Incomplete Androgen and Progesterone Suppression Following Midluteal GnRHa Prior to Controlled Ovarian Hyperstimulation for IVF-ET

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Purpose: We aimed to determine if midluteal GnRH agonist (GnRHa) use prior to controlled ovarian hyperstimulation (COH) results in uniform progesterone and androgen suppression and whether elevations of these hormones occurring early in follicular development may adversely effect the outcome of IVF-ET.

Methods: Forty-four COH cycles using midluteal GnRHa were evaluated. Serum gonadotropins (LH and FSH) and gonadal steroids (E_2 , A, P_4 , and T) were measured after 10 days of GnRHa administration [cycle day 31 (CD 31)] and again on the day of hCG administration, following COH. Cycle outcomes evaluated were the number of oocytes retrieved, morphologic grade, fertilization, implantation, pregnancy, and spontaneous abortion rates.

Results: Endogenous serum FSH was uniformly suppressed (6.32 ± 0.47 IU/L) on CD 31, however, LH was not (23.76 ± 0.76 IU/L). Five and four tenths percent of cycles demonstrated low-level P_4 elevations (≥ 0.9 ng/ml), 24.4% demonstrated serum androstenedione levels ≥ 600 pg/ml, and 39% of cycles were characterized by serum T levels ≥ 200 pg/ml despite evidence of E_2 suppression (≤ 30 pg/ml) and the absence of follicular growth by sonography. LH levels were not predictive of incomplete P_4 or androgen suppression. Elevations of either P_4 , A, or T occurring early in the follicular phase were not found to correlate with an impairment in clinical cycle outcome.

Conclusions: Midluteal GnRHa use prior to COH may result in incomplete suppression of circulating progesterone and

androgens. However, these relative elevations, occurring early in the development of the follicular cohort, did not appear to affect IVF cycle outcome adversely.

KEY WORDS: androgens; gonadotropin-releasing hormone agonist; in vitro fertilization-embryo transfer; progesterone.

INTRODUCTION

The addition of highly potent gonadotropin releasing hormone agonists (GnRHa) to controlled ovarian hyperstimulation (COH) regimens has been shown to improve in vitro fertilization-embryo transfer (IVF-ET) cycle outcomes (1–3). GnRHa suppression of bioactive LH, resulting in decreased LH-induced ovarian stromal androgen synthesis, may account for greater follicular cohort synchrony with subsequently enhanced oocyte numbers and quality after follicular aspiration (4). GnRHa initiation in the midluteal phase results in a significantly more prompt and consistent ovarian suppression when compared with follicular phase starts (1). Furthermore, prospective, randomized trials have demonstrated an improvement in pregnancy outcome with the use of midluteal GnRHa when compared with follicular phase initiation (5). Early follicular GnRHa initiation is associated with increases in ovarian androgen production, which may have detrimental effects during the early development of the follicular cohort (5). However, previous studies in normal (6) and hyperandrogenic women diagnosed with polycystic ovarian syndrome (PCOS) (7) have suggested that the dose and duration of treatment with GnRHa required to inhibit ovarian follicular estrogen may not be sufficient to suppress androgen production completely.

The purposes of this study were therefore (a) to evaluate the possibility of incomplete ovarian stromal/thecal progesterone (P_4) and androgen suppression fol-

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lowing standard treatment with GnRHa initiated in the midluteal phase, despite suppression of ovarian follicular growth as measured by transvaginal sonography (TVS) and circulating estradiol (E_2) levels and (b) to assess any potential adverse impact on oocyte development and IVF-ET cycle outcome.

MATERIALS AND METHODS

Forty-two patients undergoing 44 consecutive cycles of COH for the purpose of IVF-ET at the Center for Reproductive Medicine at the Cedars-Sinai Medical Center were studied prospectively. Subjects ranged in age from 27 to 36 years, with a mean age of 32.2 ± 0.6 years. The principal infertility diagnoses at the time of initiation of COH included tubal factor ($n = 29$), minimal endometriosis/unexplained infertility ($n = 11$), and male factor ($n = 2$). Patients with a diagnosis of PCOS defined as chronic anovulation with associated hyperandrogenism were not included in the analysis. The duration of infertility ranged from 2.2 to 7.2 years, with a mean of 3.7 ± 0.3 years.

