The Addition of Norethindrone Acetate to Leuprolide Acetate for Ovarian Suppression Has No Adverse Effect on Ovarian Stimulation

EDWARD C. DITKOFF,^{1,2} ROBERT PROSSER,¹ RALF C. ZIMMERMANN,¹ STEVEN LINDHEIM,¹ and MARK V. SAUER¹

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Purpose: Our goal was to determine if the addition of norethindrone acetate (NETA) to leuprolide acetate (LA) has an adverse effect on controlled ovarian stimulation (COH) during in vitro fertilization (IVF).

Methods: Forty-one consecutive patients undergoing COH and IVF were divided into two groups and evaluated. Group 1 consisted of 18 patients who did not become pregnant following two cycles (one of each protocol). Group 2 consisted of 23 patients who became clinically pregnant following one cycle from either protocol. The standard protocol consisted of LA (1 mg) injected subcutaneously from the first day of menses until day 8 or when ovarian suppression was evident, at which time the dose was halved and COH was initiated. The study protocol was identical except 10 mg of NETA was given orally with LA for the first 8 days. Ovarian stimulation was similar in each protocol.

Results: No adverse effect on ovarian stimulation was evident on the addition of NETA to LA. No differences were noted in days of stimulation, peak estradiol (E_2) level attained, peak E_2 -to-oocyte ratio, dosage of exogenous gonadotropins, number of aspirated oocytes, fertilization rate, or oocyte and preembryo quality.

Conclusions: The addition of NETA does not attenuate COH in women undergoing IVF.

KEY WORDS: in vitro fertilization; norethindrone acetate; ovarian stimulation; oocyte and preembryo quality.

INTRODUCTION

Suppressing the pituitary with a gonadotropin agonist (GnRHa) prior to ovarian stimulation results in a

greater number of oocytes recovered by enlarging the recruitment pool. This approach increases the number of embryos and improves embryo quality in patients undergoing IVF (1,2). Down regulation with GnRH agonist (GnRHa) requires 1 to 3 weeks and is associated with an initial agonist (stimulatory) phase that may induce ovarian follicular cysts (3,4). Pure GnRH antagonists may prevent these adverse occurrences (5,6). However, GnRH antagonist use and availability are very limited for clinical practice.

To lessen the incidence of cyst formation, progestins and oral contraceptives have also been used prior to ovarian stimulation (7–11). These protocols typically require patients to take medications for many days before beginning ovarian stimulation. Investigators have suggested that pretreatment with progestins or oral contraceptives attenuates ovarian stimulation during IVF (12). These medications are used since progestins directly inhibit GnRHa stimulation (13,14) and have a major influence on the pattern of hypothalamic GnRH secretion by centrally suppressing the release of gonadotropins (15). We recently demonstrated that the addition of norethindrone acetate (NETA) to leuprolide acetate (LA) enhances ovarian suppression and lessens ovarian cyst formation, thereby significantly decreasing the overall cost per cycle without affecting pregnancy rates (15).

The purpose of the present study was to determine if NETA used in combination with LA (NETA + LA) during the early follicular phase adversely affects clinical parameters of ovarian stimulation. Oocyte quality and preembryo quality were evaluated as representative clinical end points of the efficacy of ovarian stimulation.

¹ Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Columbia–Presbyterian Medical Center, Columbia University, College of Physicians and Surgeons, New York, New York, 10032.

² To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Division of Reproductive Endocrinol-

ogy, Columbia–Presbyterian Medical Center, PH-Room 1630 East, 622 West 168th Street, New York, New York, 10032.

MATERIALS AND METHODS

Patients. The study was reviewed and approved by the Institutional Review Board of Columbia University Medical Center. Subjects ($n = 41$) were those who either conceived after their initial cycle attempt or did not become pregnant after attempting each protocol (two unsuccessful cycles). Subjects who conceived during their second cycle were excluded since our intent was to compare pregnant or nonpregnant cycles respectively and minimize bias.

