Anti-Müllerian hormone dynamics during controlled ovarian hyperstimulation and optimal timing of measurement for outcome prediction

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BACKGROUND: Anti-Müllerian hormone (AMH) has been suggested as a marker of ovarian reserve and predictor of ovarian response to controlled ovarian hyperstimulation (COH). Several studies have demonstrated AMH changes during follicular and luteal phases during COH, but not after human chorionic gonadotrophin (hCG) administration. The objectives of this study were to investigate changes in AMH levels during the entire COH cycle and to clarify the regulatory mechanism of AMH secretion. In addition, we analyzed the COH outcome parameters to determine the optimal timing for AMH measurement to predict outcome.

METHODS: The study included 74 women who underwent in vitro fertilization (IVF) cycles with a GnRH agonist or antagonist protocol. Serum AMH and inhibin B levels were measured at baseline, Day 5 of stimulation (d5), day of hCG administration (dhCG), day of oocyte retrieval (dOPU) and 14 days after oocyte retrieval (dPO14). Follicular fluid (FF) from dominant follicles upon oocyte retrieval were also analyzed for AMH and inhibin B concentrations. AMH levels were analyzed for changes during the cycle and for correlations with COH outcome parameters.

RESULTS: Serum AMH levels decreased progressively during COH until dhCG, then increased on dOPU and further increased on dPO14. Serum and FF AMH levels and dynamic changes were not different between the GnRH agonist and antagonist cycles. Serum AMH levels on every sample day and the FF AMH levels were significantly correlated with outcomes of COH, such as dose of gonadotrophins used, estradiol level on dhCG and number of retrieved oocytes; the strength of the relationship was highest for baseline AMH.

CONCLUSIONS: The results of the present study suggest that changes in the hormonal milieu during stimulation and after the LH surge may affect AMH secretion. Serum AMH levels during COH are good markers to predict ovarian response, but the baseline serum level seems to be the most predictive marker.

Key words: anti-Müllerian hormone / inhibin B / controlled ovarian hyperstimulation / GnRH agonist / GnRH antagonist

Introduction

Anti-Müllerian hormone (AMH), also called Müllerian-inhibiting substance, is a member of the transforming growth factor-β superfamily. Recently, it has been suggested that AMH acts as an ovarian reserve marker as well as a regulator of folliculogenesis and oocyte maturation. Serum AMH concentrations during the early follicular phase of the menstrual cycle decrease consistently with age in women, and this change starts earlier than that of conventional markers such as follicle stimulating hormone (FSH), estradiol and inhibin B (de Vet et al., 2002; Shin et al., 2008). Other researchers have shown that AMH is undetectable in women after spontaneous or surgically induced menopause (La Marca et al., 2005). A recent study also showed that serum AMH levels are related to the onset of menopause and that AMH is able to specify a woman’s reproductive age more realistically than chronological age alone (van Disseldorp et al., 2008).
It has been suggested that AMH is involved in the inhibition of primordial to primary follicle growth and of follicular responsiveness to FSH (Durlinger et al., 2001, 2002). In addition, it has been demonstrated that AMH may reduce both aromatase activity and the number of LH receptors in FSH-stimulated granulosa cells (Josso et al., 1998). These studies suggest that AMH has an important role in ovarian folliculogenesis and oocyte maturation. Several studies on AMH concentration in follicular fluid (FF) have shown more directly in ovarian folliculogenesis and oocyte maturation. Several studies on the number of LH receptors in FSH-stimulated granulosa cells (Josso et al., 2005; Fanchin et al., 2007; Lee et al., 2008; Takahashi et al., 2008). However, the precise regulatory mechanism of AMH secretion and its role in folliculogenesis are still unclear. To clarify these issues, several studies have been performed on changes in serum AMH levels during controlled ovarian hyperstimulation (COH) (Fanchin et al., 2003; Eldar-Geva et al., 2005; Fanchin et al., 2005; Catteau-Jonard et al., 2007). However, most of these studies were conducted only during GnRH agonist cycles and either follicular (Fanchin et al., 2003; Eldar-Geva et al., 2005; Catteau-Jonard et al., 2007) or luteal phases (Fanchin et al., 2005) of the COH cycle. To the best of our knowledge, there have been few studies on AMH changes over the entire COH period, including days before and after human chorionic gonadotrophin (hCG) administration, and during GnRH antagonist cycles.

