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A combination of norethindrone acetate and leuprolide acetate blocks the gonadotrophin-releasing hormone agonistic response and minimizes cyst formation during ovarian stimulation

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A protocol utilizing both leuprolide acetate (LA) and norethindrone acetate (NETA) in subjects undergoing ovarian suppression prior to follicle aspiration proved more effective than LA alone in reducing the incidence of ovarian cyst formation without affecting clinical outcome. Patients (n = 105) undergoing ovarian stimulation followed by follicle aspiration and in-vitro fertilization (IVF) were prospectively randomized and studied. Study measures included ovarian suppression days, days of human menopausal gonadotrophin (HMG) stimulation, serum oestradiol concentrations, number of cycles developing de novo cysts (>15 mm), number of induced flare responses (day 8 oestradiol ≥50 pg/ml), number of office visits, total dose exogenous gonadotrophins, number oocytes retrieved, and clinical pregnancy and delivery rates per retrieval. Patients undergoing IVF received either LA alone (n = 58; controls) or LA and NETA (n = 47; study group) for the first 8 days of their cycle. Results comparing NETA/LA versus LA demonstrated: serum oestradiol 20.7 ± 3.9 versus 57.3 ± 9.4 pg/ml respectively on day 8 of ovarian suppression ($P < 0.01$); 8.6 ± 2.74 days required for ovarian suppression versus 12.3 ± 6.09 days ($P < 0.01$); and only three individuals (6.4%) using NETA/LA developed ovarian cysts >15 mm compared to 15 (25.9%) controls ($P < 0.01$). No differences were observed for days of stimulation, peak oestradiol attained, total dosage of exogenous gonadotrophins, or number of aspirated oocytes. Neither were there differences in the clinical pregnancy (26.8 versus 22.6%) nor in delivery rates (19.5 versus 20.8%). We conclude that the addition of NETA to LA enhances ovarian suppression and lessens ovarian cyst formation, thereby significantly decreasing the overall cost per cycle.

Key words: IVF/norethindrone acetate/ovarian suppression

Introduction

The use of the gonadotrophin-releasing hormone agonists (GnRHa) has become commonplace in the treatment of reproductive disorders. GnRHa are useful adjuncts in combination with menotrophins for ovarian hyperstimulation. GnRHa prevents premature luteinization and premature luteinizing hormone (LH) surges. Suppressing the pituitary with GnRHa prior to ovarian stimulation results in a greater number of oocytes recovered by enlarging the recruitment pool (Porter *et al.*, 1984; Neveu *et al.*, 1987), thus increasing the number of embryos while improving embryo quality in patients undergoing in-vitro fertilization (IVF).

However, down-regulation with GnRHa requires 1–3 weeks (Yen, 1983) and is associated with an initial agonistic (stimulatory) phase that may result in ovarian follicular cyst formation (Sampaio *et al.*, 1991). Accordingly, studies have been carried out utilizing pure GnRH antagonists to prevent these adverse occurrences (Cassidenti *et al.*, 1991). Antagonists induce immediate decreases in circulating gonadotrophin concentrations and do not exhibit agonistic effects (Ditkoff *et al.*, 1991). Unfortunately GnRH antagonists are difficult to obtain, expensive, and have potential harmful side-effects related to histamine release. Furthermore, their current use and availability is limited.

Progestins and oral contraceptives have also been used prior to ovarian stimulation (Gerli *et al.*, 1989; Kemeter and Feichtinger, 1989; Hughes *et al.*, 1992; Franco *et al.*, 1995; Gonzalez *et al.*, 1995).

Reports demonstrate that oral contraceptive use prior to ovarian stimulation minimizes the risk of hyperstimulation in high risk patients (Neal *et al.*, 1993). However, these protocols typically require patients to take medications for many days prior to starting ovarian stimulation. The use of these medications is based upon the knowledge that progestins directly inhibit GnRHa stimulation (Anderson *et al.*, 1990; Poindexter *et al.*, 1993) and have a major influence on the pattern of hypothalamic GnRH secretion by centrally suppressing the release of gonadotrophins (Hemrika *et al.*, 1993).

The purpose of this study was to determine whether norethindrone acetate (NETA) used in combination with leuprolide acetate (NETA+LA), induces down-regulation as efficiently or more so than LA alone when administered during the early follicular phase. Furthermore, we wished to determine if enhanced ovarian suppression lessens the flare effect commonly seen with GnRHa therapy as manifested by the formation of ovarian cysts.

We chose to study this protocol during the early follicular phase because it is common practice in the USA to start GnRHa at this time during the menstrual cycle, since ovulatory patients with tubal patency are not then concerned about pregnancy while taking LA.