The 41 subjects included had serum follicle stimulating hormone (FSH) values below 15 mIU/ml and were consecutively enrolled in a randomized prospective, designed schematic protocol at the time of their initial attempt at ovarian stimulation and follicle aspiration for IVF and embryo transfer (Fig. 1). Patients either injected LA (1 mg s.c.) daily during the first 8 days of their menstrual cycle or underwent our study protocol and injected LA (1 mg s.c.) and received NETA (10 mg p.o.) daily during the first 8 days of their cycle.

Eighteen (mean, 37 ± 1.1 years; range, 28–43 years) of the 41 patients (group 1) studied did not become pregnant during their first cycle and elected to undergo a second cycle after a minimum of 8 weeks passed utilizing the other protocol. These patients did not achieve pregnancy following their second cycle either. These cycles served as their own controls for comparison.

Of the remaining 41 patients, 23 became clinically pregnant (group 2) following their first cycle of IVF. Protocols were randomly determined. Eleven became

pregnant following their first cycle with NETA + LA, while the remaining 12 became pregnant following their first cycle with LA alone [mean, 36.1 ± 3.9 years; range, 31–41 years; and mean, 32.8 ± 3.5 years; range, 27–39 years; (respectively); P , NS]. Subsequently, one and two of these pregnant patients on LA and LA + NET, respectively, had first-trimester miscarriages.

Oocytes and preembryos from all 41 patients and 59 cycles were scored by one embryologist without any known clinical information. Oocytes and preembryos were graded for maturity and quality by traditional methods (17).

Indications for IVF included male factor, tubal obstruction, endometriosis, pelvic or uterine factor, and idiopathic infertility. The various infertility diagnoses were distributed equally between the patients.

Ovarian Suppression Protocol. All subjects demonstrated normal pelvic ultrasound exams immediately prior to ovulation induction. Patients were seen on the morning of the eighth day of LA + NETA or LA alone for serum E_2 and vaginal ultrasound. Ovarian suppression was defined as an E_2 level ≤ 30 pg/ml. If the serum E_2 level was ≤ 30 pg/ml, ovarian stimulation was initiated. If the serum E_2 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 was evident.

Ovarian Stimulation. All subjects underwent an identical protocol for ovarian stimulation consisting of 225 IU of human menopausal gonadotropins (hMG) injected intramuscularly daily while injecting 1 mg of

Fig. 1. Schematic protocol for 41 prospective randomized enrolled patients. This design allowed us to study both pregnant and nonpregnant patients independently with minimal bias.

LA subcutaneously. The initial visit for monitoring the stimulation occurred after 4 days of hMG therapy. If the serum E_2 was <80 pg/ml, the dose of hMG was increased to a maximum of 450 IU. If a patient recruited two or fewer follicles with a serum E_2 of ≤ 300 pg/ml, the cycle was canceled. Human chorionic gonadotropin (hCG; 10,000 IU) was administered intramuscularly when the lead follicles reached 18 mm in diameter. In general, the serum E_2 level was at least 150 pg/ml per mature lead follicle. Oocyte retrieval was scheduled 34–36 hr after hCG. Transvaginal embryo transfer occurred 48 hr after retrieval. Quantitative levels of β -hCG were performed on days 10 and 14 posttransfer. If positive (>2 mIU/ml) and rising, a level was repeated 7 days later. A vaginal ultrasound 28 days posttransfer assessed pregnancy viability.

The nonpregnant group of patients undergoing their second attempt of IVF was given a similar daily dose of human menopausal gonadotropins required during their initial cycle.

Hormone Assays. Blood was separated, and fresh sera analyzed for E_2 by a highly-specific direct radioimmunoassay (Diagnostic Products, Los Angeles, CA). The intraassay and interassay coefficients of variation were 5 and 6.2%, respectively. Assay sensitivity was 10 pg/ml.

