Growth Rate of Dominant Follicles During Controlled Ovarian Hyperstimulation

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The advent of ultrasonography provided a non-invasive means of visualizing human ovaries. Use of ultrasonographic evaluation for follicle development and ovulation has been well documented during natural cycles.1-7 Ultrasonographic evaluations of follicle growth are used to complement hormonal estimations of ovarian status in women undergoing ovulation induction for the treatment of infertility.8-11

Follicles have been reported to grow in a linear fashion during a menstrual cycle,12 and the growth rates varying from one to four millimeter per day have been documented, with some researchers reporting increases or decreases in the growth rates of ovulatory follicles during the few days leading up to ovulation.1,2,7,8,13-15

There are few data on the relationship between the rates of growth of ovarian follicles and clinical characteristics or outcome during ovulation induction for in
vitro fertilization (IVF). The objective of this study was to elucidate the correlation of the growth rates of the dominant follicles with possibly associated factors in women undergoing controlled ovarian hyperstimulation (COH).

**MATERIALS AND METHODS**

**1. Patients**

A total of 216 patients underwent 313 cycles of COH for IVF at Seoul National University Hospital from 2006 to 2009. Two hundred fifteen cycles in 144 patients underwent gonadotropin releasing hormone (GnRH) agonist long protocols, and 98 cycles in 72 patients GnRH antagonist protocols. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) were measured on the day 3 of menstrual cycle. Serial ultrasonographic measurement of the diameter of growing follicles was performed.

**2. COH protocols**

For the GnRH agonist long protocol, GnRH agonist triptorelin (Decapeptyl, 0.1 mg/d; Ferring, Malmo, Sweden) was started in the mid-luteal phase of the previous cycle. After pituitary down-regulation, the triptorelin dose was reduced to 0.05 mg/d, and recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) was added until either the leading follicle reached a mean diameter of 18 mm or two or more follicles reached a diameter of 17 mm. For the GnRH antagonist multiple-dose flexible protocol, recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) was added on the 2nd or 3rd menstrual-cycle day without previous oral contraceptive pretreatment. The GnRH antagonist cetrotelix (Cetrotide, 0.25 mg; Serono) was added daily, starting when the leading follicle reached a diameter of 14 mm and until either the leading follicle reached a mean diameter of 18 mm or two or more follicles reached a diameter of 17 mm.

For both protocols, urinary hCG (Pregnyl, 10,000 IU, IM; Organon, Oss, the Netherlands) was administered 36 hours before transvaginal oocyte retrieval. Follicle growth rate was determined by calculating the slope of the change in leading follicular diameter (in millimeters) from stimulation day 5 or 6 to hCG day: Follicle growth (mm/day) = [Leading follicle diameter at hCG day - Leading follicle diameter at stimulation day 5 or 6]/ Number of days from stimulation day 5 or 6 to hCG day.

**3. IVF/Intracytoplasmic sperm injection (ICSI)**

Retrieved oocytes were cultured for 4 to 6 hours until insemination. In the cases of ICSI, after cumulus cells were removed by hyaluronidase (Sigma, St. Louis, MO, USA), the oocytes were evaluated for their maturity using an inverted microscope (Hoffman modulation, TE2000, Nikon, Tokyo, Japan). Only metaphase II oocytes, from which the first polar body was extruded, were used for ICSI. Semen samples obtained by ejaculation in the morning of the oocytes retrieval day were liquefied at room temperature for 30 minutes, and centrifuged with SpermGrad (Vitrolife, Kungsbacka, Sweden) made of two gradient (45%/90%) at 1,500 rpm for 20 minutes. After removal of supernatant, we layered 2 mL of Universal IVF medium over the sperm pellet to centrifuge again at 1,000 rpm for 10 minutes. After washing and swim-up procedure, the only sperm pellet in the supernatant were aspirated and used for insemination. Fertilization was determined by the presence of 2 pronuclei (2PN) using an inverted microscope on the first day after insemination. Zygotes with 2PN were cultured individually in microdrops of 25 μL of growth medium, G-1™ v5 (Vitrolife) overlaid with 8 mL of mineral oil (Sigma, USA) in Falcon 1007 culture dishes (Becton Dickinson Labware, Franklin Lakes, New Zealand) at 37°C under 6% CO₂.
4. Embryo transfer (ET)

ET was performed 3 days after oocytes retrieval. Embryos were graded, according to their morphologies and cleavage rates. Embryos were graded from one to five (I, II, III, IV, V), based on number and uniformity of blastomere, and percentage of fragmentation, according to Veeck's classification system.\textsuperscript{16} We defined top-quality embryos as those of morphologic grade I/V. After embryo grading, up to 4 embryos were selected and transferred into the uterus. The luteal phase was supported daily with progesterone in oil (Progest, 50 mg; Samil, Seoul, Korea) or with 8% progesterone gel (Crinone, Serono), initially for 14 days, starting on the day of oocyte retrieval, and continuing for another 6–8 weeks in cases in which a pregnancy was achieved. Clinical pregnancy was defined by the presence of an intrauterine gestational sac with pulsating fetal heartbeats at 3 to 4 weeks after oocyte retrieval. Fertilization rate was defined as number of 2PN divided number of retrieved oocytes.

