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Ovarian stimulation by concomitant administration of cetrorelix acetate and HMG following Diane-35 pre-treatment for patients with polycystic ovary syndrome: a prospective randomized study

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BACKGROUND: Patients with polycystic ovary syndrome (PCOS) may need a longer period of pituitary downregulation to suppress the elevated serum LH and androgen levels effectively during IVF treatment using the GnRH agonist long protocol. We proposed a stimulation protocol incorporating Diane-35 and GnRH antagonist (Diane/cetrorelix protocol) and compared it with the GnRH agonist long protocol for PCOS patients. METHODS: Part I of the study was an observational pilot study to evaluate the hormonal change as a result of the Diane/cetrorelix protocol ($n = 26$). Part II of the study was a prospective randomized study comparing the Diane/cetrorelix protocol ($n = 25$) and the GnRH agonist long protocol ($n = 24$). In the Diane/cetrorelix protocol, patients were pretreated with three cycles of Diane-35, followed by 0.25 mg of cetrorelix on cycle day 3. From day 4, cetrorelix and gonadotrophin were administered concomitantly until the day of HCG injection. RESULTS: Serum LH, estradiol and testosterone levels were suppressed comparably in both protocols at the start of gonadotrophin administration. Serum LH was suppressed at constant levels without a premature LH surge in the Diane/cetrorelix protocol. The clinical results for both protocols were comparable, with significantly fewer days of injection, lower amounts of gonadotrophin used and lower estradiol levels on the day of HCG injection following the Diane/cetrorelix protocol. Furthermore, there was no significant difference in clinical pregnancy outcome between the two stimulation protocols. CONCLUSIONS: The Diane/cetrorelix protocol has a similar pregnancy outcome to the GnRH agonist long protocol for women with PCOS undergoing IVF treatment.

Key words: cetrorelix acetate/Diane-35/HMG/GnRH antagonist/PCOS

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility, characterized by chronic anovulation and hyperandrogenism (Franks, 1995). Frequently encountered endocrine features are hypersecretion of LH, hyperandrogenaemia and compensatory hyperinsulinaemia. IVF is an effective treatment after repeated failure of ovulation induction by clomiphene citrate and gonadotrophin (Dor *et al.*, 1990; Homburg *et al.*, 1993a). Tonic hypersecretion of LH is thought to be one of the major factors responsible for a high miscarriage rate, poor oocyte quality, and a low fertilization and cleavage rate in PCOS patients (Homburg *et al.*, 1988, 1993b; Smitz *et al.*, 1992; Balen *et al.*, 1993a, b). In patients undergoing IVF treatment, the elevated mean follicular phase serum LH level has a detrimental effect on the fertilization rate, cleavage rate and pregnancy outcome (Stanger and Yovich, 1985). To reduce LH concentrations throughout the follicular phase and to prevent a premature LH surge, controlled ovarian stimulation (COS) with gonadotrophin after downregulation with GnRH agonist—the GnRH agonist long protocol—is the most frequently used protocol for PCOS patients. (Smitz *et al.*, 1992; Balen *et al.*, 1993a; Homburg *et al.*, 1993a, b). Several studies have suggested that the duration of GnRH agonist administration needed to achieve pituitary suppression for PCOS patients is usually longer than that for normal ovulatory patients (Dor *et al.*, 1992; Homburg *et al.*, 1993a; Macnamee and Brinsden, 1999). In addition to elevated LH serum levels, hyperandrogenaemia and increased intrafollicular androgens were reported to be responsible for blocking

normal follicular development in PCOS patients (Hamori *et al.*, 1992). Androgens were reported to have an atretic effect on maturing follicles (Scaramuzzi *et al.*, 1977). Androgen levels are higher in women, including PCOS or non-PCOS patients, who have recurrent miscarriages than in normal fertile controls (Tulppala *et al.*, 1993; Okon *et al.*, 1998). To suppress ovarian androgens effectively, GnRH agonist downregulation longer than 2 weeks is required (Salat-Baroux *et al.*, 1988; Tanbo *et al.*, 1989; Hamori *et al.*, 1992).