Statistical Analyses. Data are expressed as mean $\pm$ SD. Data were analyzed by independent or paired t testing utilizing SPSS for MS Windows 6.0 as appropriate. Significance was defined as a P value <0.05 .

RESULTS

Table I includes data from the 18 identical IVF patients who completed their stimulation protocols and went on to embryo transfer. The data compare ovarian stimulation parameters and fertilization rates between the two protocols differing only in NETA administration. We observed no differences regarding number of stimulation days, peak estradiol, required total dose of exogenous gonadotropins, number of oocytes harvested, peak estradiol-to-number of oocyte ratio, and fertilization rate between the two groups.

When NETA + LA was administered for the initial 8 days of the follicular phase, the day 8 E_2 level was significantly less with NETA + LA than with LA alone. Furthermore, patients administering LA alone required more days of LA until they achieved ovarian suppression. One of the 18 patients developed an ovarian cyst (>15 mm), while on NETA + LA and 4 developed ovarian cysts while on LA alone.

Table I. Comparison of Controlled Ovarian Stimulation Parameters in LA Alone to LA + NETA in Identical Nonpregnant Patients

	$\bar{X} \pm SD$ (range)	
	Nonpregnant, LA alone	Nonpregnant, LA + NETA
Retrievals	18	18
Age	37 ± 1.1 (28–43)	37 ± 1.1 (28–43)
Day 8 E_2 (pg/ml)	47 ± 14 (10–201)	$15 \pm 3^*$ (10–54)
COH days	11.4 ± 0.4 (10–13)	12.4 ± 0.8 (11–15)
Peak E_2 (pg/ml)	1989 ± 272 (555–4300)	1919 ± 168 (666–3145)
Suppression days	11 ± 1.3 (8–23)	$9 \pm 0.5^*$ (8–22)
Total amps	56 ± 6 (23–97)	55 ± 6 (27–104)
No. of oocytes	12.7 ± 1.8 (3–32)	12.4 ± 1.6 (3–26)
E_2 /oocyte ratio	167 ± 15 (98–341)	187 ± 22 (103–511)
Fertilization rate (%)	70 ± 4.9	75 ± 3.6

* $P < 0.05$; significant.

Data in Table II consist of information from the 23 pregnant patients who underwent either NETA + LA or LA ovarian suppression. The data in Table II also illustrate no differences in the various parameters of ovarian stimulation between patient groups. The day 8 estradiol level was significantly lower with NETA + LA than with LA alone and patients administering NETA + LA required fewer days of medicine to

Table II. Comparison of Ovarian Stimulation Parameters in LA Alone to LA + NETA in Pregnant Patients After an Initial Cycle of IVF

	$\bar{X} \pm SD$ (range)	
	Pregnant, LA alone	Pregnant, LA + NETA
Retrievals	12	11
Age	33 ± 3.5 (27–39)	36 ± 3.9 (31–41)
Day 8 E_2 (pg/ml)	81 ± 84 (10–256)	15 ± 7 (10–25)*
COH days	11.1 ± 1.5 (9–15)	11.8 ± 1.5 (9–16)
Peak E_2 (pg/ml)	2436 ± 1163 (1142–4386)	1830 ± 1116 (634–3958)
Suppression days	13 ± 7.3	$8 \pm 0.0^*$
Total amps	36 ± 15	50 ± 18
No. of oocytes	18.3 ± 11.1	11.9 ± 7.6
E_2 /oocyte ratio	144 ± 39	167 ± 46
Fertilization rate	64 ± 19	65 ± 24

* $P < 0.05$; significant.

