The Utility of Serum Progesterone and Inhibin A for Monitoring Natural-Cycle IVF-ET¹

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Purpose: Our purpose was to assess the value of monitoring serum P and inhibin A to determine how values might improve the clinical monitoring of natural cycle in vitro fertilization (IVF)-embryo transfer (ET) patients.

Methods: All patients ($n = 26$) who underwent natural-cycle IVF-ET ($n = 35$) were analyzed. Groups were evaluated according to patients who had a spontaneous luteinizing hormone (LH) surge (group I) and women receiving human chorionic gonadotropin (hCG) who underwent subsequent oocyte aspiration (group II). Group II was further evaluated according to women who did ($n = 10$) and did not ($n = 7$) have an ET. All cycles were evaluated with serial transvaginal ultrasonography and serum estradiol, progesterone, and inhibin A. When follicle maturity was achieved, hCG, 10,000 IU, was administered intramuscularly if a LH surge was not detected. Transvaginal ultrasound-guided aspiration was performed 34–36 hr after hCG administration followed by a 48-hr transcervical ET.

Results: No differences were seen in cycles the day prior to (d-1) and the day of a spontaneous LH surge, ($n = 18$) or hCG (d-0) ($n = 17$) in group I or group II with respect to lead follicular diameter (d-1, 15.3 ± 0.6 vs. 14.2 ± 0.9 mm; d-0, 17.4 ± 0.8 vs. 17.8 ± 0.6 mm) and serum estradiol (d-1, 148 ± 15 vs. 150 ± 15 pg/ml; d-0, 218 ± 15 vs. 199 ± 16 pg/ml), respectively. However, serum progesterone was significantly elevated in group I compared with group II on d-1 (0.82 ± 0.6 vs. 0.48 ± 0.04 ng/ml; $P < 0.05$) and d-0 (1.1 ± 0.12 vs. 0.63 ± 0.08 ng/ml; $P < 0.05$). Inhibin A was significantly greater on d-1 in group I (24 ± 2.5 vs. 15 ± 2.2 pg/ml; $P < 0.05$). In group II, cycles that resulted

in an ET ($n = 10$) compared with group II cycles that did not ($n = 7$) revealed a significant difference in serum progesterone (0.51 ± 0.05 vs. 0.7 ± 0.07 ng/ml; $P < 0.05$) and inhibin A (15 ± 2.5 vs. 37.3 ± 5 pg/ml; $P < 0.05$) the day of hCG.

Conclusions: The possible application of serum progesterone and inhibin A in managing natural-cycle IVF-ET is suggested. These assays may predict women who should be set up for egg retrieval, while canceling others in spite of the absence of an LH surge.

KEY WORDS: progesterone; inhibin A; IVF-ET; predictive value.

INTRODUCTION

The use of the natural cycle for in vitro fertilization (IVF) and embryo transfer (ET) resulted in the first successful pregnancies from assisted reproduction (1). It still remains an attractive option because it avoids ovarian hyperstimulation and its inherent risks of hyperstimulation syndrome, possible inhibitory effects on embryo implantation, and multiple pregnancy (2,3). In addition to the decrease in cost, unstimulated cycles may be repeated on a monthly basis, thus enhancing the likelihood of success during a given period (4,5).

However, the number of natural-cycle IVF-ET reported to the Society for Assisted Reproductive Technology (SART) has continued to decline, from 739 initiated cycles in 1990 to 410 reported in 1994 (6,7). This may be attributed to a high cancellation rate from spontaneous luteinizing hormone (LH) surges occurring in up to 36% of initiated cycles (range, 31 to 50%) (6,7). Guidelines for using ultrasonography and serum estradiol (E_2) to monitor follicular maturity have been published (4). However, better markers demonstrating predictive value in identifying an impending LH surge would be of great value in avoiding cancellation.

Oocyte maturity parallels both the progressive antral cavity enlargement, which may be detected by ultraso-

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nography (8), and the production of E_2 by granulosa cells. However, in addition, a small but significant increase in progesterone (P) occurs 12–24 hr prior to ovulation with a continued rise following the onset of the LH surge (9). Furthermore, increasing quantities of peptides including inhibin A and activin appear to play a role in gonadotropin release and possess autocrine activity on granulosa-cell steroidogenesis (10,11). We assessed the value of monitoring serum P and inhibin A to determine how values might improve the clinical monitoring of natural-cycle IVF-ET patients.