Diane-35 (Schering, Berlin, Germany), an oral contraceptive pill (OCP) containing 2 mg of cyproterone acetate (CPA) plus 0.035 mg of ethinyl estradiol, has been widely used in treating the problems of seborrhoea and hirsutism in PCOS patients (Golland and Elstein, 1993; Prelevic *et al.*, 1993). It decreases LH production and subsequently androgen production through the inhibitory effect on the hypothalamus and pituitary gland. After three consecutive treatment cycles, serum concentrations of LH, testosterone and andostenedione returned to normal values, and sex hormone-binding globulin (SHBG) increased significantly in PCOS patients (Golland and Elstein, 1993; Prelevic *et al.*, 1993).

GnRH antagonists inhibit gonadotrophin release within several hours through binding competitively to pituitary GnRH receptors (Felberbaum and Diedrich, 1998). Without a 'flare up' effect being found with GnRH agonist, GnRH antagonist has been administered in the late follicular phase to prevent or interrupt the LH surge successfully during COS (Albano *et al.*, 2000; Olivennes *et al.*, 2000; Hwang *et al.*, 2003). If a similar application was made in PCOS patients undergoing IVF treatment, the early follicular phase high tonic LH secretion would probably still be present.

We proposed a stimulation protocol incorporating Diane-35 and GnRH antagonist in patients with PCOS who were to undergo IVF treatment. First of all, three consecutive cycles of Diane-35 pre-treatment were used in an attempt to decrease serum LH and androgens. One dose of a GnRH antagonist, cetrorelix acetate (Cetrotide; Serono, Geneva, Switzerland), was administered to augment the suppressive effect of Diane-35. Following this, cetrorelix acetate was administered concomitantly with exogenous gonadotrophin to prevent progressive tonic LH elevation in the early follicular phase and a premature LH surge in the late follicular phase. Part I of the study was an observational pilot study to evaluate the efficacy and hormonal changes associated with this protocol (Diane/cetrorelix protocol). Part II of the study was a prospective randomized controlled study to compare this protocol with the GnRH agonist long protocol. The hypothesis is that, by using the Diane/cetrorelix protocol, a similar fertilization, pregnancy and implantation rate could be achieved; and a similar serum LH and testosterone profile will be attained upon starting and during the HMG stimulation, compared with the GnRH agonist long protocol.

We tried to investigate (i) whether a similar fertilization, pregnancy and implantation rate could be achieved; and (ii) whether these two protocols attained a similar serum LH and testosterone profile upon starting and during gonadotrophin stimulation.

Materials and methods

The study was reviewed and approved by the Institute Review Board of Shin Kong Wu Ho-Su Memorial Hospital. All couples were required to sign an informed consent form. The diagnosis of PCOS included: (i) chronic anovulation manifested by the symptoms of oligomenorrhoea (>40 days per cycle), amenorrhoea or irregular menstrual cycle and confirmed by a basal body temperature chart or serum progesterone determination; (ii) ultrasonographic evidence of polycystic ovaries (Adams *et al.*, 1985), an enlarged ovary with >10 peripherally located follicles of 3–8 mm diameter around a dense central stroma; and (iii) at least one of the two hormonal abnormalities (a) normal FSH concentration (3–10 mIU/ml) and elevated LH concentration (>10 mIU/ml) or LH /FSH ratio >2; and (b) hyperandrogenaemia (serum testosterone concentrations >0.8 ng/ml). A diagnosis of congenital adrenal hyperplasia, Cushing's syndrome, androgen-producing tumours, hyperprolactinaemia and thyroid dysfunction were all excluded. The exclusion criteria included patients older than 38 years or with serum FSH levels >12 mIU/ml.

Study part I

From June 2002 to December 2002, 29 patients with PCOS were recruited from our infertility centre for this study. The age, duration of infertility and body mass index (BMI) were (mean ± SD) 31.3 ± 4.4 years old, 4.5 ± 1.2 years and 22.9 ± 2.9 kg/m^2, respectively. Before entering the IVF programme, more than six cycles of ovulation induction with gonadotrophin had been done. The patients did not take any ovulation drugs or hormones for at least 3 months prior to the trial. The stimulation protocol is shown in Figure 1. In all patients, serum concentrations of FSH, LH, estradiol (E_2),

