SHORT COMMUNICATION

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Periovulatory Serum Inhibin-A in Women with a Diminished Ovarian Reserve

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INTRODUCTION

Age has long been known to be associated with a natural decline in fertility (1). The perimenopause is characterized by a number of events that precede the complete cessation of ovarian function. These include a diminution in the number and quality of oocytes, elevations in serum gonadotropins [specifically follicle stimulating hormone (FSH)], and a decrease in the production of ovarian sex steroids (2).

The observed rise in the early follicular phase of serum FSH is one of the earliest findings of diminished ovarian reserve and precedes changes in luteinizing hormone (LH) by almost a decade (3). The increase in early follicular-phase FSH reflects a decreased negative feedback of ovarian inhibin on pituitary FSH secretion (4). Two bioactive forms of inhibin exist and are represented by divergent levels in the circulation as seen during different phases of the menstrual cycle. Inhibin-b is secreted by preovulatory follicles and is the primary inhibin during the follicular phase. Inhibin-a levels begin to rise during the late follicular phase and peak following ovulation. Inhibin-a is a product chiefly of the corpus luteum and is the primary inhibin during the luteal phase (5). Both inhibins are thought to play a role in suppressing FSH levels.

Recently, an association has been found between decreased cycle day 3 serum inhibin-b and elevations of FSH and appears to be predictive of ovarian reserve and IVF outcome (6,7). Similarly, luteal-phase levels of inhibin-a were decreased in older women and

women with elevated follicular-phase FSH concentrations (6). In contrast, periovulatory levels of inhibin-a measured in the dominant follicle appear to be similar in both older and younger women, though serum periovulatory levels of inhibin-a are elevated in older women (8). To clarify further the relationship between inhibin and folliculogenesis in the aging ovary, we evaluated patients undergoing natural-cycle in vitro fertilization-embryo transfer (IVF-ET) and assessed differences in periovulatory inhibin-a production in women with diminished ovarian reserve and normal ovulatory function.

MATERIALS AND METHODS

Forty-two patients who underwent natural-cycle IVF-ET ($n = 55$) from July 1994 to July 1997 were evaluated. Patients with abnormal semen analyses were excluded. Groups were assigned according to the following criteria: Group I (14 patients, 18 cycles), women who were known to be poor responders based on previous stimulation cycles, had a diminished ovarian reserve, were ≥ 40 years old, and/or had an abnormal CD-3 FSH (> 15 mIU/ml) and/or CD-3 estradiol (E_2) (> 50 pg/ml) level determined previously using receiver-operated curve analysis (9); Group II (28 patients, 37 cycles) consisted normal ovulatory women < 40 years old with normal CD-3 FSH and CD-3 E_2 levels.

All patients underwent natural cycle monitoring as described previously (10). Briefly, a baseline transvaginal ultrasound was performed during the first 3 days of the menstrual cycle. Patients returned 4 days before the anticipated time of ovulation for serial transvaginal ultrasonography and serum E_2 measurements. All blood was stored at -22°C for later evaluation. When follicle maturity was achieved (> 20 mm and $E_2 \geq 200$ pg/ml, > 18 mm and $E_2 \geq 250$ pg/ml, or > 16 mm and $E_2 \geq 300$ pg/ml), human chorionic gonadotropin (hCG; 10,000 IU) was administered im. If an LH surge was not detected as measured in the urine using an

LH detection kit (OvuQuick, Quidel, San Diego, CA), transvaginal ultrasound-guided aspiration was performed 34–36 hr after hCG administration. If an LH surge occurred the cycle was cancelled.

Sperm samples were obtained by masturbation and processed by a two-step centrifugation process followed by swim-up to separate spermatozoa from seminal plasma. Oocytes were inseminated after aspiration with 200,000 spermatozoa per ml. Fertilization was documented 18–22 hr following aspiration. Transcervical embryo transfer occurred 48 to 72 hr after retrieval. Serum β -hCG levels were measured 9 and 12 days after embryo transfer. Transvaginal ultrasound confirmed the viability of the pregnancy.

As expected due to the study design, there were significant differences with respect to the age and baseline serum levels of day 3 FSH and E_2 of patients in both groups: Group I, 39.7 ± 2.3 years, 18.8 ± 2.6 mIU/ml, and 84.8 ± 9.7 pg/ml, and Group II, 32.3 ± 9 years, 7.6 ± 96 mIU/ml, and 39.1 ± 4.7 pg/ml (mean $\pm$ SE; $P < 0.05$). Semen analyses were similar between groups and within normal ranges for WHO standards.