Materials and methods

Patients

The study was approved by the Institutional Review Board of Columbia University Medical Center. Subjects (n = 105) were

enrolled at the time of their initial attempt at ovarian stimulation and follicle aspiration for IVF. Patients were randomly assigned by tossing a coin to one of two groups. Indications for IVF included male factor, tubal obstruction, endometriosis, pelvic or uterine factor, and idiopathic infertility. All patients had day 3 follicle stimulating hormone (FSH) values <15 mIU/ml. The various infertility diagnoses were distributed equally between the control and study groups. Group I comprised 58 women (mean age ± SD, 35.8 ± 4.57 years; range 23–43 years) who served as controls and underwent the standard protocol. Group I patients injected LA (1 mg s.c. daily) during the first 8 days of their menstrual cycle. Group II comprised 47 women (mean age 36.7 ± 4.80 years; range 28–44 years) who served as study patients and underwent daily injections of LA (1 mg s.c.) and oral NETA (10 mg in divided doses kindly supplied as Aygestin from Wyeth-Ayerst, Philadelphia, USA) for the first 8 days of their cycle.

Ovarian suppression protocol

All subjects demonstrated normal pelvic ultrasound results immediately prior to ovulation induction. Patients were seen on the morning of day 8 of LA+NETA for serum oestradiol and vaginal ultrasound. Ovarian suppression was defined as an oestradiol level <30 pg/ml. De-novo ovarian cysts were considered enlarged if ≥15 mm maximum diameter. Regardless of the presence of an ovarian cyst, if the serum oestradiol level was ≤30 pg/ml, ovarian stimulation was initiated. If the serum oestradiol was >30 pg/ml, LA alone was continued and the patient returned 1 week later (day 15) for further monitoring or weekly thereafter until ovarian suppression (oestradiol <30 pg/ml) was evident.

Ovarian stimulation phase

All subjects underwent an identical protocol for ovarian stimulation consisting of 225 IU human menopausal gonadotrophins (HMG) injected i.m. daily while injecting 1 mg of LA s.c. The initial visit for monitoring the stimulation occurred after 4 days of HMG therapy. If the serum oestradiol was <80 pg/ml, the dose of HMG was increased to a maximum of 450 IU. If a patient had recruited two or fewer follicles with a serum oestradiol of ≤300 pg/ml, the cycle was cancelled. Human chorionic gonadotrophin (HCG, 10 000 IU) was administered i.m. when lead follicles reached 18 mm in diameter. In general the serum oestradiol value was at least 150 pg/ml per mature lead follicle. Oocyte retrieval was scheduled 34–36 h after HCG. Transvaginal embryo transfer occurred 48 h after retrieval. Quantitative measurements of β-HCG were performed on days 10 and 14 post-transfer. If positive (>2 mIU/ml) and rising, the measurement was repeated 7 days later. Pregnancy viability was assessed by vaginal ultrasound 28 days post-transfer.

Hormone assays

Blood was separated, and fresh sera analysed for oestradiol by a highly specific direct radioimmunoassay (Diagnostic Products, Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation were 5 and 6.2% respectively. Assay sensitivity was 10 pg/ml.

Statistical analyses

Data were expressed as mean ± SD, and were analysed by Levene's test for equality of variances (independent *t* testing), Fisher's exact test, and multiple regression analysis utilizing SPSS for MS Windows 6.0 as appropriate. Significance was defined as a P value <0.05.

Results

Table I compares the two groups of patients during ovarian suppression. The day 8 oestradiol value for NETA+LA administration was significantly less than LA used alone [20.7 ± 26.9 versus 57.3 ± 71.7 pg/ml ($P < 0.05$)]. Furthermore, there was a significantly lower incidence of initial agonist (flare) responses (three versus 23) and cyst formation (three versus 15) ($P < 0.05$) for the combined treatment. Patients using LA alone required more days to achieve ovarian suppression (12.3 ± 6.05 versus 8.6 ± 2.8) and more office visits during the interval of down regulation [1.6 ± 0.86 versus 1.1 ± 0.43 ($P < 0.01$)].

Table I. Comparison of leuprolide acetate (LA) alone to LA + norethindrone acetate (NETA) patients prior to ovarian stimulation

	LA alone	LA + NETA
Cycles	58	47
Days until suppression	12.3 ± 6.05 (8–29)	8.6 ± 2.8[a] (8–22)
Day 8 oestradiol (pg/ml) (mean ± SD)	57.3 ± 71.7 (10–333)	20.7 ± 26.9[b] (10–147)
Cyst cycles (*n*) (%)	15[a] (26)	3[c] (6)
Flare cycles (oestradiol >30 pg/ml) (*n*)	23[b]	4[d]
Total visits prior to ovarian stimulation	1.6 ± 0.86[c] (1–4)	1.1 ± 0.43[c] (1–3)

Values in parentheses are ranges.
[a,b,c,d,e]Values with same superscript are significantly different ($P < 0.05$).