MATERIALS AND METHODS

All patients ($n = 26$) who underwent natural cycle IVF-ET ($n = 35$) from July 1994 to January 1997 were analyzed. Cases of male-factor infertility and women 40 years of age or older who had abnormal follicle-stimulating hormone (FSH) and E_2 levels on cycle days 2–3 were excluded. Groups were evaluated according to patients who had a spontaneous LH surge and were canceled (group I) and women receiving human chorionic gonadotropin (hCG) who underwent subsequent oocyte aspiration (group II). Group II was further evaluated according to women who did ($n = 10$) and did not have an ET ($n = 7$). Three cases resulted in abnormal fertilization, while the other four did not result in any fertilization.

All patients underwent natural-cycle monitoring as described previously (4). 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 -20°C for later evaluation. When follicle maturity was achieved, hCG, 10,000 IU, was administered intramuscularly. If an LH surge was not detected as measured in the urine using an LH detection kit (OvuQuick, Quidel, San Diego, CA), which has been shown to be a reliable method of predicting the serum LH surge (12), transvaginal ultrasound-guided aspiration was performed 34–36 hr after hCG administration.

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 milliliter. Fertilization was documented 22 hr after aspiration. Transcervical ET occurred 48 hr after retrieval. Serum β -hCG levels

were measured 9 and 12 days after ET. Transvaginal ultrasound confirmed pregnancy viability.

There were no differences with respect to the age of patients in both groups: group I, 31.7 ± 1 years; group II, 32.0 ± 1.6 years (mean $\pm$ standard error [SE])($P =$ not significant). Baseline serum levels of day-3 FSH and E_2 were similar (group I, 7.2 ± 1.2 , and 42.6 ± 4.9 pg/ml, respectively; group II, 6.1 ± 1.2 and 38.0 ± 5.6 pg/mL, respectively) ($P =$ not significant), as was semen analysis (group I, $125 \pm 27 \times 10^6$ /ml and $57.1 \pm 3.3\%$ motility; group II, $106 \pm 15.9 \times 10^6$ /ml and $67.6 \pm 4.7\%$ motility) ($P =$ not significant).

Serum samples were assayed for FSH, E_2 , and P using commercially available radioimmunoassay kits. For FSH (Diagnostic Products, Los Angeles, CA), calibrated against the World Health Organization 2nd IRP-human menopausal gonadotropin (hMG), intra- and interassay coefficients of variation were 3.4 and 4.2%, respectively; E_2 (DPC, Los Angeles, CA), intra- and interassay coefficients of variation were 5.6 and 7.4%, respectively; P (DPC, Los Angeles, CA), intra- and interassay coefficients of variation were 4.0 and 5.3%, respectively. A commercially available enzyme immunoassay kit was used for dimeric inhibin A (Serotec, Oxford, UK). The intra- and interassay coefficients of variation were 5.2 and 7.1%, respectively. Statistical analysis was performed using Student's t test, chi-square analysis, and linear correlation. Significance was expressed as P less than 0.05.

RESULTS

Follicular growth correlated with a progressive rise in serum E_2 ($r = 0.46$, $P < 0.002$), P ($r = 0.47$, $P < 0.002$), and inhibin A ($r = 0.65$, $P < 0.001$). Eighteen cycles (51.4%) were canceled because of a spontaneous LH surge, while 10 cycles (59% of aspirated cycles) resulted in an ET. No differences were seen in cycles the day prior to (d-1) and the day of a spontaneous LH surge or hCG administration (d-0) in group I or group II with respect to lead follicular diameter (d-1, 15.3 ± 0.6 vs. 14.2 ± 0.9 mm; d-0, 17.4 ± 0.8 vs. 17.8 ± 0.6 mm) and serum E_2 (d-1, 148 ± 15 vs. 150 ± 15 pg/ml; d-0, 218 ± 15 vs. 199 ± 16 pg/ml), respectively. However, serum P was significantly elevated in group I compared with group II on d-1 (0.82 ± 0.6 vs. 0.48 ± 0.04 ng/ml; $P < 0.05$) and d-0 (1.1 ± 0.12 vs. 0.63 ± 0.08 ng/ml; $P < 0.05$). Inhibin A was significantly greater on d-1 in group I (24 ± 2.5 vs. 15 ± 2.2 pg/ml; $P < 0.05$) (Table I).