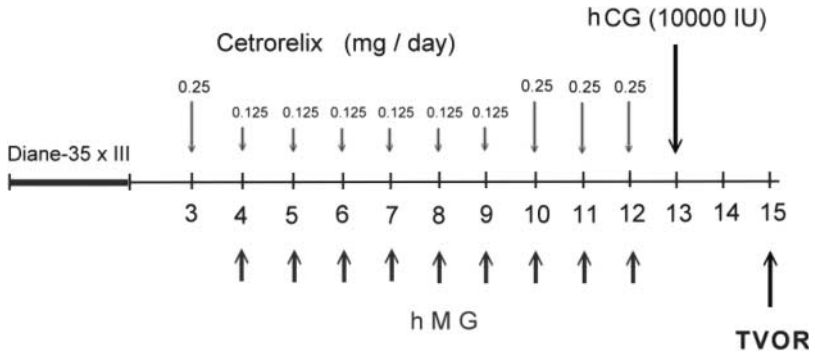

Figure 1. Treatment schema. TVOR = Transvaginal oocyte retrieval.

progesterone and testosterone were assessed on day 3 of induced or spontaneous menstruation. Diane-35 was prescribed as one tablet per day from day 5 of the cycle for 21 days. A total of three consecutive cycles were given. After pre-treatment with Diane-35, patients returned to the clinic for a hormonal profile examination, including FSH, LH, E_2 and testosterone, in the morning of day 3 of the cycle. Ultrasonography was performed to exclude the presence of any ovarian cyst >10 mm. Cetrorelix acetate was then initiated with a single dose of 0.25 mg administered s.c. between 6.00 and 8.00 p.m. on day 3 to augment the suppressive effect of Diane-35. Patients returned to the clinic the following morning (day 4) for a repeat hormonal determination. From day 4 to day 9, cetrorelix acetate was reduced to 0.125 mg/day and ovarian stimulation was initiated with 150 IU of HMG (Pergonal; Serono, Geneva, Switzerland) every day. The dose of cetrorelix acetate was increased to 0.25 mg/day from day 10 until the day before HCG (Pregnyl; NY Organon, Oss, The Netherlands) injection, and the dose of HMG was adjusted according to the patient's response (Figure 1). Serum levels of LH, FSH and E_2 were measured on days 3, 4, 10, the day of HCG injection and as indicated. HCG, 10 000 IU, was administered i.m. when at least two follicles reached 18 mm in diameter with adequate E_2 response. Transvaginal oocyte retrieval (TVOR) was performed 36 h later. ICSI was performed for all patients because our previous experience (unpublished data) and that of Stadtmauer *et al.* (2001) suggested that a number of patients with PCOS had poor fertilization rates or unexpected fertilization failure with IVF. ICSI was performed according to the method described by Van Steirteghem *et al.* (1993). The potential risks of ICSI were thoroughly explained to the patients. Embryo transfer was performed 3 days after oocyte recovery. All patients received luteal phase support with 600 mg of vaginally administered micronized progesterone (Utrogestan; Laboratoires Piette International S.A., Brussels, Belgium) daily starting from the day after oocyte retrieval. Clinical pregnancy was defined as a visible fetal heart beat on ultrasonography at 7 weeks of gestation.

Serum FSH, LH and testosterone were measured with an immunometric assay using an Immulite® kit (Diagnostic Products Corporation, Los Angeles, CA). The sensitivity for FSH was 0.1 mIU/ml. The intra- and inter-assay coefficients of variance (CVs) were 7.7 and 7.9%, respectively. For the LH assay, the sensitivity was 0.1 mIU/ml, and intra- and inter-assays CVs were 6.5 and 7.1%, respectively. For the testosterone assay, the sensitivity was 0.1 ng/ml (0.4 nmol/l), and intra- and inter-assays CVs were 4.0 and 5.6% respectively. E_2 was measured by competitive immunoassay using an Immulite® kit, with a sensitivity of 15 pg/ml (55 pmol/l), and intra- and inter-assay CVs of 6.3 and 6.4%, respectively.

Study part II

During the period of January to December 2003, PCOS patients undergoing the first cycle of IVF treatment were randomized prospectively to receive one of the two ovarian stimulation protocols. Randomization was done by opening sealed envelopes containing computer-generated block randomization numbers with a block size of 10. The laboratory staff were blinded to the stimulation protocol. The ovarian stimulation protocol in the study group was the same as in part I of the study (Diane/cetrorelix protocol). The control protocol was a GnRH agonist long protocol. A GnRH agonist, buserelin acetate (Supremon; Hoechst, Frankfurt, Germany), 500 μg/day was administrated from day 3 of induced or spontaneous menstration. After 14 days of buserelin injection, patients returned to the clinic for blood tests and the ultrasound examination to ensure pituitary downregulation. The criteria of downregulation were serum E_2 levels <50 pg/ml and the absence of ovarian cysts >10 mm in diameter. If the criteria were not met, a check-up was performed 3 days later. Buserelin was continued until the day of HCG injection, while the dosage was decreased to 250 μg/day at the beginning of HMG administration. HMG, at a dosage of 150 IU/day, was prescribed for 6 days beginning from the day of ensuing pituitary downregulation. Subsequent dosage was adjusted according to the follicular response as determined by serial ultrasound examination and serum E_2 change. The timing of HCG injection, the method of oocyte retrieval, insemination, embryo culture, embryo transfer and luteal support were the same as in part I of the study. The primary outcome measures were fertilization, pregnancy and implantation rates. The secondary outcome measures were serum LH and testosterone status upon starting and during HMG administration, and the total days of injection.