In recent years, many studies have been conducted on the basal AMH level and its association with COH outcome. It has been shown that the basal AMH level is correlated with antral follicle count (AFC), total dose of gonadotrophins used, duration of COH, estradiol level on hCG day, the number of mature follicles on hCG day and the number of oocytes retrieved (Seifer et al., 2002; van Rooij et al., 2002; Muttukrishna et al., 2004). Moreover, the AMH level was found to be positively related to pregnancy rate in in vitro fertilization and embryo transfer (IVF–ET) cycles (Hazout et al., 2004). It has been also suggested that the serum AMH level could predict poor response and ovarian hyperstimulation syndrome for IVF cycles (Nakhuda et al., 2006; Gnoth et al., 2008).

Serum AMH levels show no fluctuation throughout the menstrual cycle, and the AMH level has been correlated with ovarian response to gonadotrophin stimulation independent of the days of the menstrual cycle (La Marca et al., 2006, 2007). In another study, using different measurement timing, the AMH level during the menstrual cycle showed a correlation with the outcomes similar to that observed following COH cycles (Elgindy et al., 2008). However, during COH cycles, the serum AMH level changes throughout the cycle; thus, a correlation with COH outcomes could depend on the timing of the measurement. Only a few studies have been conducted on the correlation between COH outcomes and AMH levels measured on different stimulation days (Fanchin et al., 2003; Penarrubia et al., 2005; Silberstein et al., 2006), and these studies did not show a comparison between different measurement timings over the entire period of the COH cycle.

The objectives of this study were to investigate changes in AMH levels during the COH period, including GnRH antagonist cycles and to clarify the regulatory mechanism of AMH secretion. In addition, we analyzed the relationship between COH outcome parameters and AMH levels throughout the entire COH cycle in order to determine optimal timing for AMH measurement to predict outcome.

Materials and Methods

Subjects

A total of 74 women, aged 22–42 years, were prospectively enrolled in this study. The inclusion criteria were as follows: (i) both ovaries present with no morphological abnormalities; (ii) normal ovulatory cycle with cycle lengths between 25 and 35 days; (iii) a basal FSH (Day 3) level <10 mIU/ml; (iv) no history of poor ovarian response; and (v) no evidence of endocrine abnormalities such as hyperprolactinemia, thyroid dysfunction or polycystic ovarian syndrome as defined by the Rotterdam criteria (2004). Patients with Stage III–IV endometriosis were excluded. This study was approved by the Institutional Review Board of Seoul National University Hospital and informed consent was obtained from all patients.

COH protocols

All women received ovarian stimulation using either GnRH agonist long protocol (n = 56) or GnRH antagonist multiple-dose protocol (n = 18). For the GnRH agonist long protocol, the GnRH agonist triptorelin (Decapeptyl; Ferring, Malmo, Sweden) was started at 0.1 mg/day in the mid-luteal phase of the previous cycle. After pituitary down-regulation, on the third menstrual cycle day, the triptorelin dose was reduced to 0.05 mg/day, and recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) was added daily until the leading follicle reached a mean diameter of 18 mm or until two or more follicles reached a diameter of 17 mm. For the GnRH antagonist multiple-dose flexible protocol, recombinant FSH was started on the third menstrual cycle day without oral contraceptive pretreatment. The GnRH antagonist, cetrorelix (0.25 mg; Cetrodite, Serono), was added daily, starting when the leading follicle reached a diameter of 14 mm and ending when the leading follicle reached a mean diameter of 18 mm or when two or more follicles reached a diameter of 17 mm. For both protocols, 250 μg of recombinant human hCG (Ovidrel, Serono) was administered subcutaneously 36 h before transvaginal oocyte retrieval. Up to four embryos were transferred 3 days after oocyte retrieval. The luteal phase was supported with 50 mg of progesterone in oil (Progest; Samil, Seoul, Korea) or 8% progesterone gel (Crinone, Serono) daily, initially for 14 days starting on the day of oocyte retrieval and continuing for another 6–8 weeks in cases where a pregnancy was achieved. Pregnancy was first assessed using serum β-hCG 14 days after oocyte retrieval, and clinical pregnancy was defined by the presence of an intrauterine gestational sac with pulsating fetal heart beats 3–4 weeks after oocyte retrieval.

