Clomiphene Citrate co-treatment with low dose urinary FSH versus urinary FSH for clomiphene resistant PCOS: randomized controlled trial

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Abstract

Purpose The aim of this study was to examine the effect of clomiphene citrate [CC] co-administration during the use of exogenous low-dose urinary FSH [uFSH] for induction of ovulation in CC-resistant infertile PCOS women.

Methods In a randomised controlled setting, 174 CC-resistant infertile PCOS women were randomized into two parallel groups; Group I received CC 100 mg/day for 5 days plus uFSH 37.5 IU/day while group II received only uFSH 37.5 IU/day. Subsequent increments of uFSH by 37.5 IU/day were made according to response. Primary outcome was ovulation rate. Secondary outcomes were clinical pregnancy rates, number of follicles, endometrial thickness, and gonadotropins consumption.

Results Our results have demonstrated that group I compared to group II had significantly higher ovulation rate per intention to treat [ITT] [72.4 % vs. 34.2 %, $p < 0.001$]. Clinical pregnancy

and live birth rates were comparable between the two groups. Group I consumed significantly lower total FSH dose and needed significantly shorter stimulation duration compared to group II. **Conclusion** CC co-administered during low dose HP uFSH versus uFSH for CC-resistant PCOS yields significantly higher ovulation rate and less consumption of FSH.

Keywords Clomiphene resistant PCOS · Chronic low dose FSH · Ovulation induction · PCOS · Randomized controlled trial

Introduction

Polycystic ovary syndrome [PCOS] is the most common endocrine disorder in women. The prevalence of PCOS may be between 6 % and 10 % based on the U.S. National Institute of Health criteria, and as high as 15 % when the broader Rotterdam criteria are applied [1]. Clomiphene Citrate (CC) remains the first line treatment for ovulation induction in infertile PCOS women. Second line treatment interventions include either exogenous gonadotropins or laparoscopic ovarian surgery [2]. Failure of ovulation with clomiphene is termed “clomiphene resistance”, whereas failure of pregnancy despite ovulation with clomiphene may be considered as “clomiphene-failure” [3]. It had been estimated that between 20 and 25 % of PCOS women are resistant to CC [4]. Fixed dose, step up, step down and sequential use of CC and gonadotropins were the conventionally used gonadotropin regimens for induction of ovulation in PCOS women. In all these regimens, a high gonadotropin starting doses [150 IU/day] have been associated with increased risk of multiple pregnancies, ovarian hyperstimulation syndrome (OHSS) [5]. Subsequent efforts have resulted in the development of chronic low-dose protocol,

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Capsule Clomiphene citrate and uFSH versus uFSH alone in clomiphene-resistant PCOS patients.

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where a starting daily dose of 37.5 is incrementally increased. The chronic low dose protocol has essentially replaced the original conventional protocols [6, 7]. Monofollicular growth is the ultimate goal of the chronic low-dose step-up protocol. Unlike the conventional protocol, the low-dose protocol employs a minimum dose of gonadotropins that reaches, and not exceeding, the threshold for a single follicular growth thereby, produces monofollicular rather than multi-follicular ovulation. Having been shown to be effective in inducing monofollicular development, the low-dose protocols of FSH are, currently, the preferred second line of treatment in CC-resistant PCOS [3]. This practically eliminates the occurrence of OHSS and reduces multiple pregnancies to less than 6 % [7]. In a recent randomized trial, low dose exogenous FSH has been suggested as the first line of treatment in PCOS [8].

It has been shown, by nonrandomized studies, that gonadotropins dose can be reduced by using CC and HMG concomitantly [9] or aromatase inhibitors [AIs] and recombinant FSH sequentially [10]. Our hypothesis is that CC co-administered with low dose FSH to CC-resistant PCOS would improve ovarian response. To the best of our knowledge, there are no randomized trials evaluating the effect of CC co-administered during exogenous highly purified urinary follicular stimulating hormone [HP uFSH] treatment, using the low-dose step-up protocol, in clomiphene-resistant PCOS women. The aim of this randomized controlled trial was to study the effect of CC co-administered during a low-dose step-up protocol using HP uFSH, in CC-resistant PCOS. The primary endpoint of this trial was ovulation rate while clinical pregnancy rate and total gonadotropin dose were secondary outcome measures.