Statistical analysis

We assumed 50% of patients received the Diane/cetrorelix protocol and 50% of patients received the GnRH agonist long protocol. The sample size required would be 25 in each group in the study to give a test of significance of 0.05 and a power of 0.8 (SAS; SAS Institute, Cary, NC), assuming a similar pregnancy rate of 0.4 between these two groups. Normality of continuous variables was assessed with the Kolmogorov–Smirnov test. A paired or unpaired *t*-test was used for the statistical analysis in continuous variable with normal distribution. The Mann–Whitney test was used for variables without normal distribution. Between-groups differences in non-continuous variables were assessed with the χ^2 method and the Yates correction, if needed. A *P*-value <0.05 was considered to be significant. Analysis was performed using the SPSS statistical package window 10.0 (SPSS Inc., Chicago, IL).

Results

Hormonal change and clinical results of study part I

There were three (out of 29) cancellations before oocyte retrieval. Two were due to poor ovarian response. The other patient, because of monofollicular development, received intra-uterine insemination and achieved pregnancy.

Hormonal profiles before and after Diane-35 pre-treatment, after the first dose of cetrorelix acetate injection (day 4), after 6 days concomitant treatment of 0.125 mg/day cetrorelix acetate and HMG (day 10) and the day of HCG injection are presented in Table I. As shown, PCOS patients had higher baseline LH and testosterone levels. After 3 months of pre-treatment with Diane-35, serum LH levels declined significantly to normal values ($P < 0.001$). Before Diane-35 pre-treatment, 14 out of 29 patients had elevated serum LH levels (>10 mIU/ml) or an LH/FSH ratio >2. After OCP pre-treatment, only one patient still had abnormal serum LH levels that decreased to normal after a subsequent injection of cetrorelix acetate. The LH levels were suppressed to an even lower level after the first dose of 0.25 mg cetrorelix acetate ($P < 0.001$). Seventeen of 29 patients had abnormal serum testosterone levels, and all of them returned to normal values after pre-treatment with Diane-35 ($P < 0.001$). However, there was no further change after the first dose of cetrorelix acetate injection. Serum FSH and E_2 levels did not change significantly after Diane-35 pre-treatment, but a significant decrease was noted after the first injection of cetrorelix acetate. FSH levels increased with the injections of HMG.

Table I. Comparison of hormonal profiles at baseline (A), on day 3 of the menstrual cycle after 3 months pre-treatment with Diane-35 (B), 1 day after the first dose of 0.25 mg cetrorelix injection (day 4) (C), 6 days after concomitant treatment with 0.125 mg/day cetrorelix and HMG (day10) (D) and on the day of HCG injection (E)

	(A) Baseline ($n = 29$)	(B) After pre-treatment with Diane-35 ($n = 29$)	(C) Day 4 ($n = 29$)	(D) Day 10 ($n = 29$)	(E) Day of HCG ($n = 27$)[a]
LH (mIU/ml)[b]	10.3 ± 4.0	5.0 ± 1.7	2.8 ± 1.4	2.7 ± 1.7	2.3 ± 1.5
FSH (mIU/ml)[c]	5.8 ± 1.2	5.5 ± 1.4	4.1 ± 1.4	10.5 ± 3.2	9.9 ± 3.0
E_2 (pg/ml)[d]	48.1 ± 12.4	43.5 ± 10.5	27.5 ± 9.6		
Testosterone[e] (ng/ml)	0.76 ± 0.20	0.44 ± 0.16	0.45 ± 0.13		

Values are mean ± SD.
[a]No data available in the two poor responders.
[b]A differs from B; B differs from C at $P < 0.001$.
[c]B differs from C; C differs from D; C differs from E at$P < 0.001$.
[d]B differs from C at $P < 0.001$.
[e]A differs from B at $P < 0.001$.