Table II. Comparison of ovarian stimulation parameters and pregnancy rates between leuprolide acetate (LA) and LA + norethindrone acetate (NETA) patients

	LA alone	LA+NETA
Retrievals	53	41
No. days of ovarian stimulation	11.1 ± 1.5 (9–15)	11.8 ± 1.5 (9–16)
Peak oestradiol (pg/ml)	2073 ± 1170 (329–4203)	2124 ± 955 (526–4018)
No. of ampoules of HMG	41.6 ± 19.1 (20–104)	45.9 ± 17.9 (24–85)
Oocytes retrieved	12.8 ± 8.9 (0–28)	13.8 ± 7.6 (3–27)
No. of clinical pregnancies per retrieval	12/53= 22.6%	11/41= 26.8%
No. of deliveries/ongoing pregnancies per retrieval	11/53= 20.8%	8/41= 19.5%

HMG = human menopausal gonadotrophin.

Table II depicts ovarian stimulation parameters and pregnancy rates between groups. There were no significant differences in cancellation rates, days of ovarian stimulation, peak oestradiol value on the day of HCG administration, amount of exogenous gonadotrophins used, or number of oocytes retrieved. Furthermore, there were no differences in rates for clinical pregnancy or ongoing delivery per retrieval.

A significant correlation existed between day 8 oestradiol and the number of days required to achieve ovarian suppression ($r = 0.69$, $P < 0.05$). Patients ($n = 18$) with ovarian cysts on day 8 all had oestradiol values >30 pg/ml (mean ± SD 140 ± 97.2 pg/ml). These 18 patients also required a longer period of time for suppression (20.6 ± 0.26 days) compared to patients ($n = 87$) without cysts (9.6 ± 0.09 days).

There were no significant side-effects during the study

experienced by patients in either group related to the use of their medication.

Cysts were relatively common when NETA was not used, as demonstrated by 26% of control patients, but were present in only 6% of study patients ($P < 0.05$).

Discussion

Our data reveal that the concomitant administration of LA and NETA during the early follicular phase induces ovarian suppression more efficiently than LA alone. Evidence supporting this includes a reduced serum oestradiol on day 8 and a lower incidence of ovarian cyst formation. Study patients did not experience adverse effects during ovarian stimulation nor were pregnancy rates affected.

The close association between ovarian cyst formation and elevated estradiol concentrations on day 8 suggests that these cysts result from the initial agonist effect of LA. This was inferred since patients with pre-existing cysts during baseline scans were excluded from study. Also, patients with ovarian cysts on day 8 had associated elevated oestradiol levels and required a longer time to achieve full suppression. The decreased incidence of ovarian cyst formation in the study group is believed to be due to the known direct effect of NETA on the hypothalamic–pituitary axis (Poindexter *et al.*, 1993). NETA blocked the initial agonistic flare response from LA in all but four study patients (8.5%). Three of these four patients developed ovarian cysts and elevated oestradiol levels (118 ± 139 pg/ml).

The use of LA+NETA for down-regulation during the early follicular phase may be more efficient than using LA alone. Less LA is required, and office visits and overall cost are thus minimized. The monitoring of ovarian stimulation is simplified when ovarian cysts do not form, and protracted follow-up necessitated by cyst formation is obviated.

Other studies have utilized progestins and oral contraceptives in IVF protocols. Gerli *et al.* (1989) gave oral NETA, 10 mg daily, starting between cycle days 2–4 of the previous cycle. The NETA was used for 9–37 days and then discontinued. Ovarian stimulation was then carried out with a combination of LA and exogenous gonadotrophins until HCG was given. Hughes *et al.* (1992) gave norethisterone 10 mg/day for 14 days, beginning on day 15 of the cycle which immediately preceded the IVF cycle. On day 3 after cessation of pre-treatment with norethisterone, a GnRHa was given. HMG was prescribed 4–5 days later and subsequent doses were adjusted according to serum oestradiol and ultrasound imaging. Franco *et al.* (1995) used a protocol consisting of a low-dose contraceptive (30 μg ethinyloestradiol and 75 μg gestodene) from day 1 of the menstrual cycle preceding the cycle of ovarian stimulation and continued for 21–28 days. Ovarian stimulation was started on day 5 after discontinuation of the pill with 100 mg/day clomiphene citrate.

Our study design did not enable us to evaluate the mechanism of action of NETA nor the minimal time required for ovarian suppression. Future studies should be carried out to address these issues. We postulate that NETA prevents the initial release of gonadotrophins from the pituitary. Anderson *et al.* (1990) utilized norethindrone (NET) for cycle programming. They assessed the effects of NET on gonadotrophin secretion, its bio-availability to the ovary, and its effect on ovarian steroidogenesis *in vivo* and *in vitro*. These authors concluded that NET did not inhibit ovarian steroidogenesis but had direct effects on the hypothalamic–pituitary axis.

In summary, NETA+LA administration during the early follicular phase is useful for women undergoing ovarian stimulation. The combination of NETA and GnRHa is more efficient than GnRHa alone, since ovarian suppression occurs earlier and the incidence of ovarian cyst formation decreases significantly. Thus, the addition of NETA to LA decreases costs. Patients may ultimately prefer this approach, since suppression is more certain and the majority of patients will not develop follicular cysts.

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