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Dual suppression with oral contraceptives and gonadotrophin releasing-hormone agonists improves in-vitro fertilization outcome in high responder patients

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Certain patients have a tendency for high response to gonadotrophin therapy which is often not ameliorated with prior gonadotrophin-releasing hormone agonist (GnRHa) suppression. As a result, these patients are frequently cancelled and often experience ovarian hyperstimulation syndrome (OHSS) episodes during in-vitro fertilization (IVF)–embryo transfer cycles. Patients with polycystic ovarian syndrome (PCOS) have been noted to be particularly sensitive to exogenous gonadotrophin therapy. We have developed a protocol which is effective in improving IVF outcome in high responder patients, including those with PCOS. Oral contraceptive pills (OCP) are taken for 25 days followed by s.c. leuprolide acetate, 1 mg/day, which is overlapped with the final 5 days of oral contraceptive administration. Low-dose gonadotrophin stimulation is then initiated on the third day of withdrawal bleeding in the form of either human menopausal gonadotrophins or purified urinary follicle-stimulating hormone at a dosage of 150 IU/day. Over a 5 year period, we reviewed our experience utilizing this dual method of suppression in 99 cycles obtained in 73 high responder patients. There were only 13 cancellations prior to embryo transfer (13.1%). The clinical and ongoing pregnancy rates per initiated cycle were 46.5 and 40.4% respectively. Only eight patients experienced mild-moderate OHSS following treatment. For those patients who had undergone previous IVF–embryo transfer cycles at our centre, significant improvements were noted in oocyte fertilization rates, embryo implantation rates and clinical/ongoing pregnancy rates with this protocol. Hormonal analyses revealed that the chief mechanism may be through an improved luteinizing hormone/follicle-stimulating hormone ratio following dual suppression. An additional feature of this dual method of suppression is significantly lower serum androgen concentrations, particularly dehydroepiandrosterone sulphate.

Key words: gonadotrophin-releasing hormone agonist/high responder/in-vitro fertilization/oral contraceptives/polycystic ovarian syndrome

Introduction

It has been observed that women with polycystic ovarian syndrome (PCOS) have an increased sensitivity to gonadotrophin therapy, with consequent high rates of multiple pregnancies and ovarian hyperstimulation syndrome (OHSS) following ovarian stimulation (Wang and Gemzell, 1980). For this reason, several investigators have examined the utility of low dose gonadotrophin regimens in an effort to reduce the complication rate during ovulation induction (Seibel *et al.*, 1984; Hamilton-Fairley *et al.*, 1991; Shoham *et al.*, 1991; Dale *et al.*, 1993). This approach is predicated on the concept that patients with PCOS, due to their particular sensitivity, respond to supraphysiological gonadotrophin dosages with the recruitment of a large cohort of follicles which, with continued stimulation, results in high oestradiol responses and extraordinarily high numbers of developed follicles. The use of lower, presumably more physiological, dosages has resulted in favourable pregnancy rates, while limiting the incidence of OHSS and multiple pregnancies in this setting (Homburg *et al.*, 1995).

While multifollicular development is the goal of ovarian stimulation regimens for in-vitro fertilization (IVF) and embryo transfer, the frequently observed excessive ovarian response of patients with PCOS presents a therapeutic challenge. Patients with PCOS have been noted to have higher peak serum oestradiol concentrations, lower gonadotrophin requirements and greater numbers of oocytes retrieved than matched controls (Urman *et al.*, 1992). Proportionately higher numbers of immature oocytes, lower oocyte fertilization rates and reduced cleavage rates have also been reported (Dor *et al.*, 1990). Indeed, there is evidence which suggests that tonically elevated luteinizing hormone (LH) concentrations are associated with poorer outcomes following IVF–embryo transfer. Reduced fertilization rates and an increased incidence of spontaneous abortions have been reported when LH concentrations have been elevated during the follicular phase (Stanger and Yovich, 1985; Regan *et al.*, 1990).

The incorporation of gonadotrophin-releasing hormone agonist (GnRHa) down-regulation in stimulation protocols for PCOS patients undergoing IVF–embryo transfer offers several advantages. One of its principal benefits appears to be the reduction of LH concentrations in the follicular phase and the obviation of premature luteinization. Moreover, reducing the inordinately elevated concentrations of LH appears to improve pregnancy rates and reduce spontaneous abortion rates in PCOS patients undergoing IVF–embryo transfer (Balen *et al.*, 1993; Homburg *et al.*, 1993).

