Comparison of follicular fluid IGF-I, IGF-II, IGFBP-3, IGFBP-4 and PAPP-A concentrations and their ratios between GnRH agonist and GnRH antagonist protocols for controlled ovarian stimulation in IVF-embryo transfer patients

Young Sik Choi¹, Seung-Yup Ku¹,², Byung-Chul Jee¹, Chang-Suk Suh¹,², Young Min Choi¹,², Jung Gu Kim¹, Shin Yong Moon¹,² and Seok Hyun Kim¹,²,³

¹Department of Obstetrics and Gynecology, College of Medicine and ³Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Korea

³To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, College of Medicine, Seoul National University, 28 Yeongeon-Dong, Jongno-Gu, Seoul, 110–744, Korea. E-mail: seokhyun@snu.ac.kr

BACKGROUND: Insulin-like growth factors (IGF) and their binding proteins (IGFBP) play a major role in the autocrine and paracrine regulation of folliculogenesis. This is the first study that has compared follicular fluid (FF) IGF-I, IGF-II, IGFBP-3, IGFBP-4 and pregnancy-associated plasma protein (PAPP)-A concentrations, and their ratios, to investigate whether there was any difference in the intrafollicular microenvironment between the GnRH agonist (GnRHa) and antagonist (GnRHant) protocols for controlled ovarian stimulation (COS). METHODS: A total of 68 IVF cycles were included in this study; two groups were studied: GnRHa long protocol group (n = 36) and the flexible GnRHant multiple-dose protocol group (n = 32). FF was obtained from dominant follicles during oocyte retrieval and stored at –70°C until assayed. IGF-I, IGF-II and IGFBP-3 concentrations were measured by radioimmunoassay and IGFBP-4 and PAPP-A by enzyme-linked immunosorbent assay. RESULTS: The duration of COS was significantly longer, and total dose of gonadotrophins used, serum estradiol (E₂) levels on hCG day and the number of oocytes retrieved were significantly higher in the GnRHa long protocol group. The concentrations of FF IGF-II and IGFBP-4 were significantly higher, and the ratio of IGF-I/IGFBP-4 was significantly lower in the GnRHa long protocol group. Serum E₂ levels per mature follicle were not different between the two groups. CONCLUSIONS: Our data may indicate a difference of intrafollicular microenvironment between cycles using GnRHa long protocols and those using GnRHant protocols. However, the difference in microenvironment does not appear to result in a difference in clinical outcome.

Key words: GnRH agonist and antagonist/IGF/IGFBP/IVF-embryo transfer/PAPP-A

Introduction

GnRH agonists (GnRHa) and antagonists (GnRHant) have been widely used for the prevention of premature LH surges during controlled ovarian stimulation (COS) for IVF and embryo transfer (Porter et al., 1984; Diedrich et al., 1994). Before GnRHa suppress gonadotrophin secretion through pituitary desensitization and GnRH receptor depletion, they initially stimulate gonadotrophin secretion (flare-up effect), whereas GnRHant compete directly with endogenous GnRH for receptor binding and therefore rapidly inhibit secretion of gonadotrophins and steroid hormones. Most published studies, comparing agonist and antagonist cycles, have shown equivalent results or only slightly worse outcomes with antagonist protocols. Two meta-analyses showed significantly lower pregnancy rates in antagonist protocols (Ludwig et al., 2001; Al-Inany and Aboulghar, 2002). A meta-analysis conducted by Ludwig et al. (2001) showed significant decrease in the clinical pregnancy rate in cycles using GnRHant protocols compared to those using GnRHa long protocols. In a subgroup analysis, however, comparison of GnRHant cetrorelix with GnRHa showed no difference in the clinical pregnancy rate, while ganirelrix showed a significantly lower pregnancy rate as compared to the GnRHa. In a Cochrane review by Al-Inany and Aboulghar (2002), GnRHant showed a significantly lower pregnancy rate compared to GnRHa. In many reports, including two meta-analyses, GnRHant protocols showed a significantly lower serum estradiol (E₂) level on the day of hCG administration and resulted in a significantly lower number of
Expression of GnRH receptors has been demonstrated in numerous extrapituitary tissues (Kakar et al., 1992; Ortmann and Diedrich, 1999). GnRH can stimulate mitogen-activated protein kinase in human granulosa luteal cells and may act as an autocrine factor (Kang et al., 2000, 2001). GnRH receptor mRNA was detected in the human ovary using RT-PCR (Kakar et al., 1992; Peng et al., 1994; Minaretzis et al., 1995). Studies on the regulation of the GnRH receptor mRNA have found that GnRH up-regulates and hCG down-regulates GnRH receptor and its gene expression (Peng et al., 1994). Brus et al. (1997) suggested that GnRH receptors were predominantly found in ovarian tissue after the LH surge, and GnRH receptors could not be detected in preovulatory follicles. Therefore, although it is not clear whether GnRH receptors in the human ovary are functional, it is possible that GnRHant may disrupt autocrine and paracrine signalling of GnRH in human ovarian cells, change the intrafollicular microenvironment and account for differences in the outcomes of COS and IVF-embryo transfer compared to GnRHa.

