Serum follicle-stimulating hormone inhibition is a marker for preovulatory oocytes in in-vitro fertilization and embryo transfer

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A retrospective analysis was performed on 64 cycles stimulated with human menopausal gonadotrophin and/or pure follicle-stimulating hormone (FSH) and oestrogen (E2) levels. The increase in serum E2 on the day of HCG administration did not correlate \(r = 0.05\) with the number of preovulatory oocytes (preovs) or with an increase or decrease in serum FSH \(r = 0.31\). However, the change in serum FSH showed a significant correlation with the number of preovs \(r = -0.95, P = 0.013\). The probability of obtaining two or more preovs was relatively greater \(1.47\times\) than that of other IVF patients, which there was a drop in FSH of 5% on the day of human chorionic gonadotrophin administration.

Key words: IVF/marker for preovulatory oocytes/serum FSH inhibition

Introduction

The inability to predict oocyte maturity and to determine accurately the timing for oocyte harvest may be a critical factor responsible for failure of in-vitro fertilization (IVF) procedures. Sonographic follicular size, serum oestradiol (E2) determinations and cervical mucus assessment have been utilized widely for these purposes (Vargyas et al., 1982; Muasher et al., 1985; Laufer et al., 1986). Evidence for a correlation between follicular size and oocyte maturity has been demonstrated but has not been a sensitive predictor (Vargyas et al., 1982; Buttery et al., 1983; Kreiner et al., 1988). Likewise, serum E2 levels cannot consistently predict mature oocytes which may be associated with relatively low levels, whereas relatively high E2 levels may be associated with the presence of multiple smaller or immature follicles (Vargyas et al., 1982; Buttery et al., 1983; Mantzavinos et al., 1983; Kreiner et al., 1988). Assessment of the cervical mucus as well as the maturation index of a vaginal smear, correlates very well with ovulation in a natural cycle (Garcia, 1983). In hyperstimulated cycles, the oestrogen bioassay can be premature in predicting maturity in responders with E2 levels much higher than those normally seen in natural cycles (Kreiner et al., 1988).

Therefore, a more consistent, precise and objective index of preovulatory oocyte (preov) number is needed. Assessment of the relative follicle-stimulating hormone (FSH) suppression, presumably from follicular inhibin production during late follicular phase gonadotrophin stimulation, when inhibin appears to be maximal (McLachlan et al., 1986), may serve as such an index.

This study hypothesized that, in IVF, maximal FSH suppression may be utilized to determine the optimal time for aspiration of mature oocytes and have therefore studied FSH levels just prior to human chorionic gonadotrophin (HCG) administration, in order to evaluate its use in predicting the maturity of the oocytes obtained at harvest.

Materials and methods

Sixty-four cycles of human menopausal gonadotrophin (HMG) (Pergonal, Serono Laboratories Inc., Randolph, MA) and/or pure FSH (Metrodin, Serono Laboratories Inc., Randolph, MA), including 22 with only pure FSH and 42 with the combination stimulation cycles, were analysed retrospectively in 64 IVF patients, with respect to their serum FSH and E2 levels obtained at 08.00 hours, ~16 h after gonadotrophin injection on the day prior to and the day of administration of 10000 IU of HCG (Pregnyl, Organon, West Orange, NJ). Stimulation protocols were determined based upon the patients' previous responses or baseline hormone levels. Follicular patterns did not vary significantly between the two stimulation groups.

FSH was assayed using a quantitative radioimmunoassay kit.

Fig. 1. Percentage of increase in E2 and the number of preovulatory oocytes. The percentage of increase in serum E2 levels on the day of HCG administration does not correlate with the number of preovulatory oocytes.
D. Kreiner et al.

(Leeco, Southfield, MI) with an interassay and intrassay coefficient of variation of 4.3 and 4.8%, respectively. The E2 assay was performed using the Pantex immunodirect assay kit (Pantex, Santa Monica, CA) with an interassay and intrassay coefficient of variation of 5.7 and 5.8%, respectively. The oocytes were classified as prevulatory based on the criteria previously described (Sandow, 1983) and included metaphase II oocytes and some late metaphase I oocytes. Statistical analysis was performed using linear regression and analysis of variance.

Results

The data were analysed according to the number of preovs obtained at harvest. The percentage increase in serum E2 levels between the day before HCG and the day of HCG did not correlate \( r = 0.05 \), nor was there any significance with respect to the number of preovs (Figure 1), or the percentage of change in serum FSH \( r = 0.31; P = 0.61 \) (Figure 2). However, the percentage of change in serum FSH showed a significant inverse correlation with the number of preovs \( r = 0.95; P = 0.13 \) (Figure 3). There was a 7% decrease in serum FSH on the day of HCG administration when three or four preovs were aspirated and a 15.5% decrease when there were five or more preovs, compared with a 13.2% increase in cycles with no preovs, 11.0% increase in cycles with one preov and a 4% decrease in cycles with two preovs. The difference in the percentage of change in serum FSH was significant between those cycles with three or more preovs and those with fewer than two \( P < 0.05 \). Using a cut-off > 5% decrease in FSH as an index for the presence of two or more preovs obtained at harvest, 23/41 or 61% of cases with two or more preovs could have been predicted on the basis of the serum FSH levels alone. These results compare favorably to those obtained using the other commonly used criteria (Table I). In a study of 151 cases during the most recent IVF series, the presence of two 16-mm follicles would have correctly predicted the presence of two or more preovs in only 23/122 (19%) of cases (Kreiner et al., 1988). An E2 level > 600 pg/ml would have been predictive in only 60/122 (49%) of cases and the specific duration of the mucus shift had no statistical significance with respect to the maturation of the oocytes.