All subjects received GnRHa (Lupron; TAP Pharmaceuticals, North Chicago, IL), 1.0 mg sc qd, beginning 8 days after detection of an urinary LH surge using a urine ovulation predictor kit (Ovuquick, Monoclonal Antibodies, Sunnyvale, CA). After 10 days of GnRHa therapy, on cycle day 31 (CD 31), serum was obtained between 0800 and 0900 for measurement of circulating gonadotropins (LH and FSH), progesterone (P_4), androstenedione (A_4) and testosterone (T). Transvaginal sonography (TVS) was performed and serum E_2 assayed to ensure ovarian suppression [no ovarian cyst ≥ 10 mm in diameter and $E_2 \leq 30$ pg/ml (110 pM)]. Subjects underwent the standard COH regimen used for IVF-ET in our program, which has been previously described in detail (5). Briefly, the GnRHa dose was reduced to 0.5 mg/day sc and human menopausal gonadotropins (hMG; Pergonal; Serono Laboratories, Randolph MA), 225 IU/day, were administered intramuscularly (im) for 5 days. Follicular development was evaluated after 5 days by TVS and measurement of peripheral serum E_2 levels by rapid RIA. The hMG dosage was then individualized based on the follicular response, which was monitored at 1- to 3-day intervals. Human chorionic gonadotropin (hCG, Profasi; Serono Laboratories), 10,000 IU im, was administered when two or more follicles with a mean diameter ≥ 18 mm and a serum $E_2 \geq 500$ pg/ml (1840 pM) was achieved. Serum was obtained on the day of hCG administration for measurement of gonadotropins, P_4 , A_4 and T. Ultra-

sound-guided transvaginal ovum retrieval was performed 34–35 hr later. Oocytes were classified by morphologic grade as described previously (8). Insemination was performed 4 to 6 hr after retrieval. Fertilization was assessed morphologically as described previously (9), and transcervical intrauterine transfer of three to five embryos was performed 48 to 50 hr following oocyte retrieval. Excess embryos were cryopreserved. Progesterone in oil, 25 mg im, was administered daily beginning on the day of embryo transfer. Serum hCG levels were measured by RIA 14 days following embryo transfer.

The fertilization rate was defined as the number of embryos demonstrating morphologic evidence of two pronuclei per the number of oocytes inseminated. The implantation rate was calculated as the number of gestational sacs observed by TVS per the number of embryos transferred. Pregnancy was determined by a rising serum hCG level and evidence of a gestational sac by TVS. A spontaneous abortion (SAB) was defined as any noncontinuing pregnancy.

All sera were stored at -20°C . Hormone assays were performed by a single laboratory using commercially available RIA kits for LH and FSH (Amerlex, Eastman Kodak Corp., Rochester, NY), E_2 (Pantex, Santa Monica, CA), and P_4 (ICN Biomedical, Costa Mesa, CA). Assays for A_4 and T were performed using previously described RIA techniques in our laboratory (10). Statistical comparisons between groups were performed using the Wilcoxon sign rank sum test. Linear regression analyses were used where appropriate. P values <0.05 were considered to be significant.

RESULTS

Mean endogenous serum gonadotropin, androgen, and progesterone levels determined on cycle day 31, following 10 days of GnRHa therapy, in patients demonstrating ovarian suppression as evidenced by a serum $E_2 \leq 30$ pg/ml and the absence of ovarian cysts ≥ 10 mm by TVS are shown in Fig. 1. Endogenous serum immunoreactive FSH was uniformly suppressed (6.32 ± 0.47 mIU/ml) on CD 31, however, LH was not (23.76 ± 0.76 mIU/ml). Five and four tenths percent of cycles demonstrated low-level P_4 elevations (≥ 0.9 ng/ml) despite evidence of E_2 suppression and the absence of follicular growth. In contrast, 24.4% of cycles demonstrated serum androstenedione levels ≥ 600 pg/ml (mean, 717.6 ± 34.2 pg/ml) and 39% of cycles were characterized by serum T levels ≥ 200 pg/

Fig. 1. Serum gonadotropins, progesterone, and androgens [androstenedione (A) and testosterone (T)] on cycle day 31, following 10 days of GnRHa therapy (1 mg SC qd) in subjects demonstrating ovarian suppression as defined as a serum $E_2 \leq 30$ pg/ml and the absence of ovarian cysts ≥ 10 mm by TVS. Results are expressed as the mean $\pm$ SE.

ml (mean, 242.0 ± 8.6 pg/ml), which were suggestive of incomplete androgen suppression.