The ovarian insulin-like growth factor (IGF) system consists of IGF-I and IGF-II, their binding proteins, IGFBP-1 to -6, IGFBP protease and their two receptors in target cells (Wang and Chard, 1999; Hull and Harvey, 2001; Poretsky et al., 2005). The IGF system plays a major role in the autocrine and paracrine regulation of follicular and embryonal development. IGF-I stimulates steroidogenesis in synergy with gonadotrophins and the activity of aromatase; in addition, it also modulates the effects of FSH and LH. IGF-II is the most abundant IGF identified in human ovarian follicles (Hernandez et al., 1992; el-Roeiy et al., 1993). It plays a role in the enhancement of gonadotrophin action, granulosa cell proliferation, aromatase activity and steroidogenesis. The synthesis of IGF-II in the estrogen-dominant follicle increases dramatically at the time of follicle selection (Giudice, 2001). IGFBPs counteract the synergism of gonadotrophins and growth factors and prolong clearance time of IGFs. IGFBP-3 is a predominant IGFBP in serum and follicular fluid (FF); however, the role of IGFBP-3 in FF has not been clearly demonstrated. Although one study reported higher IGFBP-3 concentrations in estrogen-dominant follicles compared to androgen-dominant follicles (van Dessel et al., 1996), this was contradicted by another study (San Roman and Magoffin, 1993). IGFBP-4 is a low-molecular-weight IGFBP and plays a role in regulating intrafollicular bioavailability of free IGF. Specific IGFBP protease cleaves IGFBPs, allowing IGFs to bind to their receptors. Human IGFBP-4 protease, pregnancy-associated plasma protein (PAPP)-A, degrades inhibitory IGFBP and increases free IGF and E2 (Conover et al., 1999). Therefore, changes in any component of this complex IGF system could potentially affect follicular development.

Considering the slightly lower pregnancy rate, although controversial, lower serum E2 levels on the day of hCG administration and the lower number of oocytes retrieved in the GnRHant cycle as compared to the GnRHa cycle, there may be a difference in the intrafollicular IGF system which results in different outcomes for COS in IVF-embryo transfer. However, the influence of GnRHant on the human ovary and growth factors is at least partially responsible for folliculogenesis but has not been clearly demonstrated. Inhibitory effects of cetrorelix on growth factors such as epidermal growth factor (EGF), IGF-I and IGF-II and their receptors have been reported in extremely high (micromolar) concentrations under experimental conditions (Hernandez, 2000). By contrast, Weiss et al. (2003) investigated the actions of GnRHant of IGF-II, IGFBP-2 and PAPP-A in human granulosa cells from patients treated with different protocols in assisted reproduction. They concluded that GnRHant did not exert any significant effects on the IGF system of granulosa-lutein cells.