Table I. Serum E₂, P, and Inhibin A the Day Prior to and the Day of a Spontaneous LH Surge and hCG Administration

	d-1 ^a	d-0 ^b
Surge cycles (<i>n</i> = 18)		
E ₂ (pg/ml)	148 ± 15 ^c	218 ± 15
P (ng/ml)	0.82 ± 0.06 ^d	1.1 ± 0.12
Inhibin A (pg/ml)	24 ± 2.5	32 ± 6.3
Retrieval cycles (<i>n</i> = 17)		
E ₂ (pg/ml)	150 ± 14	199 ± 16
P (ng/ml)	0.48 ± 0.04 ^d	0.63 ± 0.08
Inhibin A (pg/ml)	15 ± 2.2 ^d	29 ± 5.5

^a Day prior to surge or hCG.^b Day of surge or hCG.^c Standard error.^d *P* < 0.05.

A comparison of group II cycles that resulted in an ET (*n* = 10) vs. those that did not (*n* = 7) revealed no differences in d-0 serum E₂ (178 ± 15 vs. 224 ± 33 pg/ml), but a significant difference in serum P (0.51 ± 0.05 vs. 0.7 ± 0.07 ng/ml; *P* < 0.05) and inhibin A (15 ± 2.5 vs. 37.3 ± 5 pg/ml; *P* < 0.05).

Using the 95% confidence intervals from the means of P (≥0.7 ng/ml) and inhibin A (≥18.7 pg/ml) from group I, a surge was predictive using P as early as d-1 compared with group II (76 vs. 17%; *P* < 0.05) and d-0 (100 vs. 28%; *P* < 0.05). While inhibin A was not significantly predictive of a surge (d-1, 71 vs. 33%; d-0, 88 vs. 44%; *P* = not significant), it was predictive of those that resulted in an ET compared with no ET (<18.7 pg/ml) (d-1, 90 vs. 11%, *P* < 0.05; d-0, 100 vs. 22%, *P* < 0.05).

DISCUSSION

Natural-cycle IVF-ET offers the advantage of lower cost and physical ease, thus allowing cycles to be repeated more frequently, while embryo implantation rates may be higher than in IVF-ET using controlled ovarian hyperstimulation (14). In spite of this, natural-cycle IVF-ET is offered sparingly because monitoring these cycles is problematic, due mostly to the inability to prevent a spontaneous LH surge.

Various protocols to enhance the success of natural-cycle IVF have been tried. Originally, follicle aspiration following timed serial urinary LH monitoring was attempted, but because the maturity of harvested embryos was unpredictable, this practice was abandoned (4). Other attempts included an FSH boost prior to hCG (15) combined with the administration of a

gonadotropin-releasing hormone agonist (Nal-Glu; Salk Institute, San Diego, CA) (16) to block ovulation. However, this added complexity and expense with the addition of exogenous hormone manipulations.

Seibel *et al.* (17) reported on parameters that predict success for natural-cycle IVF including cycle day-3 LH and prolactin levels. However, this and other reports (18) did not discern any differences in patients who were later canceled because of a spontaneous LH surge, or predict patients who did and did not undergo ET. Our report, although retrospective, shows a significant rise in serum P and inhibin A as early as the day prior to the spontaneous LH surge and, additionally, identified cycles that received hCG and did not result in an ET. While rises in serum P and inhibin A progressively increase during the follicular phase, reaching a midcycle peak that coincides with the gonadotropin surge (13), reflecting oocyte maturity, elevations prior to attaining a critical follicle size and serum E₂ level could also indicate premature luteinization.

In summary, these data suggest the possible application of serum markers, P and inhibin A, in managing natural cycle IVF-ET. These assays may predict women who should be set up for egg retrieval and indicate others who should be canceled in spite of the absence of an LH surge.

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