The probability of obtaining two or more preovs relative to the rest of the IVF patients was greater (1.47) when there was an FSH drop of > 5%, compared with 1.16 when E2 was > 600 pg/ml, 1.14 when two 16-mm follicles were present, 1.10 when two 14-mm follicles were present, or 1.24 when the combination of two 14-mm follicles and E2 > 600 pg/ml was present.

Discussion

These results support the hypothesis that serum FSH levels in the late follicular phase reflect follicular factors, such as inhibin or other non-steroidal factors, that maximally inhibit pituitary FSH prior to full maturation or ovulation. For the first time it can be demonstrated that serum FSH levels in the late follicular phase can be used as a test to determine optimal timing for HCG administration and oocyte retrieval. The FSH drop suggests that bioactive inhibin increases significantly - 48 h prior to achieving prevulatory or metaphase II status. As an objective assay for
the maturational state of the oocyte, this would be a very valuable tool for IVF or ovulation induction. Its sensitivity in predicting the presence of multiple mature oocytes during IVF exceeds that of ultrasound, serum E2 or cervical mucus.

Of perhaps greater interest is the finding that inhibin or other non-steroidal factors produced by the follicles appear to play a more critical role than E2 in FSH inhibition, as demonstrated by the finding that the E2 increase did not correlate with the FSH drop, whereas the number of preovs did. The immature follicles which can produce significant quantities of E2 require further FSH stimulation to continue their growth and development. Teleologically, it would appear to be advantageous for the more potent FSH inhibitor to be produced specifically by the more mature follicles that are no longer dependent on FSH. Thus, maximal FSH inhibition is accomplished at a time when the dominant follicle(s) is independent of FSH for continued maturation.

It would appear, therefore, that the timing of HCG administra-tion can be determined by serum FSH levels reflecting follicular inhibition by mature follicles of pituitary FSH secretion. Through an understanding of the physiology of folliculogenesis and the hypothalamic - pituitary - ovarian feedback loop, one can ascertain more precisely the timing for the aspiration of mature oocytes in an IVF programme.

References


In a retrospective analysis of 64 patients stimulated with human menopausal gonadotropin (hMG) and/or pure follicle stimulating hormone (FSH); 35 cycles with spontaneous luteinizing hormone (LH) surges were compared with 29 control cycles with respect to serum FSH and estradiol (E2) levels drawn on the day prior to and the day of human chorionic gonadotropin (hCG), approximately 16 hr after gonadotropin stimulation. FSH decreased significantly ($P < 0.05$) in control cycles where two or more preovulatory oocytes (preovs) were obtained, in contrast to cycles with a spontaneous LH surge, where FSH in­creased irrespective of the number of preovs. The E2 in­crease in the LH surge cycles was significantly higher ($P < 0.05$) than in the control cycles. However, the increase in E2 did not correlate with the change in FSH levels or with the number of preovs.

**KEY WORDS:** luteinizing hormone surges; follicle stimulating hormone suppression; gonadotropin stimulation.

**INTRODUCTION**

It has been demonstrated that a nonsteroidal factor (or factors) present in follicular fluid is capable of inhibiting the estrogen-induced luteinizing hormone (LH) surge (1). In the natural cycle, a spontaneous LH surge is usually triggered with estradiol (E2) levels of 200 to 300 pg/ml. However, patients un­dergoing gonadotropin stimulation, despite appar­ent supraphysiologic midcycle (E2) levels, usually fail to trigger an LH surge (2,3). This failure to elicit an LH surge in stimulated cycles is presumed to be due to excessive follicular production of this inhib­itor, gonadotropin surge inhibitory factor (GnSIF). However, many centers performing in vitro fertil­ization (IVF) have recently observed the presence of spontaneous LH surges in a subgroup of patients undergoing human menopausal gonadotropin (hMG) stimulation (4-6). In our experience these patients are characterized as being older and develop­ing fewer follicles during ovarian stimulation (7). The difficulty in these cases of spontaneous LH surges is the inability properly to time and thereby predict the presence and number of preovulatory oocytes (preovs) at ovum pickup, since the onset of the surge, unlike the timing of human chorionic go­nadotropin (hCG) administration, is unknown.

In this study, FSH suppression, presumably from follicular inhibin, was assessed during gonadotropin stimulation in the late follicular phase for its ability to predict the presence and number of preovs in cycles with and without a spontaneous LH surge.