To date, however, there are few comparative reports on the levels of FF IGF system between GnRHa and GnRHant protocols. The aim of this study was to compare the ovarian FF IGF-I, IGF-II, IGFBP-3, IGFBP-4 and PAPP-A concentrations, and their ratios, and investigate whether there was any difference in the intrafollicular microenvironment between the cycles using GnRHa long protocols and those using GnRHant multiple-dose flexible protocols for COS.

**Materials and methods**

**Patients**

Among candidates undergoing COS with GnRHa or GnRHant protocols for IVF-embryo transfer from January 2003 to December 2004, only patients aged 27–39 years, with basal FSH (day 3) concentration = 15 IU/l, no history of poor ovarian response and BMI of 16-29 kg/m² were included in this study.

The following exclusion criteria were adopted: polycystic ovarian syndrome (PCOS) defined by new diagnostic criteria proposed in Rotterdam in 2003 (Rotterdam ESHRE/ASRM-sponsored PCOS consensus working group, 2004), stage III–IV endometriosis, cycles with dominant FF contaminated with blood during oocyte retrieval and the presence of only one ovary.

A total of 68 IVF cycles (64 patients) were included in this study. Patients were divided into two protocol groups, which were determined by clinician’s preference: GnRHa long protocol group (n = 36) and GnRHant multiple-dose flexible protocol group (n = 32).

This study was approved by the Institutional Review Board for human research of Seoul National University Hospital and informed consent was obtained from all patients.

**COS protocols**

In the GnRHa long protocol, GnRHa, triptorelin 0.1 mg/day was started in the mid-luteal phase of the previous cycle. After the pituitary down-regulation, the triptorelin dose was reduced to 0.05 mg/day, and recombinant FSH (Gonal-F, Serono, Switzerland) was added until the leading follicle reached a mean diameter of 18 mm or two follicles or more reached a diameter of 16 mm. In the GnRHant multiple-dose flexible protocol, recombinant FSH (Gonal-F, Serono, Switzerland) was started on the second or third menstrual cycle day without previous oral contraceptive pretreatment. GnRH antagonist, cetrorelix 0.25 mg, was added daily, starting when the leading follicle reached a diameter of 14 mm until the leading follicle reached a mean diameter of 18 mm or two follicles or more reached a diameter of 17 mm. In both protocols, urinary hCG 10 000 IU was administered intramuscularly 36 h before transvaginal oocyte retrieval. Up to four embryos were transferred 2 or 3 days after oocyte retrieval. The embryos were graded according to their morphology and cleavage rate. We define...
high-quality embryos as those with morphologic grade I/V or II/V and four or five blastomeres on day 2 and at least seven blastomeres on day 3 after fertilization. The luteal phase was supported with progesterone in oil 50 mg or 8% progesterone gel daily, initially for 14 days starting on the day of oocyte retrieval and continued for another 6–8 weeks in cases where a pregnancy was achieved. A clinical pregnancy was defined by the presence of an intrauterine gestational sac with pulsating fetal heart beats 3–4 weeks after oocyte retrieval.

**Laboratory assay**

FF was obtained from the dominant follicle with the largest mean diameter during oocyte retrieval and centrifuged at 250 g for 15 min to separate out cellular contents and debris. FF supernatant was transferred to sterile polypropylene tubes and stored at −70°C until assayed. FF contaminated with blood was excluded. IGF-I, IGF-II and IGFBP-3 concentrations were measured by immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX, USA). The intra- and inter-assay coefficients of variation were 3.9 and 3.8% for IGF-I, 3.4 and 4.5% for IGF-II and 3.9 and 1.9% for IGFBP-3, IGFBP-4 and PAPP-A were measured by enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories). The intra- and inter-assay coefficients of variation were 3.0 and 3.4% for IGFBP-4 and 3.8 and 3.7% for PAPP-A.