Importantly, it has also been observed that women with PCOS may require a longer period of GnRHa treatment to

achieve adequate down-regulation when compared to normal women (Tanbo *et al.*, 1989). Thus, the standard mid-luteal phase GnRHa protocol may not be sufficient to normalize entirely the unfavourable hormonal milieu which may interfere with normal folliculogenesis in these women (Hamuri *et al.*, 1992).

In an effort to optimize IVF–embryo transfer outcome in this difficult group of women, we have developed a stimulation protocol which is both simple and inexpensive while achieving rapid and effective down-regulation. This protocol employs pretreatment with oral contraceptive pills (OCP) prior to the administration of GnRHa in a long protocol combined with relatively lower gonadotrophin dosages. This approach permits normalization of the LH/follicle stimulating hormone (FSH) ratio and reduces ovarian androgen concentrations, while circumventing the initial gonadotrophin flare response. Patients with PCOS, in particular, have been noted to exhibit exaggerated ovarian androgen and oestradiol responses upon institution of GnRHa therapy (Suikkari *et al.*, 1995). Importantly, this protocol can also be effectively applied in non-PCOS patients who have previously demonstrated an exaggerated response to gonadotrophin therapy.

Materials and methods

Study population

Patients undergoing IVF–embryo transfer with the dual suppression protocol consisting of OCP pretreatment prior to GnRHa suppression and low dose gonadotrophin stimulation (150 IU) between January 1, 1990 and December 31, 1994 were reviewed retrospectively. This dual suppression protocol was employed for those patients who had previously exhibited clinical features suggestive of a heightened sensitivity to exogenous gonadotrophin therapy. These criteria included previous cycles of ovulation induction or IVF–embryo transfer with peak oestradiol concentrations exceeding 2500 pg/ml in our laboratory, or evidence of a previous high response at another clinic (i.e. excessive number of oocytes retrieved and/or OHSS). For inclusion, complete data regarding basal hormonal concentrations (FSH, LH and oestradiol), stimulation characteristics and outcome parameters of previous treatment cycles were required.

Evaluation of FSH, LH, oestradiol, total testosterone, androstenedione, dihydroepiandrosterone sulphate (DHEA-S) and 17-hydroxyprogesterone (17-OHP) concentrations were performed on day 3 of a preceding menstrual cycle in ovulating women, and randomly in women with amenorrhoea. In some instances, serum that had been stored frozen at –20°C was used to complete the evaluation. Patients were classified as having PCOS if they exhibited ultrasonographic evidence of at least 10 peripherally located cystic structures 2–10 mm in diameter with an increased central ovarian stroma (Adams *et al.*, 1985) and at least two of the following clinical criteria: (i) oligomenorrhoea (menstrual cycle length >35 days) with or without accompanying obesity or hirsutism; (ii) an elevated basal LH/FSH ratio exceeding 2.5:1; or (iii) elevated total testosterone (>0.90 ng/ml) or androstenedione (>2.3 ng/ml). Patients with elevated 17-OH progesterone (>110 ng/dl) or FSH (>20 mIU/ml) concentrations were excluded. Patients were classified as 'PCO-like' if they exhibited at least one of these criteria (ultrasonographic or clinical). Patients were classified as idiopathic high responders if they failed to exhibit any of these criteria.

Treatment

Patients began combination OCP containing 1 mg norethindrone and 35 μg ethinyl oestradiol (Ortho-Novum 1/35®; Ortho Pharmaceutical Co., Raritan, NJ, USA) following onset of a spontaneous menstrual period or progestin-induced withdrawal bleeding. Oral contraceptives were taken daily for 25 days. Leuprolide acetate (Lupron®; TAP Pharmaceutical, Deerfield, IL, USA) was started on day 21 at a daily dosage of 1 mg s.c., overlapping the contraceptive pill for 5 days. Low dose gonadotrophin stimulation [150 IU of human menopausal gonadotrophins (HMG) or purified FSH] was initiated on the third day of subsequent withdrawal bleeding, at which time the dose of leuprolide acetate was decreased to 0.5 mg/day. Daily monitoring of oestradiol concentrations and follicular development commenced on cycle day 6. Ultrasound evaluations were performed with a General Electric RT 3600 real-time ultrasound (General Electric, Rancho Cordova, CA, USA) equipped with a 7.5 MHz transvaginal probe. The daily gonadotrophin dosage was decreased in an incremental step-down fashion once initial follicular recruitment was established (oestradiol ≥200 pg/ml).