Materials and methods

This randomized controlled trial was carried out between January 2010 and September 2012. It included a total of 174 cases with CC-resistant PCOS. We included in the trial only women undergoing their first treatment cycle using this protocol. The diagnosis of PCOS was based on Rotterdam criteria [11]. We diagnosed CC-resistance if ovulation was absent at CC dose of 150 mg/day [5, 12]. Informed written consent was obtained from all patients. The protocol of this study was approved by our Institutional Review Board.

Inclusion criteria were: CC-resistant PCOS women, age between 18 and 38 years, fertile semen according to WHO standards [13] as regard sperm concentration, motility and Kruger's strict criteria for sperm morphology, patent fallopian tubes proved by hysterosalpingography and/or dye test with laparoscopy within the preceding 6 months, no history of previous genital surgery and no history of treatment by exogenous gonadotropin. Exclusion criteria were: presence of any infertility factors other than anovulatory PCOS, use of

medications known to alter insulin secretion or action either currently or within the previous 3 months, presence of other endocrinopathies.

Two hundred and thirty CC-resistant PCOS patients were approached in a single private infertility center. Out of these, 192 met the inclusion criteria and 18 refused to participate in the study. We ended up with 174 who were randomized. Randomization was done by computer generated random numbers and group allocation was done by simple random allocation rule. Couples were randomly allocated into either CC-HP uFSH (87 cases, group I) or HP uFSH (87 cases, group II). A professional statistician performed randomization and a nurse coordinator implemented patient enrollment and group assignment for the study. Neither the clinicians nor couples were blinded to patients' allocation. Concealment of patients' allocation was performed through sequentially numbered opaque and sealed envelopes. Patients were evaluated clinically for blood pressure, body mass index [BMI], abdominal circumference (AC), evidence of hyperandrogenism {acne or hirsutism [defined as Ferriman-Gallwey score >8]}; and transvaginal ultrasound (TVS) for ovarian pattern and volume. For each patient, fasting blood glucose and insulin were measured, to determine insulin resistance, fasting triglycerides, high density lipoprotein (HDL), free testosterone, and basal FSH and LH were assayed. On the day of human chorionic gonadotropin (hCG) injection, serum samples were collected for measurement of serum E2 and FSH.

Starting from the 3rd day of the cycle (spontaneous or progesterone withdrawal), group I received CC oral tablets (Clomid, 50 mg tablets Global Napy Pharmaceuticals 2nd Industrial zone 6th October City, Egypt) in 100 mg daily doses for 5 days plus intramuscular (IM) injection of 37.5 IU/day HP uFSH (Fostimone, IBSA CH 6903 Lugano, Switzerland) from the 3rd to the 13th cycle day. Group II was given highly purified (HP uFSH) only in the same daily doses and for the same duration. Cycle monitoring by TVS was started on the 11th of the cycle. At the first follow-up; if the follicle diameter was medium sized [12–15 mm] the starting dose was maintained. Should the growing follicle[s] was smaller; an increment of 37.5 IU was made. The lowest dose that achieved significant follicular increase was maintained until hCG ovulation trigger. Subsequent visits were scheduled according to ovarian response until the leading follicle mean diameter reached ≥ 18 mm. At this time ovulation was triggered by injection of hCG 10,000 IU [Choriomon, IBSA, Switzerland] and regular sexual relation advised.

Cycle cancellation due to non-response was done when no evidence of any follicle >12 mm mean diameter by the 28–35th day of stimulation. Ovulation was documented by TVS 7 days after ovulation triggering and confirmed by assessing mid-luteal progesterone. Values ≥ 5 ng/mL were considered adequate for confirmation of ovulation. The end points of the treatment cycle were either menstruation or clinical pregnancy,

number of large follicles (≥ 16 mm) and medium sized follicles (12–15 mm), and endometrial thickness at the day of hCG injection. Clinical pregnancy was defined by intrauterine gestational sac observed by an ultrasound scan 2 weeks after a positive pregnancy test in urine or blood. Primary outcome measure was ovulation rate. Secondary outcome measures were clinical pregnancy rate per woman randomized (CPR). Predictors of ovulation were studied by comparing ovulatory and anovulatory cases regarding all cycle features and calculating the area under curve using ROC curve.