**Statistical analysis**

The sample size was calculated to compare differences regarding IGF-I, IGF-II, IGFBP-3, IGFBP-4 and PAPP-A levels in the FF. Power analysis showed that at least 29 patients in each group were needed to achieve 80% power at a 5% significance level using a two-sided equivalence test.

Data were analysed with the SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). The results were compared between the two groups and statistically analysed using the Student’s t-test or chi-square test where appropriate. Linear regression analysis was used to detect any correlation between FF IGF-I, IGF-II, IGFBP-3 or IGFBP-4 concentrations and serum E2 levels.

**Results**

There were no significant differences in clinical characteristics such as age, BMI and basal serum FSH levels between the two groups. The two groups were also comparable for the cause of infertility (Table I).

The duration of COS was significantly longer (11.9 ± 2.9 days versus 7.8 ± 1.1 days, P < 0.001) in the GnRHa long protocol group compared to that of the GnRHant protocol group. Total dose of gonadotrophins used (32.2 ± 11.3 ampules versus 26.8 ± 7.8 ampules, P = 0.028), serum E2 levels on hCG day (2516.1 ± 2041.8 pg/ml versus 1585.8 ± 1162.3 pg/ml, P = 0.031), the number of mature follicles ≥11 mm on hCG day (12.4 ± 4.9 versus 9.3 ± 4.6, P = 0.009) and the number of oocytes retrieved (13.4 ± 6.8 versus 8.5 ± 5.1, P = 0.002) were significantly higher in the GnRHant long protocol group when compared to those of the GnRHa group. Other outcomes such as fertilization rate, the number of high-quality embryos, pregnancy and implantation rates were not significantly different between the two groups. Although serum E2 levels on the day of hCG were significantly higher in the GnRHa long protocol group, serum E2 levels per mature follicle ≥11 mm were not different between the two groups (Table II).

The concentrations of FF IGF-II (888.4 ± 137.5 versus 805.8 ± 172.9 ng/ml, P = 0.032) and IGFBP-4 (36.9 ± 8.7 versus 30.3 ± 6.7 ng/ml, P = 0.001) were significantly higher in the GnRHa long protocol group. There were no significant differences in concentrations of IGF-I, IGFBP-3 and PAPP-A between the two groups. The ratio of IGF-I/IGFBP-3 (4.7 ± 1.9 versus 5.7 ± 2.2, P = 0.045) was significantly lower in the GnRHa long protocol group. There were no significant differences in the ratios of IGF-I/IGFBP-3, IGF-II/IGFBP-3, IGF-II/IGFBP-4 and IGFBP-4/PAPP-A between the two groups (Table III).

There were no significant correlations between FF IGF-I, IGF-II, IGFBP-3 or IGFBP-4 concentrations and serum E2 levels per mature follicle on hCG day (Figure 1).

**Discussion**

There are few comparative reports of the IGF system evaluating the levels of FF between the two protocols, the GnRHa long protocol and the GnRHant multiple-dose flexible protocol. Available studies report on findings from in vitro investigations. This study is the first in vivo study that compared the ovarian intrafollicular IGF-I, IGF-II, IGFBP-3, IGFBP-4 and PAPP-A concentrations, and their ratios, between the cycles using GnRHa long protocols and those using GnRHant multiple-dose flexible protocols for COS.