5. Statistical analysis

Correlations between growth rates of the dominant follicles and possibly associated factors were analyzed. Correlations between different parameters were determined by bivariate correlation analysis and are expressed as Pearson's correlation coefficients. The statistical

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (Mean±SD)</th>
<th>GnRH agonist long protocol (Mean±SD)</th>
<th>GnRH antagonist multiple-dose flexible protocol (Mean±SD)</th>
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<tbody>
<tr>
<td>Screening</td>
<td></td>
<td></td>
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<tr>
<td>Age (yr)</td>
<td>35.0±4.4</td>
<td>34.6±4.4</td>
<td>35.9±4.4</td>
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<tr>
<td>Body mass index (kg/m\textsuperscript{2})</td>
<td>21.8±3.7</td>
<td>21.5±3.1</td>
<td>22.6±4.6</td>
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<tr>
<td>Basal LH (mIU/mL)</td>
<td>3.3±1.8</td>
<td>3.1±1.7</td>
<td>3.6±1.8</td>
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<tr>
<td>Basal FSH (mIU/mL)</td>
<td>5.0±3.3</td>
<td>4.2±2.2</td>
<td>6.8±4.4</td>
</tr>
<tr>
<td>Basal E\textsubscript{2} (pg/mL)</td>
<td>32.7±29.5</td>
<td>33.8±30.0</td>
<td>30.2±28.2</td>
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<tr>
<td>Treatment</td>
<td></td>
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<tr>
<td>Duration of stimulation (day)</td>
<td>9.5±1.9</td>
<td>10.0±1.7</td>
<td>8.5±1.9</td>
</tr>
<tr>
<td>No. of ampules of FSH</td>
<td>30.8±13.9</td>
<td>32.1±12.9</td>
<td>28.0±15.6</td>
</tr>
<tr>
<td>E\textsubscript{2} on day of hCG (pg/mL)</td>
<td>1,925.0±1,351.0</td>
<td>2,075.1±1,404.8</td>
<td>1,410.1±1,035.0</td>
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<td>Outcome</td>
<td></td>
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<tr>
<td>Follicle growth rate (mm/day)</td>
<td>1.5±0.3</td>
<td>1.4±0.3</td>
<td>1.5±0.4</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>9.4±6.5</td>
<td>9.9±6.6</td>
<td>8.1±6.2</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>53.4±27.7</td>
<td>51.8±27.9</td>
<td>56.7±27.3</td>
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<tr>
<td>Clinical pregnancy rate (%)</td>
<td>25.2</td>
<td>27.4</td>
<td>20.4</td>
</tr>
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</table>

GnRH, gonadotropin releasing hormone; SD, standard deviation; LH, luteinizing hormone; FSH, follicle stimulating hormone; E\textsubscript{2}, estradiol; hCG, human chorionic gonadotropin.

software package SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis, and results were considered statistically significant at \( p \)-values of <0.05.

**RESULTS**

Mean age of the patients was 35.0±4.4 years. Mean body mass index (BMI) was 21.8±3.7 kg/m². Mean basal serum LH, FSH, and E2 levels were 3.3±1.8 mIU/mL, 5.0±3.3 mIU/mL, and 32.7±29.5 pg/mL, respectively. There were no significant differences in these variables between two protocols. Mean duration and total dosage of gonadotropin used were 9.5±1.9 days, 30.8±13.9 ampoules, respectively. In GnRH agonist long protocol, mean duration and total dosage of gonadotropin used were 10.0±1.7 days and 32.1±12.9 ampoules, and in GnRH antagonist protocol, 8.5±1.9 days and 28.0±15.6 ampoules, respectively. Mean E2 on the day of hCG was 1,867.0±1,334.9 pg/mL in total, 2,075.1±1,404.8 pg/mL in GnRH agonist long protocol, and 1,410.1±1,035.0 pg/mL in GnRH antagonist protocol, respectively. The mean growth rate of dominant follicles was 1.5±0.3 mm/day in total, 1.4±0.3 mm/day in GnRH agonist long protocol, and 1.5±0.4 mm/day in GnRH antagonist protocol, respectively. The mean number of oocytes retrieved was 9.4±6.5 in overall, 9.9±6.6 in GnRH agonist long protocol and 8.1±6.2 in GnRH antagonist protocol. The fertilization rate and clinical pregnancy rate were 53.4±27.7% and 25.2%, respectively (Table 1).