Serum and FF collection

Blood samples were obtained on the first and fifth days of FSH administration (baseline and d5), the day of hCG administration (dhCG), the day of oocyte retrieval (dOPU) and 14 days after OPU (on the day of pregnancy test, dPO14) (Fig. 1). Serum was separated and frozen in aliquots at −20°C for subsequent centralized analysis. FF was obtained from dominant follicles with the largest mean diameters during OPU and centrifuged at 500g for 15 min; FF supernatant was stored at −20°C until assayed. FF contaminated with blood was excluded.

Hormonal measurements in serum and FFs

AMH and inhibin B concentrations were measured from all serum samples and FF by enzyme-linked immunosorbent assay (Immunotech, Marseille, France, for AMH; Diagnostic Systems Laboratories, Webster, TX, USA, for inhibin B). For AMH, intra- and inter-assay coefficients of variation were 4.6 and 8.0%, respectively, with a sensitivity of 0.098 ng/ml. For
inhibin B, intra- and inter-assay coefficients of variation were 5.6 and 7.6%, respectively, with a sensitivity of 7 pg/ml.

Statistical analysis
Sample size was calculated using data of a previous study (Lee et al., 2008), and at least 72 subjects (54 for the GnRH agonist group and 18 for the GnRH antagonist group) were necessary to achieve 80% power at a 5% significance level using a two-sided equivalence test of continuous variables. A 1:3 ratio was used for calculation because patient distribution in our center showed a 1:3 ratio of GnRH antagonist to agonist cycles.

Longitudinal changes in serum AMH and inhibin B levels during the IVF cycle, as well as FF AMH and inhibin B levels, were analyzed. Correlations between serum and FF AMH and inhibin B levels and COH outcomes were analyzed. Statistical analysis was performed using Wilcoxon signed-rank test, Fisher’s exact test, Mann–Whitney U test or Spearman’s correlation analysis, as appropriate. The statistical software package SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis, and results were considered to be statistically significant at P-values of < 0.05.

Results
Clinical characteristics of the study subjects and outcomes of COH
Clinical characteristics of the study subjects and COH outcomes are shown in Table I. Comparing the characteristics and COH outcomes between the GnRH agonist and GnRH antagonist cycles, no significant differences were observed except in the duration of COH and the total dose of gonadotrophins used.

Dynamics of AMH and inhibin B concentrations
Serum AMH levels decreased progressively during COH until hCG day, then increased on OPU day, and further increased on Day 14 after OPU. Serum and FF AMH levels and dynamic changes were not different between the GnRH agonist and antagonist cycles. The longitudinal serum concentration changes were statistically significant during the entire COH cycle (P < 0.001; Fig. 2). Inhibin B levels increased during COH and reached a peak on hCG day, then abruptly decreased on OPU day, and further decreased on Day 14 after OPU. All changes had statistical significance. The GnRH agonist and antagonist cycles showed similar dynamic changes. Serum and FF inhibin B levels were not different between the agonist and antagonist cycles except for the baseline level (Table II).

Correlations between AMH, inhibin B and the outcomes of COH
Serum AMH levels on every sample day and FF AMH level were significantly correlated with AFCs and with the outcomes of COH, such as the dose of gonadotrophins used, serum estradiol level on hCG day, number of follicles on hCG day, number of retrieved oocytes and number of fertilized oocytes. The strength of the relationship between baseline AMH level and COH outcomes was highest.
Figure 2 Dynamics of AMH concentration during COH. All longitudinal serum concentration changes reached statistical significance \((P < 0.001)\). Values are mean (error bar: standard error of mean).