Adjustments in gonadotrophin dosage were individually tailored according to each patient's follicular response, although most patients received ~50% (75 IU) of their original gonadotrophin dosage daily in the latter part of their stimulation cycle. The timing of human chorionic gonadotrophin (HCG) administration was based on several parameters, including the lead follicular diameter(s), serum oestradiol concentration, rate of rise of serum oestradiol and oocyte or embryo quality in the patient's previous cycle(s), when available. In general, HCG was administered in a dosage of 3300 to 10 000 IU i.m. once two or more follicles reached 17 mm in diameter. Ultrasound-guided vaginal oocyte retrievals were scheduled 35 h following HCG administration. Leuprolide acetate was discontinued on the day of HCG administration.

All oocytes were inseminated with a concentration of 150–200×10^3/ml washed motile spermatozoa ~5 h after oocyte aspiration. In the event of oligozoospermia, asthenoteratozoospermia, or history of prior fertilization failure, micromanipulation techniques (subzonal insemination, intracytoplasmic sperm injection) were undertaken as previously reported (Cohen *et al.*, 1991; Palermo *et al.*, 1995). Oocyte culture media consisted of 50–100 μl drops of human tubal fluid with 10–15% patient's serum under mineral oil.

On the day after insemination, oocytes were examined for the presence of two pronuclei. After fertilization, ensuing embryos were incubated in a 5% CO_2 environment at 37°C. Selective assisted hatching was performed on the day of embryo transfer, based on clinical criteria (i.e. age, zona thickness) as previously described (Cohen *et al.*, 1992). Embryo transfers were performed ~72 h after oocyte retrieval. All patients undergoing embryo transfer received short courses of tetracycline (250 mg q.i.d. for 4 days) and methylprednisolone (16 mg p.o. daily for 4 days) starting on the day of oocyte retrieval (Cohen *et al.*, 1990). In addition, supplemental progesterone-in-oil (25–50 mg i.m. daily) was administered daily, starting on the day after oocyte retrieval and continued until either a negative pregnancy test or sonographically confirmed embryonic viability was noted.

Laboratory evaluation

All hormonal evaluations were performed in duplicate by radioimmunoassays utilizing commercial kits: FSH, LH (Binax, Detroit, MI, USA); oestradiol, 17-hydroxyprogesterone (Pantex, Santa Monica, CA, USA); DHEA-S, androstenedione (Diagnostic Systems Laboratories, Webster, TX, USA); and total testosterone (Diagnostic Products Corp., Los Angeles, CA, USA). Interassay and intra-assay

coefficients of variation did not exceed 11% for any of these assays at concentrations within usual working ranges.

Statistics

Comparison of means between the three groups was undertaken utilizing one-way analysis of variance (ANOVA) and post-hoc paired comparisons with Tukey's HSD. Comparison of rates between the three groups was performed by χ^2. Comparisons of means and rates of the same patient population evaluated at two different time points were undertaken with paired *t*-tests and McNemar tests respectively. Fisher's exact test was carried out in lieu of the McNemar test when appropriate. One-way analysis of variance (ANOVA) in a repeated measures design was used when there were more than two measurements in the same patient population. Statistical significance was defined by P values <0.05. These data are represented as means ± SD.

Results

A total of 99 IVF-embryo transfer cycles utilizing the dual method of suppression combined with low dose gonadotrophin therapy in 73 high responder patients was analysed. Patients were equally distributed into protocols involving either HMG or purified FSH. There were 13 cycles (13.1%) which were cancelled prior to embryo transfer. These included three cycles in which all embryos were electively cryopreserved following oocyte retrieval due to a high risk for hyperstimulation. Three cycles were cancelled prior to oocyte retrieval for high response and four cycles were cancelled for poor response. The development of a dominant follicle, concern for a possible deep venous thrombosis and inadequate serum concentrations of HCG post HCG administration accounted for the remaining three cancellations. Mean oestradiol at the time of HCG administration was 2111 pg/ml. The overall oocyte fertilization rate was 47.4%. The clinical pregnancy rates per initiated cycle, retrieval and transfer were 46.5, 51.7 and 59.0% respectively. The ongoing pregnancy rates (i.e. pregnancies which continued to the second trimester with a viable fetus on ultrasound) per initiated cycle, retrieval and transfer were 40.4, 44.9 and 51.3% respectively. There were a total of three spontaneous abortions, two blighted ova and one ectopic pregnancy in this series. The multiple pregnancy rate of 60.0% may, in part, be attributed to the relatively high embryo implantation rate (number of detected fetal heart beats per embryos transferred) of 28.7%. There were 15 twin pregnancies, eight triplet pregnancies and one quadruplet pregnancy in addition to 16 singleton pregnancies which were ongoing. There were eight episodes of OHSS noted, all of which were classified as mild-moderate in severity (Golan *et al.*, 1989). One hospitalization for OHSS was required without any specific interventions needed other than observation.