The relationship between serum immunoreactive LH measured on day 31, following 10 days of GnRHa begun in the midluteal phase, and serum P₄, A, and T levels was examined using multiple stepwise linear regression analysis. Serum LH levels were not found to correlate with either serum P₄ levels ($r = 0.24$, $P = 0.33$) or A levels ($r = 0.06$, $P = 0.73$). However, LH levels on day 31 were found to be somewhat predictive of serum T levels ($r = 0.38$, $P = 0.08$), prior to initiating COH.

The relationships between serum E_2 , P₄ and androgen levels (A and T) on day 31 and those following COH on the day of hCG administration were also assessed using the linear regression model. Neither E_2 , P₄, nor T levels on day 31 were found to be predictive of those seen on the day of hCG ($r = 0.27$, $P = 0.16$), whereas A levels on day 31 were somewhat predictive of those seen on the day of hCG ($r = 0.37$, $P = 0.07$). In contrast, both serum A and serum T levels measured on the day of hCG were strongly predicted by E_2 ($r = 0.86$, $P < 0.0001$, for A and $r = 0.71$, $P < 0.0001$, for T).

The effect of incompletely suppressed serum P (≥ 0.9 ng/ml), A (≥ 600 pg/ml), and T (≥ 200 pg/ml) on day 31 following midluteal GnRHa initiation on cycle outcome following COH is shown in Fig. 2. Neither elevated androgen (A, T) nor P₄ levels occurring on day 31 were predictive of cycle outcome

as determined by oocyte number, morphology, fertilization, implantation, pregnancy, or abortion rates.

DISCUSSION

The use of GnRHa as an adjunct to COH for the purpose of IVF-ET has been shown to result in improved cycle characteristics including reduced cancellation rates caused by premature LH elevations as well as increased oocyte numbers and quality for use in IVF (3, 4). Midluteal-phase GnRHa initiation has been shown to improve pregnancy outcome compared with follicular-phase initiation in prospective, randomized trials (5). Early follicular GnRHa initiation, due to the initial "flare" of endogenous gonadotropins, is associated with increases in ovarian androgen production which may have detrimental effects during the early development of the follicular cohort (5, 11). In contrast, GnRHa begun in the midluteal phase results in a more rapid and pronounced suppression of bioactive LH and may suppress early follicular-phase ovarian stromal/thecal androgen production (4).

Ovarian E_2 production has been used as the primary determinant of adequate suppression of the pituitary-ovarian axis following GnRHa prior to initiating COH (1). Prior dose-response studies using leuprolide acetate have suggested that serum estradiol and the initial serum LH response to exogenous GnRHa could be maximally suppressed by the lowest dosage examined

Fig. 2. The effect of incompletely suppressed serum androstenedione ($A \geq 600$ pg/ml), testosterone ($T \geq 200$ pg/ml), and progesterone ($P_4 > 0.9$ ng/ml) on day 31 following midluteal GnRH α initiation (1 mg Sc, qd, $\times 10$ days) on the cycle outcome following COH. * $P = ns$.

(2.5 μ g/kg/day) in normal ovulatory women (7). However, maximal serum testosterone suppression required doses as high as 20 μ g/kg/day for up to 3 months and was found to correlate with suppression of basal LH (7). Our results confirm these earlier findings in that, following 10 days of GnRH α treatment begun in the midluteal phase at a dose sufficient to suppress E_2 to castrate levels, up to 25% of cycles were characterized by serum A_4 levels ≥ 600 pg/ml, and approximately 40% of cycles had serum testosterone levels well above castrate levels (≥ 200 pg/ml) (14). Although immunoassayable basal serum LH has been suggested to correlate with serum testosterone levels in hyperandrogenic women (7), subsequent studies have shown that

immunoreactive serum gonadotropin levels are not a useful marker of bioactive LH suppression as determined by in vitro rat Leydig cell assay (15, 16). More recently, however, GnRH binding sites have been identified in the rat gonad and direct inhibition of steroidogenesis by GnRH α binding has been observed in vitro (13), further clouding the role of immunoreactive versus bioactive gonadotropins in control of ovarian stromal steroidogenesis. Although serum immunoreactive LH was not suppressed after 10 days of GnRH α therapy in this study, there was no correlation observed between LH levels and the levels of circulating androgens.