Biochemical analysis and hormone assay

- 1- Determination of serum glucose and lipid profile levels: Glucose was measured according to the method of Trinder [14]; triglycerides according to the method of Wahlefeld et al. [15] and HDL was detected based on the method of Assmann et al. [16]. All kits were provided by Spinreact [SPAIN]. Low density lipoprotein [LDL] cholesterol was calculated using the formula of Friedewald et al. [17]. Lipid profile and glucose levels were measured using screen plus spectrophotometer.
- 2- Determination of serum insulin levels: Insulin was assayed by solid phase enzyme—linked immunosorbent assay ELISA technique by a commercially available kits from Immunospec (UK). The assay system utilizes one antibody for solid phase (micotiter wells) immobilization and another anti-insulin antibody-enzyme (horseradish peroxidase) conjugate solution. The sensitivity of this assay was found to be $2 \mu\text{U/mL}$. The coefficient of variation of this method is $<2\%$. Insulin resistance was calculated according to the formula of Homeostasis Model Assessment for Insulin Resistance (HOMA-IR): Fasting insulin concentration $\mu\text{U/mL} \times \text{fasting glucose (mg/dL)}/405$. Insulin resistance was considered present when the HOMA-IR value >2.5 [18].
- 3- Determination of serum free testosterone levels: Free testosterone levels were determined using ELISA technique by commercially available kits from (LUCIO-Medical, Germany) [19]. The sensitivity of this assay was found to be 0.06 pg/mL . The intra-assay and inter-assay variation of the assay was $<10\%$. Free testosterone level $\geq 6 \text{ pg/mL}$ was considered abnormally elevated and evidence of hyperandrogenaemia.

Metabolic syndrome was diagnosed according to the criteria of National Cholesterol Education Program-Adult Treatment Panel III (Modified NCEP-ATP III) [20].

Statistical analysis and sample size

IBM SPSS statistics 20 for Windows (SPSS, Chicago, IL, USA) and Microsoft Excel 2003 were used for statistical analysis. Non-normally distributed continuous variables were

log transformed before statistical analysis. Student's t-test, χ^2 -test, Fisher's exact test were used where appropriate. ROC was used for ovulation predictors. $P \leq 0.05$ was considered statistically significant. For sample size calculation, Epi Info 2000 version 1.1 2001 program [CDC Atlanta, GA, USA] was used. Assuming ovulation rate difference of 20 between the CC-uFSH and the FSH arms (a pilot study finding) for a power of 80 and alpha 0.05, 160 cases were required. A larger sample size (174) was recruited to compensate for dropouts.

Results

As shown in Table 1, the baseline features of the 2 arms of the trial (group I & group II) are comparable regarding the mean values for age, infertility duration, BMI, AC, ovarian volume, basal FSH, LH, LH/FSH ratios, free testosterone, fasting insulin, fasting glucose, HDL, triglycerides and HOMA-IR. Also the percentages of infertility types (primary vs. Secondary), menstrual patterns [amenorrhea, oligomenorrhea, or dysfunctional uterine bleeding (DUB)], hyperandrogenism, insulin resistance and metabolic syndrome were comparable between the two arms. Furthermore, the distribution of different anovulatory phenotypes of PCOS, according to Moran et al., [phenotypes: A = anovulation + hyperandrogenism + PCO, B = anovulation + hyperandrogenism, D = anovulation + PCO] were comparable between the two study groups [21]. The treatment outcome measures are shown in Table 2. Flow chart for the study recruitment process is summarized in Fig. 1.