Baseline inhibin B concentration was significantly correlated with AFC and COH outcomes (Table III). Baseline inhibin B concentration was significantly correlated with AFC and COH outcomes except for serum estradiol level on hCG day. D5 and dhCG inhibin B levels showed significant correlation with AFC and all parameters of COH outcomes. Inhibin B levels on dOPU and dPO14 showed no correlation with any of the above parameters except AFC for dOPU. FF inhibin B correlated significantly with AFC, dose of gonadotrophins used, number of follicles and number of retrieved oocytes (Table IV).

Discussion

In the present study, serum AMH concentration was decreased gradually until the day of hCG triggering and showed its minimum level on the hCG day. This finding confirms the findings of a previous study that demonstrated a gradual decrease in serum AMH level during follicular phase COH (Fanchin et al., 2003). A study which compared the changes in follicular phase serum AMH levels between spontaneous cycles and ovulation-induction cycles using exogenous FSH showed that serum AMH levels did not change in the spontaneous cycles but progressively decreased in the FSH-treated cycles (La Marca et al., 2004). Thus, the decrease in serum AMH concentration during the follicular phase of the COH cycle, as shown in the present study, originates from characteristics of COH cycles, as opposed to spontaneous cycles.

There are several possible causes for the gradual decrease of serum AMH levels during the follicular phase of the COH cycle. First, FSH could have a negative role on AMH secretion. Baarends et al. (1995) reported that FSH may suppress the expression of AMH and AMH type II receptor (AMHRII) in adult rat ovaries. In addition, other investigators have shown that FSH treatment significantly reduces AMH expression in cultured granulosa cells (Pellatt et al., 2007). However, the mechanism of how FSH affects AMH secretion remains unclear. Second, increased serum estradiol levels during COH could have a negative effect on AMH secretion. A previous study that analyzed the relationship between FF AMH and estradiol level demonstrated a negative correlation (Andersen and Byskov, 2006). In the present study, serum estradiol levels on hCG day showed a significant correlation with the decrease in serum AMH until the hCG day \((\Delta d3-dhCG)\). Although the causality of this relationship between estradiol increase and AMH decrease is not clear from the present data, a previous experimental study showed that estradiol could inhibit the expression of AMH and AMHRII in pre-antral and small antral follicles, supporting a causal relationship between estradiol and AMH (Baarends et al., 1995). Third, the decrease in AMH levels during COH could be explained by the decrease in small antral follicles. Fanchin et al. (2003) showed that serum AMH is significantly correlated with the decrease in the number of small antral follicles. The authors suggested that the serum AMH decrease during COH reflects the reduction in the number of small antral follicles. Other investigators have also shown a relationship between serum AMH changes and small follicles with diameters of 2–5 mm (Catteau-Jonard et al., 2007). In the present study, we did not count the number of small antral follicles, but the AMH decrease preceding hCG day showed a significant positive correlation with the number of follicles compared with other sample days. The reduction in AMH between baseline and hCG day \((\Delta d3-dhCG)\) was also significantly correlated with AFC and COH outcomes (Table III).

Table II Comparison of serum and FF Inhibin B concentrations during COH according to stimulation regimens.