A total of 22 patients met all the criteria for PCOS; 30 patients exhibited at least one criterion and were therefore classified as 'PCO-like'; 21 patients did not exhibit any PCOS criteria and were classified as idiopathic high responders. The basal hormonal profiles of these subgroups are presented in Table I. PCOS patients had significantly higher serum LH, total testosterone and androstenedione concentrations than either 'PCO-like' or idiopathic high responder patients. In addition, PCOS patients had significantly higher follicular phase 17-OHP levels than idiopathic high responder patients. There were no significant basal hormonal differences between the 'PCO-like' and idiopathic high responder subgroups.

Stimulation characteristics and clinical outcome did not appear to vary among the three subclassifications (Table II). Although there appeared to be slightly better oocyte fertilization and implantation rates, as well as clinical and ongoing pregnancy rates, in the idiopathic high responder group, none of these was statistically significant.

An attempt was made to compare results of the dual suppression protocol with previous IVF–embryo transfer cycles undertaken at our centre. Comparison of the results of the dual suppression protocol with those of previous cycles occurring at other centres was not performed because of the potential for variability in treatment approaches and laboratory assays. Therefore not all patients in our series are included in this portion of the analysis. In all, 38 high responders were identified who had undergone the dual suppression protocol and at least one previous cycle at The Center for Reproductive Medicine and Infertility at The New York Hospital–Cornell Medical Center. In no instance did any patient receive initial gonadotrophin dosages higher than our standard protocol (300 IU). These included 20 cycles in which patients received 150 IU of gonadotrophins daily after standard GnRHa suppression, nine cycles in which patients received 225 IU of gonadotrophins daily after standard GnRHa suppression and 18 cycles in which patients received 300 IU of gonadotrophins after standard GnRHa suppression. In five cycles, a combination of clomiphene citrate and 150 IU of gonadotrophins was employed. One cycle involved initiation of 300 IU of gonadotrophins after the dual method of suppression and one additional cycle involved 150 IU of gonadotrophins in a pure gonadotrophin protocol. The cumulative results of these previous cycles were poor, with a high cancellation rate (48.1%) as well as low clinical pregnancy (11.1%) and ongoing pregnancy (7.4%) rates per initiated cycle (Table III). In cycles which culminated in oocyte retrieval and embryo transfer, there was a significant difference in the oocyte fertilization rate and embryo implantation rate when compared with the dual suppression–low dose gonadotrophin cycles ($P < 0.01$).

To examine the issues of the dual method of suppression in comparison with standard GnRHa suppression without the confounding issues of gonadotrophin dosage, we identified 16 patients who received the same low gonadotrophin dosage in two cycles, one with and one without oral contraceptives. These 16 patients underwent 21 cycles with dual suppression and had undergone 20 previous cycles with standard GnRHa suppression at our centre (Table IV). A clinical advantage was observed for the dual suppression protocol in terms of the oocyte fertilization rate, embryo implantation rate and clinical/ongoing pregnancy rate per initiated cycle ($P < 0.05$).

We analysed the peripheral hormonal status of these high responder patients to elucidate potential mechanisms whereby benefit might accrue from the dual method of suppression. There were 35 patients who had serum tested at baseline and on day 3 following both a standard GnRHa suppression and the dual method of suppression (Table V). Three patients who had previous cycles at our centre did not undergo a protocol

Table I. Basal hormonal profile of high responder patients according to polycystic ovary (PCO) subclassification. Values are means ± SD