The CC-uFSH group compared with uFSH group, as shown in Table 2, received significantly lower total FSH dose [IU] (532.5 ± 315 vs. 1057.5 ± 585) ($P < 0.001$), had shorter stimulation duration (days) (12.34 ± 4.5 vs. 18.42 ± 6.2) ($P < 0.001$), produced less number of medium sized follicles (1.1 ± 0.98 vs. 1.8 ± 2.03) ($p = 0.005$) but comparable large follicles (1.6 ± 1.03 vs. 1.5 ± 1.5) ($p = 0.62$) endometrial thickness (mm) (10.6 ± 2.5 vs. 11.1 ± 1.8) ($p = 0.15$), higher hCG-day E2 (pg/mL) (465.49 ± 377.95 vs. 296.88 ± 255.45) ($p = 0.02$), but lower hCG day serum FSH (mIU/mL) (4.79 ± 1.88 vs. 5.5 ± 1.65 , $p = 0.02$) higher ovulation rate (72.4 % vs. 34.2 %). The relative risk (RR) and the 95 % confidence interval [95 % CI] for the probability of ovulation using the CC and uFSH were (RR: 2.1 and 95 % CI, 1.5 to 2.9, $p < 0.0001$). However, clinical pregnancy rate (CPR), whether per woman randomized or per cycle started, was comparable (Table 2).

Predictors of ovulation

Comparing ovulatory and anovulatory cases in the whole trial (Table 3), we found that anovulatory cases had significantly higher proportions of DUB (26.9 %, vs. 11.4 %) and amenorrhea (11.2 vs. 3.2 %), lower proportion of oligomenorrhea (61.9 vs. 85.4 %, $p = 0.002$), higher BMI (34.8 ± 5.41 vs. 32.97

Table 1 Patients baseline clinical and endocrine characteristics

	All cases	Group I (CC + HPuFSH)	Group II (HPuFSH)	<i>p</i>
Number	174	87	87	
Age (year) ^a	24.8±4.4	24.8±4.7	24.7±4.3	0.88 ^c
Infertility Type ^b				0.11 ^d
Primary	112(64.2)	50(57.4)	61(70.2)	
Secondary	62 (35.8)	37 (42.5)	26 (29.8)	
Duration (year) ^a	3.25±3.67	3.24±3.5	3.26±3.85	0.97 ^c
Menstrual patterns ^b				0.88 ^c
Amenorrhea	6 (3.4)	3 (3.4)	3 (3.4)	
Oligomenorrhea	150 (86.2)	74 (85.1)	76 (87.5)	
DUB	18 (10.5)	10 (11.5)	8 (9.1)	
Hyperandrogenism ^b	119(68.3)	61(70.1)	58 (66.7)	0.74 ^d
BMI ^a	33.2±5.5	33.3±5.4	33.2±5.7	0.92 ^c
PCOS Phenotype				0.48 ^e
A	137(78.7)	70(80.4)	67(77.2)	
B	25(14.4)	13(14.9)	12(13.7)	
D	12(6.9)	4(4.7)	8(9.1)	
AC ^a	104.8±12.78	102.2±10.6	105.8±14.7	0.12 ^c
Ovarian volume (cm ³) ^a	14.01±5.46	13.76±5.90	14.27±5.0	0.53 ^c
FSH (mIU/mL) ^a	5.4±1.7	5.4±1.75	5.40±1.72	0.97 ^c
LH (mIU/mL) ^a	6.1±3.9	5.58±4.2	6.53±3.7	0.11 ^c
LH/FSH ratio ^a	1.1±0.66	1.02±0.57	1.2±0.72	0.07 ^c
Free Testosterone (pg/mL) ^a	1.93±3.0	1.6±2.6	2.2±3.2	0.17 ^c
Fasting insulin(uU/mL) ^a	13.7±11.7	11.5±12.2	14.4±12.6	0.12 ^c
Fasting glucose(mg/dL) ^a	100.35±18.8	99.9±15.6	100.6±21.4	0.80 ^c
HDL(mg/dL) ^a	39.7±15.5	40.7±16.7	38.8±14.6	0.42 ^c
Triglycerides (mg/dL) ^a	135.7±71.5	138.2±68.13	132.8±75.0	0.61 ^c
HOMA-IR ^a	3.2±3.1	2.8±2.6	3.4±3.4	0.19 ^c
IR present ^b	72(41.4)	31(35.6)	41(47.3)	0.16 ^d
MS present ^b	90(51.7)	46(53.8)	44(50.5)	0.87 ^d