Table III. Comparison of Oocyte and Embryo Quality in LA Alone to LA + NETA in Both Pregnant and Nonpregnant Patients

	X ± SE			
	Nonpregnant	Nonpregnant	Pregnant	Pregnant
	NETA + LA (n = 18)	LA alone (n = 18)	NETA + LA (n = 11)	LA alone (n = 12)
Oocytes				
Immature	1.6 ± 0.6	2.1 ± 1.4	2.3 ± 1.3	2.7 ± 1.9
Mature	12.3 ± 7.2	11.4 ± 0.4	11.1 ± 7.7	16.8 ± 0.3
Embryos				
Poor-quality	1.8 ± 1.5	2.8 ± 2.5	1.6 ± 0.9	2.7 ± 2.9
Average-high quality	4.0 ± 1.6	3.5 ± 1.7	3.9 ± 1.3	4.8 ± 0.8
Transferred	4.1 ± 1.2	3.8 ± 1.3	4.0 ± 1.2	4.7 ± 0.7
Frozen	8.0 ± 1.4 (n = 6)	11.3 ± 1.9 (n = 3)	8.5 ± 3.7 (n = 4)	11.6 ± 5.4 (n = 5)

achieve ovarian suppression. One of the 11 pregnant patients developed an ovarian cyst (>15 mm) on NETA + LA, while 4 of 12 pregnant patients formed a cyst on LA.

Table III compares oocyte and embryo quality from nonpregnant and pregnant patients undergoing either protocol. The number of preembryos transferred and cryopreserved in each group is included. The data revealed no differences in oocyte and embryo quality between the two protocols.

DISCUSSION

It is now common practice to start GnRHa during the first few days of the menstrual cycle. This allows baseline FSH and E₂ levels to be defined and, in ovulatory patients with tubal patency, obviates fears of initiating LA during a pregnancy cycle.

We were not surprised with the results since overall clinical pregnancy rates per retrieval were similar (22.6 vs 26.8%) in our earlier study comparing each protocol (16). Previously, we demonstrated both NETA and GnRHa were more efficient than GnRHa alone for inducing ovarian suppression, with hypogonadal states occurring earlier and the incidence of ovarian cyst formation decreasing between patient groups.

Other studies have utilized progestins and oral contraceptives in IVF protocols. Gerli *et al.* gave oral NETA, 10 mg daily, starting between cycle day 2 and cycle day 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 gonadotropins until hCG was given. They concluded there was no correlation

between the duration of NETA suppression and the number of days of gonadotropin therapy needed to reach hCG administration. Similar to our findings, they also found ovarian response was not affected by NETA administration.

Hugues *et al.* gave norethisterone, 10 mg/day for 14 days, beginning on day 15 of the cycle, which immediately preceded the IVF attempt. The third day after cessation of pretreatment with norethisterone, GnRHa was given. Four to five days later, hMG was prescribed and subsequent doses were adjusted according to serum E₂ and ultrasound imaging. They concluded that in progestogen-pretreated women, the serum E₂ level during the flare-up period could be utilized as a reliable index to predict subsequent effectiveness of ovarian stimulation with hMG.

Gonzalez *et al.* studied whether the use of norethisterone acetate in the previous cycle affects the ovarian response to GnRHa in flare-up protocols and COH in older patients. They found that the administration of norethisterone acetate did not affect ovarian response.

Anderson *et al.* utilized norethindrone (NET) for cycle programming and assessed the effects of NET on gonadotropin secretion, its bioavailability to the ovary, and its effect on ovarian steroidogenesis in vivo and in vitro. These authors concluded NET did not inhibit ovarian steroidogenesis but had direct effects on the hypothalamic–pituitary axis.

Our results are compatible with these from previous studies. We also noted similarities in days of stimulation, peak E₂ levels, number of oocytes recovered, peak E₂-to-oocyte ratio, amount of menotropins required, fertilization rates, and appearances of oocyte and preembryo quality among out patient groups. Reduced serum E₂ on day 8, earlier ovarian suppression, and

less ovarian cyst formation following NETA + LA confirmed our earlier findings. Furthermore, the data reveal no adverse effect on the end results of ovarian stimulation, those being oocyte and preembryo quality.

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