Hormone	PCOS patients (n = 22)	'PCO-like' patients (n = 30)	Idiopathic high responders (n = 21)
FSH (mIU/ml)	7.4 ± 2.2	8.0 ± 3.0	8.6 ± 2.5
LH (mIU/ml)[a]	25.0 ± 10.0	14.8 ± 8.7	12.9 ± 5.9
Oestradiol (pg/ml)	44.4 ± 22.5	38.7 ± 15.0	46.2 ± 18.5
Total testosterone (ng/ml)[a]	0.58 ± 0.23	0.34 ± 0.14	0.26 ± 0.09
DHEA-S (ng/ml)	2061 ± 1151	1723 ± 753	1675 ± 569
Androstenedione (ng/ml)[b]	2.25 ± 0.55	1.61 ± 0.56	1.46 ± 0.48
17-hydroxyprogesterone (ng/dl)[c]	51.1 ± 20.0	40.9 ± 19.8	31.7 ± 10.9

PCOS = polycystic ovarian syndrome; FSH = follicle stimulating hormone; LH = luteinizing hormone; DHEA-S = dihydroepiandrosterone sulphate.
[a]PCOS group differs from both 'PCO-like' and high responder groups at $P < 0.01$.
[b]PCOS group differs from 'PCO-like' and high responder groups at $P < 0.05$ and $P < 0.01$ respectively.
[c]PCOS group differs from high responder group at $P < 0.05$.

Table II. Stimulation characteristics and outcome of in-vitro fertilization–embryo transfer cycles according to polycystic ovarian syndrome (PCOS) subclassification. Where appropriate, values are mean ± SD

Characteristic	PCOS patients (n = 22)	'PCO-like' patients (n = 30)	Idiopathic high responders (n = 21)
No. of cycles	37	35	27
No. of cancellations[a]	5 (13.5)	4 (11.4)	4 (14.8)
Age (years)	32.5 ± 4.7	32.7 ± 3.9	33.1 ± 2.6
Duration of stimulation (days)	9.5 ± 1.8	9.5 ± 1.5	9.6 ± 1.3
No. of gonadotrophin ampoules	18.0 ± 4.5	18.1 ± 3.4	17.8 ± 3.1
Oestradiol at time of HCG (pg/ml)	2067 ± 864	1956 ± 847	2381 ± 560
No. of oocytes retrieved	15.9 ± 6.5	15.9 ± 7.7	15.3 ± 4.8
Oocyte fertilization rate	0.43 ± 0.24	0.46 ± 0.28	0.56 ± 0.21
Mean no. of embryos transferred	3.43	3.16	3.26
Implantation rate (%)	26.2	25.3	36.0
No. of biochemical pregnancies	7	1	4
No. (%) of clinical pregnancies[b]	15 (40.5)	15 (42.9)	16 (59.3)
No. (%) of ongoing pregnancies[b]	13 (35.1)	13 (37.1)	14 (51.9)
No. (%) with OHSS[c]	3 (8.1)	3 (8.6)	2 (7.4)

[a]Cancellations include all terminated cycles prior to embryo transfer.
[b]Clinical pregnancy and ongoing pregnancy rates were determined per initiated cycle.
[c]All cases of OHSS were classified as mild-moderate in severity.
HCG = human chorionic gonadotrophin; OHSS = ovarian hyperstimulation syndrome.

employing GnRHa suppression and therefore were not analysed. Serum FSH concentrations were significantly suppressed from baseline levels following both standard GnRHa suppression and the dual suppression protocol. However, serum LH concentrations were significantly suppressed only in the dual suppression protocol. Oestradiol, total testosterone and androstenedione concentrations were significantly suppressed in both the standard GnRHa and dual suppression protocols. For these latter hormonal parameters, there seemed to be slightly greater (although not significant) suppression with the dual OCP/leuprolide protocol. Likewise, serum DHEA-S values were also significantly reduced from baseline after both the GnRHa and dual suppression protocols, with the dual suppression protocol producing lower DHEA-S concentrations when compared to GnRHa alone.

Discussion

High responder patients are characterized by heightened sensitivity to exogenous gonadotrophin therapy manifested by recruitment of large numbers of follicles, rapid oestradiol responses and a significant cycle cancellation rate due to the potential risk of hyperstimulating during IVF–embryo transfer attempts. Even for those patients who progress to oocyte retrieval and embryo transfer, appreciably lower fertilization, implantation and ongoing pregnancy rates are seen. It is not entirely clear whether the impairment in oocyte fertilization rates is attributable to physician decisions on the timing of HCG administration or to the hormonal follicular microenvironment in these patients. As a large number of oocytes are often retrieved, a relatively normal number of embryos is still available for transfer in most instances. However, despite this, the embryo implantation rate (an appropriate indicator of IVF–embryo transfer efficiency) is persistently low. Clearly, embryonic health and viability are also reduced in these high responder patients undergoing standard stimulation protocols.