<table>
<thead>
<tr>
<th></th>
<th>GnRH agonist cycles ((n = 56))</th>
<th>GnRH antagonist cycles ((n = 18))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibin B on baseline ((pg/ml))</td>
<td>15.7 ± 3.3</td>
<td>39.3 ± 4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inhibin B on d5 ((pg/ml))</td>
<td>719.2 ± 127.0</td>
<td>818.7 ± 181.2</td>
<td>0.296</td>
</tr>
<tr>
<td>Inhibin B on dhCG ((pg/ml))</td>
<td>1887.1 ± 246.6</td>
<td>1310.8 ± 297.2</td>
<td>0.450</td>
</tr>
<tr>
<td>Inhibin B on dOPU ((pg/ml))</td>
<td>334.8 ± 110.9</td>
<td>155.1 ± 50.4</td>
<td>0.312</td>
</tr>
<tr>
<td>Inhibin B on dPO14 ((pg/ml))</td>
<td>33.8 ± 2.4</td>
<td>34.3 ± 3.9</td>
<td>0.676</td>
</tr>
<tr>
<td>FF Inhibin B ((pg/ml))</td>
<td>40 064.9 ± 5878.5</td>
<td>33 914.5 ± 4542.1</td>
<td>0.837</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Baseline, the first day of gonadotrophin treatment; d5, the fifth day of gonadotrophin treatment; dhCG, the day of hCG administration; dOPU, the day of oocyte retrieval; dPO14, the 14th day after oocyte retrieval.
### Table III Correlations of serum and FF AMH concentrations with AFC, and COH outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>d5</th>
<th>dhCG</th>
<th>dOPU</th>
<th>dPO14</th>
<th>FF</th>
<th>Δd3-dhCG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFC</td>
<td>0.541</td>
<td>&lt;0.001</td>
<td>0.476</td>
<td>&lt;0.001</td>
<td>0.537</td>
<td>&lt;0.001</td>
<td>0.533</td>
</tr>
<tr>
<td>Dose of gonadotrophins used</td>
<td>-0.515</td>
<td>&lt;0.001</td>
<td>-0.425</td>
<td>&lt;0.001</td>
<td>-0.496</td>
<td>&lt;0.001</td>
<td>-0.444</td>
</tr>
<tr>
<td>Serum estradiol on dhCG</td>
<td>0.577</td>
<td>&lt;0.001</td>
<td>0.500</td>
<td>&lt;0.001</td>
<td>0.460</td>
<td>&lt;0.001</td>
<td>0.299</td>
</tr>
<tr>
<td>No. of follicles ≥ 11 mm on dhCG</td>
<td>0.670</td>
<td>&lt;0.001</td>
<td>0.582</td>
<td>&lt;0.001</td>
<td>0.605</td>
<td>&lt;0.001</td>
<td>0.498</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>0.641</td>
<td>&lt;0.001</td>
<td>0.510</td>
<td>&lt;0.001</td>
<td>0.572</td>
<td>&lt;0.001</td>
<td>0.474</td>
</tr>
<tr>
<td>No. of fertilized oocytes</td>
<td>0.558</td>
<td>&lt;0.001</td>
<td>0.455</td>
<td>&lt;0.001</td>
<td>0.534</td>
<td>&lt;0.001</td>
<td>0.491</td>
</tr>
</tbody>
</table>

r, Spearman correlation coefficient; Baseline, the first day of gonadotrophin treatment; d5, the fifth day of gonadotrophin treatment; dhCG, the day of hCG administration; dOPU, the day of oocyte retrieval; dPO14, the 14th day after oocyte retrieval; FF, follicular fluid; Δd3-dhCG, the difference between d3 and dhCG AMH levels.

### Table IV Correlations of serum and FF inhibin B concentrations with AFC, and COH outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>d5</th>
<th>dhCG</th>
<th>dOPU</th>
<th>dPO14</th>
<th>FF</th>
<th>Δd3-dhCG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFC</td>
<td>0.254</td>
<td>0.035</td>
<td>0.274</td>
<td>0.023</td>
<td>0.579</td>
<td>&lt;0.001</td>
<td>0.282</td>
</tr>
<tr>
<td>Dose of gonadotrophins used</td>
<td>-0.454</td>
<td>&lt;0.001</td>
<td>-0.411</td>
<td>&lt;0.001</td>
<td>-0.475</td>
<td>&lt;0.001</td>
<td>-0.048</td>
</tr>
<tr>
<td>Serum estradiol on dhCG</td>
<td>0.203</td>
<td>0.090</td>
<td>0.444</td>
<td>&lt;0.001</td>
<td>0.585</td>
<td>&lt;0.001</td>
<td>0.132</td>
</tr>
<tr>
<td>No. of follicles ≥ 11 mm on dhCG</td>
<td>0.331</td>
<td>0.005</td>
<td>0.541</td>
<td>&lt;0.001</td>
<td>0.773</td>
<td>&lt;0.001</td>
<td>0.082</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>0.281</td>
<td>0.017</td>
<td>0.494</td>
<td>&lt;0.001</td>
<td>0.763</td>
<td>&lt;0.001</td>
<td>0.194</td>
</tr>
<tr>
<td>No. of fertilized oocytes</td>
<td>0.320</td>
<td>0.007</td>
<td>0.514</td>
<td>&lt;0.001</td>
<td>0.613</td>
<td>&lt;0.001</td>
<td>0.235</td>
</tr>
</tbody>
</table>