**MATERIALS AND METHODS**

In a retrospective analysis of 64 patients stimu­lated with hMG (Pergonal, Serono Laboratories, Inc., Randolph, MA) and/or pure FSH (Metrodin, Serono Laboratories Inc., Randolph, MA), 35 cy­cles with spontaneous LH surges were selected for comparison with 29 randomly selected control cy­cles within the same IVF series. All patients had regular menstrual cycles with diagnoses of tubal factor, male factor, endometriosis, and unexplained infertility evenly distributed between the two groups. Patients were stimulated on days 3 and 4 of the menstrual cycle with either 4 ampoules of pure FSH or 2 ampoules of hMG and 2 ampules of FSH. On days 5 to 8 the patients were stimulated with 2 ampoules of FSH or hMG, depending on their rise in E2 and growth in follicular size. Patients were given hCG if the leading follicle was >= 16mm, if the E2 rate of rise was rapidly increasing or plateauing, or if the E2 level was >425 pg/ml in the presence of a >= 14-mm follicle. Their serum E2, FSH, and LH were assayed from blood specimens at 8 AM, ap­proximately 16 hr after gonadotropin injection on the day prior to and on the day of administration of hCG (Pregnyl, Organon, West Orange, NJ) (10,000 IU). Radioimmunoassay of E2 was performed using a Pantex immunodirect assay kit (Pantex, Santa Monica, CA). The interassay and intraassay coeffi­cient of variation for E2 was 5.7 and 5.8%, re­spectively. For FSH the coefficient was 4.3 and 4.8%, respectively, using a quantitative radioimmunoas­say kit (Leeco, Southfield, MI). LH was also as­sayed with this kit; the coefficient of variation was 4.1 and 7.1 %, respectively. The data were analyzed according to the number of preovs obtained at har­vest. The classification of a mature oocyte was based on the presence of a germinal vesicle and an extruded polar body within 12 hr of harvest (8). In this study an LH surge was defined as a level >60 IU/liter. Harvest was performed 16 hr early if the E2 level dropped after hCG administration and was performed approximately 34.5 hr after hCG admin­istration if there was an E2 rise. Ovulation was rarely noted in these cases. Statistical analysis was performed by linear regression and analysis of vari­ance.

**RESULTS**

FSH decreased significantly ($P < 0.5$) on the day of hCG administration in control cycles where two or more preovs were obtained. This FSH decrease inversely correlated in a significant fashion with the number of preovs ($r = -0.95, P < 0.01$) (Fig. 1). In contrast to control cycles, in those cycles with a spontaneous LH surge the FSH increased irrespec­tive of the number of preovs ($r = 0.56, P = 0.33$). This difference in the percentage of change in FSH levels between the control group (- 13.6%) and the
spontaneous LH surge group (+11.6%) was statistically significant ($P = 0.001$). The increase in E2, however, was significantly greater ($P = 0.04$) in the spontaneous LH surge group (65.1%) than in the control group (31.9%) (Fig. 2), but the increase in E2 did not correlate with the change in FSH ($r = 0.31$) levels or with the number of preovs ($r = 0.05$) in either group.

**DISCUSSION**

These results demonstrate that in the absence of an LH surge, FSH is suppressed independent of E2 levels approximately 48 hr prior to ovum pickup in the presence of, and in proportion to the number of, preovs obtained at harvest. Thus, it appears that there is an additive effect from follicular inhibin which is maximal at this time (9). However, in the absence of the usually observed inhibition of LH surges in stimulated cycles, there is an associated absence of FSH suppression, which was seen even when multiple preovs were obtained. Three possible explanations for this exist. (a) The follicular production of the nonsteroidal factors, inhibin and GnSIF, may be integrally related in that one does not occur without the other, i.e., if they are co-produced through cleavage of a common prohormone. (b) Not exclusive of a, the absence of these nonsteroidal factors may indicate poor health of the follicular apparatus despite apparent maturity. (c) Since the rate of E2 rise was significantly greater in the group with spontaneous LH surges, the absence of FSH and LH inhibition may reflect premature stimulation of the pituitary prior to adequate production of the inhibitors. The finding that an absence of inhibition occurs in the presence of multiple preovs suggests that this cannot be the only explanation.

It appears from these results that FSH suppression may be used as a marker for the number of mature oocytes only in the absence of a spontaneous LH surge. Perhaps this reflects the relative health of these mature follicles.

Additionally, it may be concluded that it is the rate of the rise of E2, rather than the absolute E2 level, that appears to trigger an LH surge.

**REFERENCES**

2. Schenken RS, Hodgen GO: Follicle stimulating hormone-induced ovarian hyperstimulation in monkeys: Blockade of the luteinizing hormone surge. JCEM 1983; 57:50
5. Rossavik IK, Gibbons WE: Growth curve analyses of follicular growth in the in vitro fertilization program. Fertil Steril 1986; 45: 834