It has long been recognized that patients with PCOS often have an exquisite sensitivity to exogenous gonadotrophin therapy. It is not entirely clear whether this tendency towards a high response is due simply to the availability of an increased number of follicles in the gonadotrophin-sensitive pool or whether the granulosa cells themselves respond in an enhanced

Table III. Analysis of outcome of in-vitro fertilization–embryo transfer cycles in high responder patients who had undergone a previous cycle at The New York Hospital–Cornell Medical Center. Where appropriate, values are mean ± SD

Characteristic	Dual suppression (n = 38)	Previous cycles (n = 38)
No. of cycles	51	54
No. (%) cancellations[a]	3 (5.9)	26 (48.1)
Age (years)	33.0 ± 3.4	32.2 ± 3.4
Duration of stimulation (days)	9.3 ± 1.4	8.9 ± 0.9
No. of gonadotrophin ampoules	17.3 ± 3.3	16.8 ± 4.3
Oestradiol at time of HCG (pg/ml)	2227 ± 827	2621 ± 927
No. of oocytes retrieved	16.2 ± 6.9	19.9 ± 7.2
Oocyte fertilization rate[d]	0.45 ± 0.25	0.28 ± 0.20
Mean no. of embryos transferred	3.12	3.12
Implantation rate[e] (%)	26.7	5.3
No. of biochemical pregnancies	8	2
No. (%) of clinical pregnancies[b]	24 (47.1)	6 (11.1)
No. (%) of ongoing pregnancies[b]	20 (39.2)	4 (7.4)
No. (%) with OHSS[c]	3 (5.9)	2 (3.7)

[a]Cancellations include all terminated cycles prior to embryo transfer.
[b]Pregnancy rates were determined per initiated cycle.
[c]All cases of OHSS were classified as mild-moderate in severity.
[d]$P < 0.01$. (Paired t-test comparing first cycles of each protocol.)
[e]$P < 0.01$. (Fisher's exact test comparing first cycles of each protocol.)
Cancellation rates and pregnancy rates were not compared in this analysis.
HCG = human chorionic gonadotrophin; OHSS = ovarian hyperstimulation syndrome.

Table IV. Analysis of in-vitro fertilization–embryo transfer outcome of high responder patients who had undergone low-dose gonadotrophin (150 IU) cycles following both standard gonadotrophin-releasing hormone agonist (GnRHa) suppression and the dual method of suppression at The New York Hospital–Cornell Medical Center. Where appropriate, values are mean ± SD

Characteristic	Dual suppression (n = 16)	GnRHa suppression (n = 16)
No. of cycles	21	20
No. (%) of cancellations[a]	0 (0.0)	4 (20.0)
Age (years)	33.6 ± 2.9	31.8 ± 3.2
Duration of stimulation (days)	9.0 ± 1.3	8.7 ± 1.0
No. of gonadotrophin ampoules	17.0 ± 3.0	16.3 ± 1.7
Oestradiol at time of HCG (pg/ml)	2080 ± 830	2436 ± 783
No. of oocytes retrieved	15.6 ± 7.0	19.1 ± 5.2
Oocyte fertilization rate[d]	0.37 ± 0.22	0.24 ± 0.13
Mean no. of embryos transferred	2.94	2.80
Implantation rate[e] (%)	23.5	4.8
No. of biochemical pregnancies	3	2
No. (%) of clinical pregnancies[b,f]	10 (47.6)	2 (10.0)
No. (%) of ongoing pregnancies[b,f]	8 (38.1)	2 (10.0)
No. (%) with OHSS[c]	3 (5.9)	2 (4.0)

[a]Cancellations include all terminated cycles prior to embryo transfer.
[b]Pregnancy rates were determined per initiated cycle.
[c]All cases of OHSS were classified as mild-moderate in severity.
[d]$P = 0.01$. (Paired t-test comparing first cycles of each protocol.)
[e]$P < 0.01$. (Fisher's exact test comparing first cycles of each protocol.)
[f]$P < 0.05$. (McNemar's test either: (i) comparing first cycles, or (ii) excluding patients with discordant results).
HCG = human chorionic gonadotrophin; OHSS = ovarian hyperstimulation syndrome.

manner. We, as well as several investigative teams (Smitz *et al.*, 1990; MacDougall *et al.*, 1992), have observed the early recruitment of large numbers of small follicles (in the range of 20–40 total), identifiable ultrasonographically as early as the fourth day of gonadotrophin stimulation in PCOS patients. On the other hand, a few recent reports utilizing in-vitro cell culture methods have demonstrated greatly enhanced oestradiol production in response to FSH by granulosa cells from patients with PCOS compared with controls (Andreani *et al.*, 1994; Mason *et al.*, 1994).