r, Spearman correlation coefficient; Baseline, the first day of gonadotrophin treatment; d5, the fifth day of gonadotrophin treatment; dhCG, the day of hCG administration; dOPU, the day of oocyte retrieval; dPO14, the 14th day after oocyte retrieval; FF, follicular fluid; Δd3-dhCG, the difference between d3 and dhCG inhibin B levels.
larger than 11 mm on the day of hCG. This finding also supports previous results that suggested a relationship between serum AMH and changes in follicle distribution during COH. In the present study, serum AMH levels gradually decreased prior to the hCG day during COH, and the degree of this decrease was significantly correlated with COH outcomes. These results suggest that serum AMH secretion is regulated by hormonal changes during folliculogenesis, and its change could reflect ovarian response to stimulation. Further research is necessary to elucidate the mechanism of AMH regulation during the follicular phase of folliculogenesis.

An interesting finding of the present study is that serum AMH level shows its minimum level on hCG day and re-increases on OPU day. This result suggests that the initial changes in AMH secretion after hCG injection are related to pre-ovulatory granulosa cell adaptation after the LH surge. Although the exact mechanism of these initial changes is still unclear, several hypotheses can be suggested. First, the hormonal environment that triggers granulosa cells to reduce AMH secretion during COH may be absent or reversed after hCG injection. Elevated FSH and estradiol levels, possible causes of the AMH decrease, may be reduced after hCG injection. Second, the follicular microenvironment, which changes after the LH surge, may trigger granulosa cells to increase AMH secretion. After the LH surge, a number of critical changes take place in granulosa cells until ovulation occurs. These changes could cause AMH secretion to be different from that during the late follicular period before the LH surge. It has been shown that suppression of vascular endothelial growth factor (VEGF) decreases AMH expression in the primate ovary (Thomas et al., 2007). Expression of VEGF increases as follicles develop into pre-ovulatory follicles, and this increase of VEGF in pre-ovulatory follicles could trigger granulosa cells to increase AMH secretion. This finding suggests that an increase in VEGF after the LH surge could result in an increase in AMH secretion. Third, hCG itself, which increase after exogenous hCG injection, may induce AMH secretion in pre-ovulatory granulosa cells. A positive correlation between serum hCG and AMH levels during the luteal phase of COH, as observed in a previous study, supports this hypothesis (Fanchin et al., 2005). This increase of the AMH level after the LH surge cannot be explained by a decrease of small antral follicle number. Serum level of AMH does not solely reflect the number of small antral follicles, but also reflects the production of AMH by granulosa cells of mature follicle. As we demonstrated in a previous study, serum levels of AMH on the oocyte retrieval day significantly correlates with the FF AMH levels, which means that serum AMH reflects intrafollicular AMH production as well as number of follicles (Lee et al., 2008).

The early luteal changes in AMH levels during COH shown in the present study differ from the results of previous research. One study on AMH dynamics during the luteal phase of COH observed a decline in AMH levels from the hCG day until 4 days after hCG, after which levels began to increase (Fanchin et al., 2005). The difference between previous work and the present study is sampling timing. We measure AMH levels on the OPU day, and the AMH level reflects early pre-ovulatory changes of AMH secretion; in contrast, the previous study measured AMH levels 4 days after hCG injection, which reflects the early luteal phase after ovulation. Combining the results from the previous and present studies, we suggest that the initial pre-ovulatory changes of granulosa cell function may disappear as luteinization progresses, causing AMH levels to decrease after the early pre-ovulatory period. Therefore, although there is not sufficient data to draw a firm conclusion, we can suggest that there could be different microenvironments between the pre-ovulatory and early luteal follicles.