No significant correlation was found between growth rates of the dominant follicles and age (\( r = -0.019, p = 0.743 \)), BMI (\( r = 0.012, p = 0.839 \)), Basal LH (\( r = 0.046, p = 0.414 \)), FSH (\( r = 0.079, p = 0.165 \)), and E2 (\( r = 0.001, p = 0.980 \)), retrieved oocytes (\( r = -0.071, p = 0.701 \)), and fertilization rates (\( r = -0.022, p = 0.701 \)), and neither in GnRH agonist or GnRH antagonist protocol (Table 2).

**DISCUSSION**

Ovarian follicles comprise oocytes surrounded by granulosa cells. Follicular growth is achieved by a small increase in oocyte volume and predominantly by the

<table>
<thead>
<tr>
<th>Table 2. Correlation between follicular growth rate and other factors</th>
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<tr>
<td><strong>Correlation with follicular growth rate</strong></td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td><strong>r</strong></td>
</tr>
<tr>
<td>Age (yr)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
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<tr>
<td>Basal LH (mIU/mL)</td>
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<tr>
<td>Basal FSH (mIU/mL)</td>
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<tr>
<td>Basal E2 (pg/mL)</td>
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<tr>
<td>No. of oocytes retrieved</td>
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<tr>
<td>Fertilization rate (%)</td>
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GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, estradiol. \(^*\) \( r \) = partial correlation coefficient adjusted by duration of stimulation and number of ampoules of FSH.

proliferation of surrounding gonadotropin-responsive granulosa cells and the expansion of antral cavity. A small proportion of follicles develop into healthy antral follicles. These follicles are highly sensitive and responsive to FSH. Majority of recruited follicles enter into atresia. During a spontaneous menstrual cycle, a single antral follicle is selected and becomes dominant, whereas the growth of multiple follicles is supported by exogenous gonadotropins in assisted reproduction cycles. The follicular diameter increases to approximately 20 mm before ovulation.

The occurrence of elevated FSH levels in women of older reproductive age may lead to advancement of normal dominant follicle growth, as in older ovulatory women who showed shorter follicular phase and overall cycle length. Previous studies suggested that this shorter length in old subjects results from earlier dominant follicle selection, independent of hormonal influences. Our present study demonstrates that no significant correlations between age of patients and follicular growth rate. These results suggest that age has no significant effect in regard to follicular growth rate in COH cycle with pituitary suppression.

The negative impact of obesity in the outcome of assisted reproductive technology has been suggested by multiple reports. Obese patients undergoing COH are known to have increased FSH requirement, fewer collected oocytes, frequent cycle cancellation, lower pregnancy rate, and increased miscarriage rate. However, they did not address the correlation between the BMI and follicular growth rate. While being obese, one can speculate, might have associated with ovarian follicle growth, our data showed that the follicular growth rate was not affected by BMI.

We intended to search for any correlation between follicular growth rate and basal hormone level, ovarian response or fertilization rate, however, no significant correlation was observed. Although our results suggest that the follicular growth rate has no correlation with them in COH cycles, these factors may have significance in natural cycle, which is subject to future investigations.

In conclusion, our retrospective study observed that the growth rates of dominant follicles have no correlation with clinical characteristics or outcome variables in COH cycles using GnRH agonist or GnRH antagonist.

Acknowledgement

The authors would like to express our deepest gratitude to Sun Kyung Oh, Hee Sun Kim, Moon Ju Kang, Sung Ah Kim for their laboratory support of this study.

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ultrasonography and total serum estrogen in human meno-
Abstract

Objective: To evaluate if there is any correlation between the growth rate of dominant follicles and clinical characteristics or outcome variables in women undergoing controlled ovarian hyperstimulation (COH).

Methods: This study was performed in 313 in vitro fertilization (IVF) cycles. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) were measured on day 3 of menstrual cycle, and serial ultrasonographic measurement of the diameter of growing follicles was performed. The growth rates of dominant follicles calculated by diameter difference divided by days were correlated with clinical characteristics and outcome variables.

Results: There was no significant difference in the growth rate of the dominant follicles between gonadotropin releasing hormone (GnRH) agonist and antagonist cycles. No significant correlation was found between the growth rates and evaluated factors such as age, body mass index, LH, FSH, E2, retrieved oocytes and fertilization rate.

Conclusion: The Growth rate of dominant follicles seems to show an independent feature of basal characteristics and ovarian response.

Key Words: Follicle growth, Controlled ovarian hyperstimulation