In the present study, we identified that there were significant differences in COS outcomes between the two protocols studied. Significant differences were noted between the GnRHa long protocol and the GnRHant multiple-dose flexible protocol, and these included the duration of COS, serum E2 levels on the day of hCG administration and the number of oocytes retrieved between cycles. However, there were no significant differences in fertilization rate, the number of high-quality embryos, pregnancy and implantation rates. Although implantation and pregnancy rates were not significantly different in this study, because of the small sample size, we could not rule out that the non-significant differences seen in pregnancy and implantation rates could be attributed to a type II error. Regarding sample sizes, 1251 patients in each group would achieve 80% power at a 5% significance level using a two-sided equivalence test of proportions when the pregnancy rate in each group is 30% and the maximum allowable difference between pregnancy rates that still results in equivalence is 5%. This is not realistic for a single centre. Therefore, further multi-center studies in a larger scale will be necessary to confirm our results.
Table II. Outcomes of controlled ovarian stimulation and IVF-embryo transfer between the two groups

<table>
<thead>
<tr>
<th>Outcome</th>
<th>GnRH agonist long protocol (n = 36) (Mean ± SD)</th>
<th>GnRH antagonist protocol (n = 32) (Mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of controlled ovarian stimulation (days)</td>
<td>11.9 ± 2.9</td>
<td>7.8 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total dose of gonadotrophins used (amp.)</td>
<td>32.2 ± 11.3</td>
<td>26.8 ± 7.8</td>
<td>0.028</td>
</tr>
<tr>
<td>Serum estradiol levels on hCG day (pg/ml)</td>
<td>2516.1 ± 2041.8</td>
<td>1585.8 ± 1162.3</td>
<td>0.031</td>
</tr>
<tr>
<td>No. of follicles ≥11 mm on hCG day</td>
<td>12.4 ± 4.9</td>
<td>9.3 ± 4.6</td>
<td>0.009</td>
</tr>
<tr>
<td>Serum estradiol per follicle ≥11 mm on hCG day (pg/ml)</td>
<td>2105.5 ± 134.2</td>
<td>200.3 ± 123.8</td>
<td>NS</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>13.4 ± 6.8</td>
<td>8.5 ± 5.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>64.8</td>
<td>71.3</td>
<td>NS</td>
</tr>
<tr>
<td>Number of embryos cryopreserved (2PN)</td>
<td>1.8 ± 3.2</td>
<td>0.7 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Number of high-quality embryos</td>
<td>1.0 ± 1.2</td>
<td>1.1 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Number of transferred embryos</td>
<td>3.2 ± 1.0</td>
<td>3.3 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy rate per cycle (%)</td>
<td>7/36 (19.4)</td>
<td>11/32 (23.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>10/117 (8.5)</td>
<td>16/107 (15.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

High-quality embryos: morphologic grade I/V or II/V and four or five blastomeres on day 2 and at least seven blastomeres on day 3 after fertilization.

Table III. Comparison of follicular fluid concentrations of IGF-I, IGF-II, IGFBP-3, IGFBP-4, PAPP-A and their ratios between the two groups

<table>
<thead>
<tr>
<th>Concentration</th>
<th>GnRH agonist long protocol (n = 36) (Mean ± SD)</th>
<th>GnRH antagonist protocol (n = 32) (Mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I (ng/ml)</td>
<td>160.8 ± 44.6</td>
<td>165.9 ± 59.2</td>
<td>NS</td>
</tr>
<tr>
<td>IGF-II (ng/ml)</td>
<td>888.4 ± 137.5</td>
<td>805.8 ± 172.9</td>
<td>0.032</td>
</tr>
<tr>
<td>IGFBP-3 (ng/ml)</td>
<td>3,167.6 ± 488.4</td>
<td>2,983.7 ± 574.4</td>
<td>NS</td>
</tr>
<tr>
<td>IGFBP-4 (ng/ml)</td>
<td>36.9 ± 8.7</td>
<td>30.3 ± 6.7</td>
<td>0.001</td>
</tr>
<tr>
<td>PAPP-A (mIU/ml)</td>
<td>0.53 ± 0.25</td>
<td>0.50 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>IGFBP-4/PAPP-A (× 10^4)</td>
<td>176.7 ± 545.0</td>
<td>137.6 ± 231.3</td>
<td>NS</td>
</tr>
<tr>
<td>IGF-II/IGFBP-3 (× 10^3)</td>
<td>5.1 ± 1.4</td>
<td>5.5 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>IGF-II/IGFBP-4</td>
<td>4.7 ± 1.9</td>
<td>5.7 ± 2.2</td>
<td>0.045</td>
</tr>
<tr>
<td>IGFBP-3/IGFBP-2</td>
<td>28.3 ± 3.3</td>
<td>27.1 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>IGFBP-4/IGFBP-2</td>
<td>25.2 ± 6.3</td>
<td>27.3 ± 5.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