Serum samples were assayed for FSH, E_2 , and progesterone (P) using commercially available radioimmunoassay kits. For FSH (Diagnostic Products, Los Angeles, CA), calibrated against the WHO 2nd IRP-hMG, intra- and interassay coefficients of variation (CV) were 3.4 and 4.2%, respectively; for E_2 (DPC, Los Angeles, CA), intra- and interassay CV were 5.6 and 7.4%, respectively; and for P (DPC), intra- and interassay CV were 4.0 and 5.3%, respectively. A commercially available EIA kit was used for dimeric inhibin-A (Serotec, Oxford, UK). Intra- and interassay CV were 5.2 and 7.1%, respectively.

All cycles were included for analysis. The day prior to a spontaneous surge and the day of administered hCG was defined as d-0. Serum levels are expressed as d-1, d-2, and d-3 relative to that day. Statistical analysis was performed using the Student *t* test, chi-square analysis for categorical comparisons. Significance was expressed as $P < 0.05$. Results are expressed as means $\pm$ standard errors (SE).

RESULTS

The occurrence of a spontaneous surge and the date for administering hCG were noted to be advanced in Group I compared to Group II (9.2 ± 2.5 vs 11.3 ± 2.5 days; $P < 0.05$). Peak serum E_2 (d-0) (288 ± 27.5 vs 236 ± 16 pg/ml) and P (d-0) (0.98 ± 0.26 vs 0.68

± 0.07 ng/ml) were similar in both groups (P , NS). However, inhibin-a was significantly higher in Group I compared to Group II on d-2 (20 ± 3.8 vs 12.2 ± 2.9 pg/ml) and d-0 (40 ± 5.8 vs 25.1 ± 3.2 pg/ml) ($P < 0.05$). Figure 1 shows the mean serum concentrations of E_2 , P, and inhibin-a relative to the day prior to a spontaneous LH surge or hCG administration.

The numbers of oocytes and embryos transferred were similar (Group I, 1.6 ± 0.3 and 1.0 ± 0.2 , respectively; Group II, 1.7 ± 0.4 and 1.1 ± 0.1 ; respectively; P , NS). Pregnancy outcomes were similar between groups [Group I, 5.6% (1/18 per initiated cycle) and 12.5% (1/7 per retrieval); Group II, 13.5% (5/37 per initiated cycle) and 26.3% (5/19 per retrieval); P , NS].

DISCUSSION

Ovarian aging is manifested as a decrease in fecundity rates, diminished responses to COH, decreased oocyte quality, and lower IVF success rates. This process occurs over time, with subtle markers of ovarian aging and chronological age that include a selective rise in early follicular serum FSH and E_2 and a shortened follicular phase (1,2,6). This is consistent with our data, where women ≥ 40 who are known poor responders and/or have increased FSH have a significantly shorter follicular phase. These early alterations in ovarian function proceed other known changes in LH, luteal-phase length, and corpus luteal progesterone production, which occur much later in the climacteric (3), demonstrating a slow, progressive process.

Recently, alterations in inhibin production have also been shown to be associated with elevated follicular-phase FSH concentration (6). It has been suggested that reduced serum inhibin may be a more sensitive indicator of a diminished "ovarian reserve" than pituitary FSH secretion (7). Nonetheless, like FSH, preantral follicular inhibin-b and late corpus luteal inhibin-a represent early markers of ovarian dysfunction. On the other hand, steroidogenesis by the dominant follicle produces similar peak periovulatory serum E_2 levels in those with a diminished "ovarian reserve" and their young counterparts. Moreover, there appears to be compensated periovulatory serum inhibin-a production in those with a diminished "ovarian reserve." This is similar to the findings of others (8), though the physiological significance is unclear.

Unlike the decrease in inhibin-a and -b in ovarian aging, which is thought to be a reflection of fewer primordial to early antral follicles and altered corpus luteal function, the selected dominant follicle is still

Fig. 1. Serum concentration of estradiol, progesterone, and inhibin-a relative to an LH surge or hCG administration.

capable of periovulatory inhibin-a production, and in fact compensated production seems to occur. Though this in itself may represent altered ovarian function, like corpus luteal P, it may be one of the last changes to occur prior to menopause (11).

In conclusion, many events occur in the ovarian aging process. These events are slow and occur over many years, finally leading to menopause. While early follicular- and late luteal-phase inhibin production may be one of the earliest manifestations, this report demonstrates that periovulatory inhibin-a is a much later physiological process.

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