Serum levels of inhibit B during COH increased during stimulation, reached a peak on the hCG day and abruptly decreased after hCG injection. This dynamic change in the inhibit B serum level agrees with the results of previous studies (Lockwood et al., 1996; Hohmann et al., 2005). The increased serum level during stimulation may reflect follicle growth in response to exogenous FSH administration (Welt et al., 2001). After hCG injection, this stimulatory effect from FSH disappeared, and the serum level decreased. Inhibit B has been presented as a potential predictive marker of ovarian response to COH (Seifer et al., 1997; Urbansek et al., 2005). In the present study, serum inhibit B levels on each sampling day correlated with most COH outcome parameters, and the strength of the relationship was the lowest at the baseline level. Because of these characteristics of inhibit B as a response marker after stimulation, its practical value as a predictive marker before stimulation is reduced (Corson et al., 1999; Creus et al., 2000).

Recently, serum AMH levels have been used as markers of COH outcomes such as the total dose of gonadotrophins used, estradiol level on hCG day, number of mature follicles on hCG day and number of oocytes retrieved (Seifer et al., 2002; van Rooij et al., 2002; Muttukrishna et al., 2004). However, few studies have been conducted on the optimal timing of AMH measurement for prediction of COH outcome. Most studies were conducted on basal serum AMH level before gonadotrophin administration. Penarrubia et al. (2005) showed that the serum AMH level on stimulation Day 5 is a better predictor of cycle cancellation than the basal AMH level. Silberstein et al. (2006) demonstrated that the AMH level on the d5CG correlated with COH outcomes, including the number of mature follicles, number of oocytes retrieved, estradiol levels and embryo morphology score. Comparisons with other COH days were not performed. In the present study, AMH levels on every sample day were significantly correlated with COH outcomes, but the strength of correlation with all outcome parameters was highest for the basal AMH level. As well as providing the strongest correlation with COH outcomes, the timing is more optimal with measuring baseline AMH. Baseline AMH is measured before gonadotrophin administration, thus we can adjust the gonadotrophin starting dose depending on the basal AMH level. If serum AMH levels on d5 or dhCG are used as predictive markers, they are significantly correlated with COH outcomes, but gonadotrophin dose adjustment is less effective for control of ovarian response.

There was no difference in serum AMH levels on each sampling day during COH and no difference in FF AMH levels between the GnRH agonist and antagonist cycles. This finding confirms previous study results showing that in vivo or in vitro steroidogenesis is not affected by treatment with either GnRH agonist or antagonist (Ortmann et al., 2001; Weiss et al., 2001) and that intrafollicular cytokine and hormone levels are similar between the GnRH agonist and antagonist cycles (Asimakopoulos et al., 2006; Lee et al., 2008). The baseline serum inhibit B level differed between the GnRH agonist and antagonist cycles, but this difference disappeared after stimulation was started. FF inhibit B levels were also similar in the two regimens.
The difference in baseline serum levels was due to the pituitary down-regulation effect of the GnRH agonist. In the GnRH agonist cycle, pituitary function is already down-regulated before stimulation; thus, the relatively lower FSH level could cause relatively lower inhibin B levels. This effect of pituitary down-regulation disappeared after stimulation; therefore, we can say that serum inhibin B levels were also similar during COH between the two regimens. This finding also confirms that there was no difference in folliculogenesis between the two regimens. On the basis of the results of the present and previous studies, we suggest that no significant difference in follicular microenvironments exists between the two protocols, including secretion of AMH and inhibin B. However, the relatively small sample size of the present study could reduce the statistical power, thus further studies on a larger scale could be necessary to confirm this result.

In conclusion, there were no differences in serum AMH dynamics during COH nor in FF AMH levels between the GnRH agonist and antagonist cycles. Serum AMH levels gradually declined during the follicular phase of the COH cycle and were then increased on the OPU day after hCG administration, which suggests that changes of hormonal milieu after an LH surge may affect AMH secretion. Serum AMH levels during COH and the FF AMH level are both good candidate markers to predict ovarian response to COH, but the baseline serum level before gonadotrophin administration seems to be the most predictive marker.

Authors’ roles


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