Figure 2 shows the trend of serum LH during the period of cetrorelix acetate administration. From the day of concurrent cetrorelix acetate and HMG administration (day 4), LH concentrations remained relatively constant between 2 and 3 mIU/ml. Premature LH surge did not occur in any patients in this study.

Table II summarizes the results of the 26 completed cycles. There were 11 clinical pregnancies. The clinical pregnancy rates per initiated cycle and per embryo transfer were 37.9 and 42.3%, respectively. One pregnancy ended in miscarriage at 9 weeks of gestation. There were 10 deliveries including seven singletons born at term and three sets of twins born at 37, 36 and 34 weeks of gestation respectively. There were two episodes of moderate overian hyperstimulation syndrome (OHSS), according to the classification proposed by Golan *et al.* (1989). No hospitalization was required.

Clinical results of study part II

A total of 60 patients were assessed for eligibility. Among them, two refused to participate and two did not meet the inclusion criteria. A total of 56 patients were randomized to receive either the Diane/cetrorelix protocol ($n = 27$) or the GnRH agonist long protocol ($n = 29$). In patients receiving the Diane/cetrorelix protocol, one patient was cancelled due to poor ovarian response and the other dropped out of treatment for personal reasons. In the GnRH agonist long protocol group, two were converted to intra-uterine insemination due to inadequate ovarian response (<3 matured follicles developed) and three cancellations were made after TVOR for fear of severe OHSS. All the embryos were frozen in these three patients. Finally, there were 25 completed cycles (out of 27) in the study group and 24 cycles (out of 29) in the control group (Figure 3).

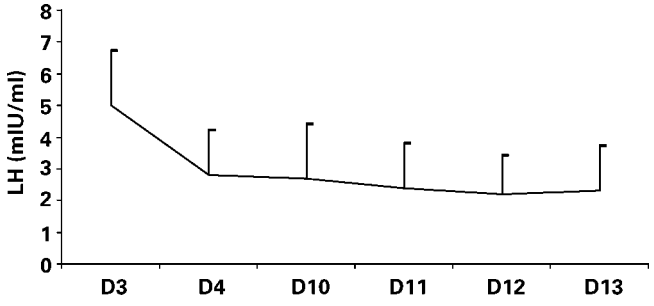

Figure 2. Serum LH levels relative to the cycle in part 1 study. D = day, D4, the day of starting HMG.

The patients' characteristics revealed no significant difference between the two groups in age, duration of infertility, BMI and basal hormonal levels, as shown in Table III. The serum LH, E_2 and testosterone levels on the day of starting HMG stimulation were comparable between the two groups. There was a trend of progressive lowering of serum LH levels in the GnRH agonist long protocol group. After 6 days of HMG stimulation, the serum LH levels were marginally significantly lower in the GnRH agonist long protocol group compared with the Diane/cetrorelix group ($P = 0.05$). On the day of HCG injection, the serum LH levels were significantly lower in the GnRH agonist long protocol group. In the Diane/cetrorelix group, the serum LH levels were relatively constant during the HMG stimulation. There was no significant difference in LH levels on the day HMG was started compared with 6 days after HMG administration, and between 6 days after HMG administration and the day of HCG injection in the Diane/cetrorelix group. Similarly, there

Table II. Patient and cycle characteristics in part I of the study

Variables	Values
No. of patients	29
No. of cycles	29
No. of cancellations	3
Duration of HMG stimulation(days)	9.4 ± 1.9
No. of HMG (ampoules)	21.3 ± 5.5
E_2 on the day of HCG (pg/ml)	1966 ± 674
No. of oocytes retrieved	15.5 ± 6.9
No. of oocytes fertilized	11.7 ± 6.6
Fertilization rate (%)	76(68 to 84)
No. of embryos transferred[a]	3(2–4)
Implantation rate (%)	18.7(8.6 to 28)
Pregnancy rate per started cycle (%)	37.9%(17 to 59)
Pregnancy rate per embryo transfer (%)	42.3(19 to 66)
Miscarriage rate (%)	9.1(−11 to 29)
OHSS (%)	7.7(−3.3 to 19)

OHSS = ovarian hyperstimulation syndrome.
Where appropriate, values are mean ± SD.
Values in parentheses are the 95% confidence interval.
[a]Median (range).