It has also been speculated that intra-ovarian androgens may serve as substrates for aromatization and that FSH (in concert with other regulatory peptides) may stimulate granulosa cell aromatase activity (Franks and Mason, 1991), which is thought to be ordinarily deficient in the 'resting' polycystic ovary (Erickson *et al.*, 1979). Further modulatory agents may also include growth factors such as insulin and IGF (insulin-like growth factor) I and II, which are known to have enhancing effects on granulosa cells (Adashi *et al.*, 1985; Erickson *et al.*, 1990; DiBlasio *et al.*, 1994). In particular, an important feature of PCOS is the frequent presence of elevated concentrations of circulating insulin (Chang *et al.*, 1983; Pasquali *et al.*, 1983) which may act as a gonadotrophic hormone in the enhancement of ovarian steroidogenesis, aromatase activity and induction of LH receptors (Poretsky and Kalin, 1987).

The characteristics of patients who have heightened responses to gonadotrophins and do not have PCOS have been less well described. It has been recently noted that certain patients with ultrasonographic evidence of PCO without any clinical or biochemical evidence of PCOS may continue to manifest exaggerated responses to gonadotrophin therapy (Wong *et al.*, 1995). We have also observed several patients in our 'PCO-like' group who demonstrated previous high responses and had as their only manifestation isolated polycystic morphology on ultrasonography. On the other hand, there appear to be patients who have tendencies for high responses who do not manifest any indications of PCOS at all.

There is mounting evidence that elevated follicular concentrations of LH have an adverse effect on follicular and oocyte development and that suppression of LH concentrations improves IVF–embryo transfer outcome in PCOS patients. A recent report observed that there was a significant inverse correlation between the baseline LH/FSH ratio and the percentage of mature oocytes in PCOS patients undergoing an IVF–embryo transfer protocol without GnRH analogue (Tarlatzis *et al.*, 1995). In addition, the mean LH/FSH ratio was higher in women who miscarried than in women who had a live birth. The administration of GnRHa in the long protocol seemed to reverse the detrimental effects on oocyte development; however, a continued greater potential for miscarriage persisted. Other authors have concluded that adjunctive GnRHa may result in a reduction in the rate of spontaneous abortion as well as improve both the oocyte fertilization rate and pregnancy rate in PCOS women undergoing IVF–embryo transfer (Homburg *et al.*, 1993). On the other hand, a recent well-controlled study failed to demonstrate a benefit of GnRHa presuppression in women undergoing low dose ovulation induction who had a history of recurrent miscarriages, PCO and hypersecretion of LH (Clifford *et al.*, 1996).

The duration of administration of GnRHa may play a role in its effectiveness in improving IVF–embryo transfer cycle outcome in PCOS patients. Not all investigators have clearly demonstrated advantages with pituitary desensitization with

Table V. Comparison of hormonal profiles at baseline (A) and on day 3 of the treatment cycle of high responder patients who underwent cycles following both standard gonadotrophin-releasing hormone agonist (GnRHa) suppression (B) and the dual method of suppression (C). Values are mean ± SD

Hormone	(A) Baseline (n = 35)	(B) GnRHa suppression (n = 35)	(C) Dual suppression (n = 35)
FSH (mIU/ml)[a]	7.9 ± 2.9	6.1 ± 2.1	6.2 ± 1.8
LH (mIU/ml)[b]	16.8 ± 9.3	17.3 ± 7.7	11.4 ± 5.1
Oestradiol (pg/ml)[a]	42.5 ± 15.6	27.9 ± 27.0	19.4 ± 8.4
Total testosterone (ng/ml)[a]	0.37 ± 0.18	0.24 ± 0.18	0.19 ± 0.15
DHEA-S (ng/ml)[c]	1762 ± 666	1568 ± 646	1264 ± 547
Androstenedione (ng/ml)[a]	1.84 ± 0.55	1.61 ± 0.57	1.52 ± 0.47

[a]A differs from both B and C at $P < 0.01$.
[b]A differs from C at $P < 0.01$; B differs from C at $P < 0.01$.
[c]A differs from both B and C at $P < 0.01$; B differs from C at $P < 0.01$.
FSH = follicle stimulating hormone; LH = luteinizing hormone; DHEA-S = dihydroepiandrosterone sulphate.