We also noted persistent low-level elevations in serum progesterone in 5.4% of cycles following 10 days of GnRHa therapy despite evidence of E_2 and follicular suppression. Again, although serum immunoreactive LH was not suppressed on cycle day 31, there was no correlation observed between LH and progesterone levels. However, as has been observed previously in the case of circulating androgens, the duration and dosage of GnRHa required to achieve maximal suppression of serum progesterone to castrate levels may be greater than those required to suppress estradiol (14).

The possibility exists that incomplete suppression of circulating A, T, and P_4 levels following pituitary downregulation by GnRHa therapy as evidenced by suppressed circulating gonadotropin levels may be accounted for by a significant adrenocortical contribution to these circulating steroid hormones. However, previous investigations have shown that prolonged administration of the GnRHa, leuprolide acetate, at a dose of 100 μ g daily for 28 days to both hyperandrogenic and normal women has been shown to decrease C-21, Δ^4 steroids of ovarian origin to castrate levels (12). In addition, as observed in the current study, a strong correlation was observed between ovarian follicular estradiol production and measured circulating A, T, and P_4 levels in response to gonadotropin stimulation. This observation suggests that ovarian follicular steroid production may be of greater significance. As another alternative to incomplete suppression, the initial agonistic response associated with GnRHa initiation in the midluteal phase resulting in marked elevations of endogenous pituitary gonadotropins has been implicated in potential "rescue" of corpus luteum (CL) steroidogenesis. This could explain elevations in serum progesterone persisting into the follicular phase (13).

Following 10 days of GnRHa administration begun in the midluteal phase, a significant number of cycles demonstrated incomplete androgen suppression despite evidence of complete E_2 suppression and the absence of follicular growth by TVS. However, we found no evidence of an adverse effect of incomplete androgen suppression on early follicular growth as determined by the number or morphology of the oocytes retrieved or their ability to fertilize. It is interesting to note that all pregnancies occurred in cycles characterized by incomplete androgen suppression at the time of hMG initiation for COH, although this difference was not statistically significant. Both A4 and T levels were found to be strongly predictive of follicular E_2 measured on the day of hCG administration, prior to ovum retrieval. Previous investigators

have noted a decreased ovarian response to hMG following prolonged treatment (3–4 weeks) with LA, required to "fully suppress" the ovarian axis (17), although serum androgens were not evaluated. In this study, incomplete androgen suppression appeared to be a marker for normal subsequent ovarian response to COH.

Persistent low-level elevations in serum P_4 were found much less frequently than elevations in serum androgens observed following midluteal GnRHa pituitary downregulation. Progesterone elevations prior to initiating COH were not predicted by immunoassayable LH levels, androgen elevations, or subsequent E_2 production. We were unable to demonstrate an adverse effect of P_4 elevations occurring early in the development of the follicular cohort on subsequent follicular development as determined by the number or morphology of the oocytes retrieved. Fertilization rates were similar in cycles with and without incompletely suppressed P_4 following GnRHa. This mirrored the results noted in the case of incomplete androgen suppression. Although "rescue" of the CL by elevations of endogenous gonadotropins resulting from midluteal GnRHa initiation resulting in "persistent follicular luteinization" has been suggested potentially to affect cycle outcome (13), the low incidence of such elevations observed in the current study does not allow for meaningful comparisons of pregnancy or miscarriage rates.

In summary, midluteal GnRHa use prior to COH may result in incomplete suppression of androgens and P_4 , despite the presence of indicators routinely used to indicate ovarian suppression ($E_2 \leq 30$ pg/ml and absence of follicular growth by TVS). However, incomplete circulating androgen suppression prior to COH is not associated with adverse outcomes in IVF-ET and may be an indicator of a normal follicular response to gonadotropin induced COH. The incidence of persistent elevations in serum P_4 following midluteal GnRHa appears to be quite low and of questionable clinical significance to subsequent cycle outcome in IVF-ET.

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