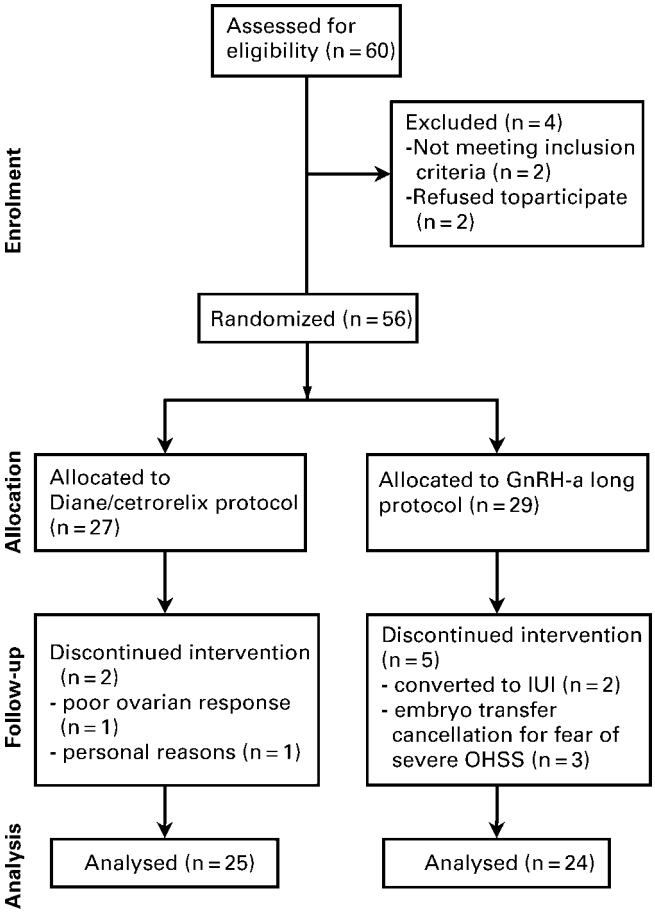

Figure 3. Flow diagram of the steps through the randomized controlled trial.

was no significant difference in LH levels between the day HMG was started and 6 days after HMG administration in the GnRH agonist long protocol. However, a statistically significant difference was found between 6 days after HMG administration and the day of HCG injection in the GnRH agonist long protocol group ($P < 0.05$). No premature LH surge occurred in either patient group. The mean duration of buserelin injection before the start of HMG stimulation was 18.5 ± 3.1 days in the GnRH agonist long protocol group.

Table IV summarizes the clinical characteristics of the two groups of patients. Total days of injections, ampoules of HMG used and serum E_2 on the day of HCG injection were significantly higher in the GnRH agonist long protocol group than in the Diane/cetrorelix group. The duration of HMG stimulation, number of oocytes retrieved, the number of oocytes fertilized, fertilization rate, clinical pregnancy rate and implantation rate were similar between the two groups. The incidence of OHSS was similar, with no severe OHSS occurring in any of the patients who completed the treatment cycle. In the GnRH agonist long protocol group, the three patients who cancelled the embryo transfer did not develop severe OHSS. The numbers of miscarriages during the first trimester were one and two in the Diane/cetrorelix group and GnRH agonist long protocol group, respectively. At the time of writing, there was one singleton delivered at term, and six single and two sets of twin pregnancies ongoing smoothly in the Diane/cetrorelix group. In the GnRH agonist long protocol group, one singleton was born at term and one set of twins were born at 34 weeks of pregnancy. The other four single and two sets of ongoing twin pregnancies were progressing well.

Table III. Patients' characteristics and hormonal concentrations of the two groups of patients in part II of the study

	Diane/cetrorelix ($n = 27$)	GnRH agonist long protocol ($n = 29$)	*P*-value
Age (years)	31.4 ± 3.5	31.7 ± 3.7	NS
Duration of infertility (years)	4.4 ± 1.9	4.4 ± 1.6	NS
Body mass index (kg/m^2)	23.2 ± 2.8	23.4 ± 2.9	NS
LH (mIU/ml)			
Baseline	10.9 ± 4.2	11.3 ± 4.5	NS
The day HMG started	2.7 ± 1.4[a]	2.3 ± 1.2[c]	NS
6 days after HMG administration	2.7 ± 1.7[b]	1.9 ± 1.3[d]	0.05
The day of HCG injection[e]	2.1 ± 1.5	1.3 ± 0.7	<0.05
FSH (mIU/ml)			
Baseline	5.8 ± 1.2	5.4 ± 1.7	NS
E_2 (mIU/ml)			
Baseline	51.4 ± 15.2	49.5 ± 16.4	NS
The day HMG started	28.7 ± 9.9	28.4 ± 8.2	NS
Testosterone (ng/ml)			
Baseline	0.81 ± 0.35	0.77 ± 0.39	NS
The day HMG started	0.46 ± 0.16	0.46 ± 0.12	NS
6 days after HMG administration	0.55 ± 0.18	0.58 ± 0.17	NS
The day of HCG injection[e]	0.74 ± 0.20	0.73 ± 0.18	NS

