Hormone Release from Cultured Luteinized-Granulosa Cells Mimics Differences Seen in Vivo in Patients Undergoing IVF-ET

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Objectives: Previous research from this laboratory has suggested that a relationship exists between the increase in circulating progesterone concentrations at the time of hCG administration and cycle outcome in patients undergoing IVF. Progesterone (P) increases of threefold or better within the 24-h period surrounding hCG administration appeared to be associated with a higher pregnancy rate. These data suggest a functional difference in the luteinized-granulosa of patients undergoing IVF. To test this hypothesis:

Design: A split-split plot arrangement of treatments with two cell sources under five hormonal stimulations at four time points.

Methods: Luteinized-granulosa cells (LGC) were collected from patients with either a normal increase (≥threefold = NC) in circulating P (n = 4) or those with lower P increases (AC; n = 4). The cells were isolated by Ficoll gradient centrifugation and then cultured in 24-well culture plates using a modified media 199 containing 100 mIU/ml of hMG, FSH, LH, hCG, or a nonhormonal control to stimulate steroid-hormone production. At time points 2, 4, 6, and 8 days, media from each well was frozen for later hormone assay and replaced with fresh media containing the same stimulating factor. After all the media had been collected, P and estradiol (E2) released into the media were measured using radioimmunoassay.

Results: Results indicate a higher media concentration of P (P < 0.001), but not E2 (P = 0.254), from NC, regardless of hormone stimulation or time in culture, when compared to the media from AC. Media concentrations of E2 were affected by a cell source by hormone stimulation by time interaction (P < 0.001) with varying effects. Media from NC maintained a constant E2 of between 1000–3000 pg/ml over the 8-day period (P = 0.163). However, media from AC demonstrated a stimuli-dependent E2 release (P < 0.001) ranging from < 1000 to over 11,000 pg/ml.

Conclusions: These data appear to support the existence of functionally different populations of luteinized-granulosa cells from patients undergoing IVF-ET.

KEY WORDS: luteinized-granulosa; culture; progesterone, IVF cycle outcome.

INTRODUCTION

The use of in vitro fertilization-embryo transfer (IVF-ET) to treat infertility in humans is 15 years old. During that time period, improvements have been made in stimulation protocols, retrieval methods, culturing procedures, and transfer techniques. Yet pregnancy rates at the most successful IVF-ET centers are no greater than 50%, and the national average remains at approximately 20% (1). While much work still remains in perfecting the technical aspects of IVF-ET, another means of improving pregnancy rates is the use of patient selection criteria which identifies those patients, or patient cycles, which have limited chances of success, and canceling or modifying the cycle prior to embryo transfer.

A number of recent studies have suggested various means of predicting cycle outcome prior to oocyte retrieval (2–5). A review of the literature suggests the majority of these predictive techniques are based on various hormone concentrations at set stages during follicular stimulation (6–9), with the largest number of studies examining the predictive...
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value of progesterone (P) concentrations at the time of human chorionic gonadotropin (hCG) administration. Several recent studies have suggested a correlation between circulating concentrations of P and IVF-ET cycle outcome based on a single blood draw on the day of hCG administration (10–13). However, evidence exists which disputes this correlation (14–16).

In 1988, Hsuing et al. (17) examined the rise in P concentrations after hCG administration and found a correlation between the increase seen in P concentrations 20–34 h after hCG administration and cycle outcome. Recent work from our laboratory also suggests that the hCG-induced rise in circulating P concentrations after hCG administration might be useful in predicting cycle outcome (18). We found that patients with a threefold or greater increase in circulating P from 12 h pre- to 12 h post-hCG administration had a significantly increased pregnancy rate (46%) when compared to those patients whose P increased less than threefold over the same 24-h period (14%).

In addition to its potential usefulness in predicting cycle outcome, these data also appear to have identified two distinct populations of follicles: (1) follicles which respond “normally” to the administration of hCG by initiating luteinization and increasing P secretion; (2) follicles which have only a minimal P response to hCG stimulation. In an effort to determine if such differences exist, luteinized-granulosa cells (LGC) were obtained from the follicular aspirate of women who were undergoing IVF-ET for infertility and whose hCG-induced rise in circulating P had been determined. The cells were then placed in culture under various hormonal stimuli for 9 days. Cellular response to stimulation was measured as the release of the hormones P and estradiol (E₂) into the culture media.

MATERIALS AND METHODS

Selection of Patients for Inclusion in Study

Luteinized-granulosa cells were obtained from the follicular aspirates of patients undergoing IVF-ET for treatment of infertility (IRB approved). The patients had previously been down regulated with leuprolide acetate, then stimulated with human menopausal gonadotrophins (hMG) followed by 5000 IU of hCG to trigger oocyte maturation. As in the previous study (18), circulating P concentra-

tions were determined at 12 h before and 12 h after hCG administration, and the rise in P concentration was calculated. As described above, this value appears to be correlate with cycle outcome. Patients whose P increases threefold or greater over the 24-h period exhibit significantly increased pregnancy rates (46%) when compared to those patients with less than a threefold increase over the same 24-h period (14%). It also represents a relative value between the two time points, rather than an absolute P concentration. Therefore this value appears to be independent of the effects of follicle number while measuring a response of the female reproductive system (possibly a response of the granulosa) to hCG administration.

Using the in vivo P data, the patients where separated into the two groups which had earlier been associated with cycle outcome: those with less than a threefold increase (AC) versus those with a threefold or greater increase (NC). Luteinized-granulosa cells obtained from a total of four patients in each group were included in the present study.

Acquisition of Luteinized-Granulosa Cells

Luteinized-granulosa cells were collected at oocyte retrieval and cultured based on the technique of Tapanainen et al. (19). Oocytes were recovered via transvaginal ultrasound guided follicular aspiration. Once oocytes had been removed to culture, aspirates of mature follicles (as judged by follicular size of >15 mm and location of a visually mature, metaphase II oocyte) were transferred to a 10-ml centrifuge tube (Falcon; Cockeysville, MD) and centrifuged at 300  g for 10 min. The supernatant was discarded and the pellet resuspended and washed twice in fresh medium 199 (Irvine Scientific; Irvine, CA). The final pellet was resuspended in 5 ml of media containing 0.1% collage-nase-dispase (Sigma Chemical; St. Louis, MO) and centrifuged at 300 × g for 10 min. The supernatant was discarded and the pellet resuspended and washed twice in fresh medium 199 (Irvine Scientific; Irvine, CA). The final pellet was resuspended in 5 ml of media containing 0.1% collage-nase-dispase (Sigma Chemical; St. Louis, MO) and incubated at 37°C for 30 min. The suspension was transferred to a 10-ml centrifuge tube containing 1 ml of fetal calf serum (FCS; Irvine Scientific) and centrifuged for 5 min at 300 × g. The pellet was resuspended in 3 ml of fresh medium 199 and the suspension transferred to a 10-ml centrifuge tube containing 3.5 ml of Ficoll (Sigma Chemical). The LGC were separated from other cell types by centrifugation at 600 × g for 10 min. The LGC were collected at the media-Ficoll interface, while other cell types collected at the bottom of the tube. The layer containing the LGC was removed to a sepa-