were centrifuged at 1,300 g for 10 minutes and then stored at -80°C until the assay.

IVF and subsequent culture of embryos were performed as previously reported.\textsuperscript{23} In all of study population, ejaculated sperms were used for fertilization. The mean of total motile sperm count was 54.9×10^6. Intracytoplasmic sperm injection (ICSI) was used in six cycles. Individual embryo quality were evaluated and graded by morphological criteria:

- Grade A: symmetrical blastomers and no fragmentation.
- Grade B: slightly uneven blastomers and <20% fragmentation.
- Grade C: uneven blastomers and >20% fragmentation.
- Grade D: uneven blastomers and >50% fragmentation.

Good-quality embryos were defined as those with morphologic grade A or B. The embryo score was also calculated by multiplication of morphological grade of embryo (grade A=4, grade B=3, grade C=2, grade D=1) by the number of blastomeres.

The levels of GDF-9 in FF were estimated by a sandwich enzyme-linked immunosorbent assay (ELISA) using commercial monoclonal antibody (E0427Hu; Uscn Life Science Inc., Wuhan, China). The samples were incubated with microplate wells pre-coated with an antibody specific to GDF-9. After 60 minutes incubation and washing, monoclonal anti-human beta2-microglobulin antibody labeled with horseradish peroxidase was added to the wells and in-