Values are mean ± SD.
[a,c] Comparison of LH levels between the day HMG started and 6 days after HMG administration by paired *t*-test, NS in both[a,c].
[b,d] Comparison of LH levels between 6 days after HMG administration and the day of HCG injection by paired *t*-test, $P < 0.05$ in[d], but NS in[b].
[e] Data were only available in 25 patients in the Diane/cetrorelix group.

Table IV. Cycle characteristics of the two groups of patients completing the treatment in part II of the study

	Diane/cetrorelix	GnRH agonist long protocol	*P*-value
No. of started cycles	27	29	
No. of cancellations	2	5	
Duration of HMG stimulation (days)	9.9 ± 2.1	10.8 ± 2.2	NS
Total days of injection	11.9 ± 2.1	30.4 ± 3.9	<0.05
Amount of HMG (ampoules)	21.6 ± 6.3	25.9 ± 7.2	<0.05
E_2 on day of HCG injection (pg/ml)	2159 ± 690	2633 ± 775	<0.05
No. of oocytes retrieved	16.3 ± 6.4	17.6 ± 5.9	NS
No. of oocytes fertilized	11.3 ± 5.4	12.2 ± 4.2	NS
Fertilization rate (%)	68.7 (62 to 75)	69.8 (65 to 75)	NS
No. of embryos transferred[a]	3.0 (2 to 4)	3.0 (2 to 4)	NS
Implantation rate (%)	18 (7.7 to 28)	17.7 (7.6 to 28)	NS
Pregnancy rate (%) per started cycle	37 (18 to 57)	34.5 (16 to 53)	NS
Pregnancy rate (%) per embryo transfer	40 (19 to 61%)	41.7 (20 to 63)	NS
Miscarriages (%)	10 (−13 to 33)	20 (−16 to 50)	NS
OHSS (%)	8.0 (−3.6 to 20)	8.3 (−3.4 to 19)	NS

OHSS = ovarian hyperstimulation syndrome.
Where appropriate, values are mean ± SD.
Values in parentheses are the 95% confidence interval.
[a]Median (range).

Discussion

The incorporation of a GnRH agonist in the COS protocols to suppress elevated LH and androgen levels and prevent a premature LH surge appears to improve the pregnancy rate and reduce the miscarriage rate in PCOS patients undergoing IVF treatment (Smitz *et al.*, 1992; Balen *et al.*, 1993a; Homburg *et al.*, 1993a+b. To accomplish the suppressive effect on serum LH and androgen, we attempted to use Diane-35 and GnRH antagonist to treat the patients with PCOS who were undergoing IVF treatment. On the day of starting HMG stimulation, the serum LH, testosterone and E_2 concentrations were comparable between the two groups of patients, suggesting similar pituitary suppression. The mean duration of buserelin injection before the start of HMG stimulation was 18.5 ± 3.1 days in the GnRH agonist long protocol, in contrast to 1 day of cetrorelix injection in the Diane/cetrorelix protocol. The major drawback of the study protocol was long-term administration of Diane-35. Our results showed that the mean serum LH and testosterone concentrations decreased to normal levels after 3 months of Diane-35 pre-treatment, which is similar to previous studies (Golland and Elstein, 1993; Prelevic *et al.*, 1993). One injection of 0.25 mg of cetrorelix acetate before the start of ovarian stimulation by HMG further lowered the serum LH levels. The minimal duration of OCP pre-treatment required to correct the abnormal serum hormonal profile has not been well studied. Elkind-Hirsch *et al.* (2003) reported that concurrent administration of GnRH antagonist and gonadotrophin from the early follicular phase appears to be effective in PCOS women undergoing ovulation induction and intrauterine insemination. They found that serum LH and FSH levels decreased significantly 2.5–3 h after the second dose of ganirelix (Antagon; Organon Pharmaceutical Inc., West Orange, NJ) administration in 18 PCOS patients. The ganirelix was given on the morning of day 2 of menstruation after administration of 21 days of OCP containing 0.15 mg of desogestrol and 35 mg of ethinyl oestradiol (Desogen; Organon Pharmaceutical Inc.). Follitropin-β and ganirelix was started (morning ganirelix and evening follitropin-β) upon LH suppression and continued concurrently until the day of HCG injection. These patients then underwent intra-uterine insemination. The overall clinical pregnancy rate was 44.4%, with an ongoing pregnancy rate of 27.8% (Elkind-Hirsch *et al.*, 2003).