IGF, insulin-like growth factor; IGFBP; insulin-like growth factor binding protein; PAPP-A, pregnancy-associated plasma protein-A.

Although it could not be confirmed in the case of cetrorelix by Ludwig et al., two recent meta-analyses, including five prospective randomized trials comparing GnRHa with GnRHant for COS in IVF-embryo transfer patients, showed lower pregnancy rates in cycles using GnRHant (Ludwig et al., 2001; Al-Inany and Aboulghar, 2002). It is still unclear whether this is a result of a detrimental effect of the GnRHant on the oocytes or a detrimental effect on the endometrium or perhaps staff lacking experience with the new protocols. Hernandez (2000) suggested that GnRHant might have detrimental effects on endometrial receptivity by impairment of growth factors such as IGF-I. Others argued against such an effect and blamed a clinician learning curve for lower pregnancy rates in the newly introduced GnRHant treatments (Mannaerts and Godon, 2000). Recently, in fact, there have been some reports with comparable or even better pregnancy rates in the antagonist cycles (Roulier et al., 2003; Berger et al., 2004). The present study supports latter findings. Some animal studies suggested that GnRHant might disturb early folliculogenesis and embryonic development (Funston and Seidel, 1995; Raga et al., 1999); however, human studies have found no disturbance in the growth of follicles obtained from patients treated with the GnRHant ganirelix and recombinant FSH (de Jong et al., 2001). It is impossible to completely exclude any negative effects of GnRHant on human reproductive function such as folliculogenesis and endometrial receptivity.

Expression of GnRH and the GnRH receptor has been reported in a number of reproductive tissues including the ovary, uterus and placenta (Kakar et al., 1992; Ortmann and Diedrich 1999). GnRH is able to stimulate mitogen-activated protein kinase in human granulosa luteal cells and may act as an autocrine factor (Kang et al., 2000, 2001). In in vitro studies, GnRHant represses cell growth by decreasing the synthesis and the growth stimulatory effects of IGFs (Emons et al., 1993). Thus, it is possible that GnRHant may disrupt autocrine or paracrine signalling of GnRH in cells in these reproductive tissues. If GnRHant can inhibit IGF, a direct inhibition of IGF by the GnRHant can affect folliculogenesis, implantation and embryo development.

In this study, we observed that there were significant differences in the IGF system such as IGF-II, IGFBP-4 concentrations and the ratio of IGF-II/IGFBP-4 between the two groups. Considering that serum E2 levels per mature follicle and the concentrations of PAPP-A were not different between the two groups, the effect of higher IGF-II concentrations appeared to be inhibited by higher IGFBP-4 in the GnRHa long protocol group compared to the GnRHant group. Therefore, steroidogenesis of dominant follicles in cycles using GnRHant was not different from that in cycles using GnRHa; this may indicate that lower serum E2 levels during cycles using GnRHant are not because of direct ovarian action of GnRHant. There have been some reports on the comparison of steroidogenesis
between GnRHa and GnRHant (Lin et al., 1999; Ortmann et al., 2001; Weiss et al., 2001). These reports showed that treatment with either GnRHα or GnRHant did not significantly affect steroidogenesis in vivo or in vitro, which was in line with this study. A previous in vitro cell culture study suggested that GnRHant did not affect IGF-II, IGFBP-2 or PAPP-A of the granulosa-lutein cell and therefore was unlikely to impair ovarian function (Weiss et al., 2003). These findings are in agreement with our study for PAPP-A but not for IGF-II.