GnRHa administered in standard protocols (Dor *et al.*, 1992). One group has suggested that at least 4 weeks of pretreatment with GnRHa is required to normalize serum LH, testosterone and androstenedione concentrations in PCOS patients (Tanbo *et al.*, 1989). A comparison of a long (30 day) and short (15 day) protocol of pituitary desensitization with GnRH analogues prior to gonadotrophin stimulation for IVF–embryo transfer in PCOS patients revealed similar numbers of oocytes and embryos as well as oocyte fertilization rates and pregnancy rates for both protocols (Salat-Baroux *et al.*, 1988). However, the greater duration of pituitary desensitization resulted in lower amounts of circulating androgens, most notably androstenedione, on the day of oocyte retrieval. In addition, these latter patients demonstrated a less pronounced polycystic follicular response and a decreased tendency towards hyperstimulation.

In addition, there may be other potential actions of the dual suppression protocol specifically related to the oral contraceptive element which modifies ovarian responsiveness. Oral contraceptives have often been used in the past in attempts to programme ovarian stimulation cycles for IVF–embryo transfer (Gonen *et al.*, 1990). One programme noted that, when applied to all candidates, the profound suppression of the pituitary and ovary following oral contraceptive treatment resulted in inadequate responses in certain patients, necessitating longer administration or increasing doses of gonadotrophins (Benadiva *et al.*, 1988). An interesting recent report also showed that progesterone pretreatment prior to a short-term GnRHa 'flare-up' protocol results in a significantly lower gonadotrophic response (particularly LH) as well as a blunted initial oestradiol flare response (Cedrin-Durnerin *et al.*, 1996).

Use of the dual method of suppression results in significantly lower oestradiol, total testosterone, DHEA-S and androstenedione concentrations at the onset of gonadotrophin stimulation in high responders undergoing IVF–embryo transfer. As opposed to GnRHa suppression in a standard protocol, the dual method of suppression also results in significantly lower serum LH values. DHEA-S concentrations are also significantly further suppressed with the dual suppression method than with GnRHa alone. One possible explanation for this latter finding is that with further suppressed oestrogen concentrations there may be less inhibition of 3β-hydroxysteroid dehydrogenase activity (Byrne *et al.*, 1986). Similar results have been noted in anovulatory women with elevated DHEA-S concentrations who had been on GnRHa therapy for 3 months (Gonzalez *et al.*, 1991).

Although limited by a retrospective design, this study suggests clinical benefits from the use of the dual method of suppression–low dose gonadotrophin stimulation protocol for high responders undergoing IVF–embryo transfer. The efficacy of this protocol appears to remain consistent across the subclassification categories of high responders and includes patients with PCOS, those with limited PCO characteristics and also patients with a history of idiopathic high response. Our comparison of the subgroup of high responder patients who were previously treated at our centre provides some insights. In particular, the 16 patients who had both standard GnRHa suppression and the dual method of suppression followed by administration of the same low dosage of gonadotrophin allowed for comparisons of two cycles in the same group of patients in which the only variable differing was the use of oral contraceptive pretreatment. In this latter group of patients, use of the dual method of suppression resulted in improved oocyte fertilization, embryo implantation and clinical pregnancy rates. Use of the dual method of suppression combined with a low dose gonadotrophin stimulation protocol also allowed for a low cancellation rate in patients who have exhibited high responses previously with standard gonadotrophin dosages following GnRHa long protocol suppression. We customarily employ a very conservative approach in cycles which portend a high response by withholding HCG and cancelling the oocyte retrieval altogether. We believe that it is for this specific reason that we did not demonstrate differences in the incidence of OHSS. Due to high embryo implantation rates, the multiple pregnancy rate was high with this protocol. We believe that this can be modulated by restricting the number of embryos transferred. Use of the dual method of suppression also allows an opportunity for programming of cycle initiation and, in addition, provides a relatively simple way of initiating GnRHa therapy in high responder oligo-ovulatory or anovulatory women. Our data support the need for a prospective randomized study comparing

equal periods of hypothalamo-pituitary suppression with either GnRHa alone or with a combination of oral contraceptives followed by a GnRHa in high responder patients undergoing IVF–embryo transfer.

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