The fertilization, pregnancy and implantation rates of the two methods of ovarian stimulation were comparable. The serum LH levels during HMG stimulation were relatively constant in the Diane/cetrorelix group. There was a trend of progressive deeper serum LH suppression during the GnRH agonist long protocol. It could contribute to the higher amount of HMG used, the higher serum E_2 on the day of HCG injection and three embryo transfer cancellations for fear of severe OHSS in this group of patients. Both protocols used in this study worked well with regard to prevention of a premature LH surge.

For non-PCOS patients undergoing IVF treatment, many reports have mentioned the application of a GnRH antagonist either by multiple dose daily administration or by a single dose injection in the late follicular phase to prevent a premature LH surge successfully, with satisfactory clinical results (Albano *et al.*, 2000; Olivennes *et al.*, 2000; Hwang *et al.*, 2003). However, there are few reports regarding GnRH antagonist application during IVF cycles in PCOS patients. Craft *et al.* (1999) reported seven patients with polycystic ovary (seven IVF cycles) using cetrorelix acetate in combination with clomiphene citrate and FSH. Cetrorelix acetate was administered daily until the leading follicle reached 14 mm in size. Three pregnancies were achieved from six completed cycles, with one ectopic pregnancy, one miscarriage and one live birth. There was one report retrospectively comparing 13 cycles of IVF/ICSI in PCOS patients using the leuprolide long protocol and 18 cycles using the ganirelix protocol. Ganirelix was given when the leading follicle reached 13–14 mm. The pregnancy rate was comparable,

while the peak E_2 and total days of injections were significantly higher in the leuprolide long protocol (Abae *et al.*, 2002). The endocrine feature of the early follicular phase in PCOS patients treated by the traditional GnRH antagonist protocol is not well studied. It might be possible that high tonic LH hypersecretion is still present. Unsuppressed LH levels during the early follicular phase have been speculated to be related to a lower pregnancy rate consistently observed in the GnRH antagonist group during phase 3 comparative trials between agonist and antagonist (Kolibianakis *et al.*, 2003). Albano *et al.* (1997) reported one premature LH surge in seven patients treated with 0.1 mg of cetrorelix acetate in the late follicular phase. Therefore, we thought that 0.125 mg/day would be enough to maintain low serum LH levels in the early follicular phase, which was shown to be between 2 and 3 mIU/ml in this study. The advantage is the decrease in patients' cost. On day 7 of HMG administration, cetrorelix acetate was increased to 0.25 mg/day in order to prevent a premature LH surge.

CPA, the progestational agent in Diane-35, possesses both anti-gonadotrophic and anti-androgenic properties that produce an inhibition of ovarian androgen production and a competitive inhibition of androgen receptors on the skin (Golland and Elstein, 1993; Prelevic *et al.*, 1993). In addition to the skin, androgen receptors have been reported to be present in the human ovary and endometrium (Horie *et al.*, 1992; Apparao *et al.*, 2002). Apparao *et al.* (2002) have demonstrated an elevated endometrial androgen receptor expression in women with PCOS, which may reduce the endometrial receptivity. Favourable effects, in terms of three pregnancies in eight PCOS women, within 3 months after discontinuation of Diane-35 therapy have been reported (Prelevic *et al.*, 1989).

In summary, this study demonstrates that the incorporation of Diane-35 and cetrorelix acetate into the COS protocol for patients with PCOS undergoing IVF treatment could achieve a degree of pituitary suppression similar to that of the GnRH agonist long protocol at the start of HMG stimulation. The fertilization, pregnancy and implantation rate were similar to those of the GnRH agonist long protocol, with lower amounts of HMG used and lower serum E_2 levels on the day of HCG injection. The total days of injections are fewer, while long-term Diane-35 administration is the major disadvantage. Further study is needed to clarify the minimal duration of Diane-35 pre-treatment.

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