It would be logical that higher FF IGF-I/IGFBP-4 ratio in GnRHant protocol group results in more bioactive IGF-I and higher serum E2 levels per mature follicle, which was not observed in our study. Although it cannot be clearly explained, some explanations can be possible. Firstly, IGF–II, rather than IGF-I, is the important factor in the human dominant follicles, which is supported by evidence indicating that IGF-II is the most abundant IGF in human ovarian follicle. Secondly, women with Laron-type dwarfism, which was characterized by IGF-I deficiency responded to exogenous gonadotropin stimulation with the production of multiple, mature follicles with good estrogen production (Dor et al., 1992). Thirdly, since there can also be a role of other inhibitory IGFBPs in intrafollicular IGF system, an increase of this ratio in GnRHant protocol group may not truly reflect the increase in the bioactive IGF-I.

Recombinant FSH increases the expression of follicular PAPP-A, and it may influence positively the follicular microenvironment (Choi et al., 2003). In our study, recombinant FSH and/or human menopausal gonadotrophin were administered for COS. Since there was no significant difference in the types of gonadotrophins used between the two groups, and this finding was not observed in our study, it would be unlikely that the types of gonadotrophins used influenced our results.

During COS, FF composition shows dynamic changes as individual cells of the ovarian follicle respond to the exogenous gonadotrophins by secreting different hormones, growth factors and cytokines, which are likely to influence, either directly or indirectly, oocyte viability and developmental potential. Several endocrine parameters, ovarian local regulatory factors, such as E2, P4, leptin (Agarwal et al., 1999; Dorn et al., 2003), cyclin D2 (Robker and Richards, 1998), EGF receptor (Maruo et al., 1993), inhibin-α (Pellicer et al., 1994) and vascular endothelial growth factor (Doldi et al., 1997) in serum, FF or granulosa cells obtained from patients undergoing COS for IVF-embryo transfer have been reported to reflect follicular health positively or negatively. Since we investigated
only the microenvironment related to the IGF system in the present study, the effects of other growth factors and cytokines should be elucidated further in future studies.

Several limitations of the present study should be mentioned. First, this was an observational study and not a randomized controlled trial; hence, patients were selected to undergo different GnRH analogue treatments, and there may be confounding variables that may influence the results. However, the similarity in clinical characteristics such as age, BMI, basal serum FSH levels and the distribution of infertility causes supported that selection bias would be minimal. Second, FF specimens of a single follicle may not truly reflect granulosa or thecal cell production. Third, serum LH levels and FF androgen or estrogen levels were not measured in this study, narrowing the conclusions of the study. There can be an impact of the androgenic environment on FF IGF bioavailability, and we do not know if there is a correlation between the serum E2 levels on the day of hCG and the local E2 levels in this dominant follicle. Fourth, although we are concerned about microenvironment of dominant follicle which is most likely to contain a mature oocyte, results of this dominant follicle may not reflect the other follicles in the ovary. Fifth, in Table III, although the P values showed statistical significance, the mean and SD showed a wide variance of results and a large overlap between the two groups, weakening the clinical significance of these differences.

In conclusion, FF IGF-II and IGFBP-4 concentrations and the ratio of IGF-I/IGFBP-4 were significantly different when the cycles using GnRHa long protocols and those using GnRHant multiple-dose flexible protocols were compared, which may indicate a difference of follicular microenvironment between the two protocols. However, the difference of the microenvironment does not appear to result in a difference in clinical outcomes.

Acknowledgements
This work is supported by grant No. 04-2004-048-0 from the Seoul National University Hospital Research Fund.

References
Follicular fluid IGF system in IVF-ET patients


Submitted on November 17, 2005; resubmitted on March 1, 2006; accepted on March 2, 2006