^a Mean ± SD^b *n* (%)^c *T*-test^d Fisher's exact test^e χ^2 -test

±5.33, $p=0.033$), higher percentage of IR (61.9 vs. 31.25 %, $p=0.0002$) and lower proportion of protocol I (CC-Ufsh) (21 % vs. 55.7 %, $p<0.001$). All other features: Age, infertility type or duration, AC, ovarian volume, LH/FSH ratio, hyperandrogenism/hyperandrogenaemia, PCOS phenotypes, mean HOMA-IR, fasting blood glucose, fasting insulin, HDL and percentage of MS, were comparable. Using ROC curve showed that only protocol 1 significantly predicted ovulation {Area under curve (AUC)=0.644, 95 % CI=0.51–0.77, $p=0.05$ }, whereas BMI (AUC=0.616, 95 % CI = 0.40–0.82, $p=0.39$), menstrual pattern (AUC=0.55, 95 % CI=0.39–0.72, $p=0.45$) and ratio of insulin resistance (AUC=0.56, 95 % CI=39–73, $p=0.47$) did not predict ovulation.

Discussion

Our study has shown that CC co-treatment with uFSH has significantly reduced the duration and total dose of uFSH.

Furthermore, CC co-treatment with uFSH was associated with significantly lower number of medium sized follicles. On the other hand, ovulation rate was significantly higher in the CC-FSH group. In the meantime, number of large follicles (≥ 16 mm), endometrial thickness and CPR were not compromised in CC-uFSH group compared with uFSH group.

The discrepancy between ovulation and conception rates in CC-FSH arm (87 vs. 29) was greater than in the FSH arm (31.5 vs. 28.5). This discrepancy, between ovulation and pregnancy rates, may be explained by the peripheral anti-estrogenic effects of CC at the level of the endometrium and cervical mucus or by hypersecretion of LH [22]. Another possible explanation from this study is the higher number of follicles and a trend, though insignificant, to thicker endometrium in the FSH arm compared with CC-uFSH arm. Meanwhile, we observed a lower trend of insulin resistance in group I compared to group II, though not reaching a statistically significant level. This could have been expected to contribute to the higher ovulation rate in this group.

Table 2 Cycle features and outcome

	All cycles <i>n</i> =159	Group (I) Per Protocol <i>n</i> =82	Group (II) per protocol <i>n</i> =77	Difference between means (95 % CI) & RR (95 % CI)	<i>P</i>
Total uFSH Dose (IU) ^a	787.5±540	532.5±315	1057.5±585	−525.0±66.3 (395.1 to 654.9)	<i>P</i> <0.001 ^c
Stimulation Duration (Days) ^a	15.4±6.2	12.34±4.5	18.42±6.2	−6.08±0.85 (4.4 to 7.75)	<i>P</i> <0.001 ^c
Large Follicles(≥16 mm) ^a	1.5±1.3	1.6±1.03	1.5±1.5	0.10±0.20 (−0.49 to 0.29)	0.62 ^c
Medium-sized Follicles(12–15 mm) ^a	1.5±1.6	1.1±0.98	1.8±2.03	−0.72±0.25 (0.2 to 1.2)	0.005 ^c
hCG-day FSH (mIU/mL) ^a	5.2±1.72	4.79±1.88	5.5±1.65	−0.72±0.31 (0.11 to 1.32)	0.02 ^c
hCG Day-E2 (pg/mL) ^a	386±332.5	465.49±377.95	296.88±255.45	168.6±51.49 (−269.5 to −67.69)	0.0013 ^c
End. Thickness (mm) ^a	10.8±2.5	10.6±2.5	11.1±1.8	−0.50±0.34 (−0.18 to 1.18)	0.15 ^c
Ovulation Rate / per woman randomised ^b	96/174 (55.1)	63/87(72.4)	30/87(34.2)	2.1(1.5 to 2.9)	<i>P</i> <0.001
Ovulation Rate / per cycle ^b	96/159 (60.3)	72/82 (87.8)	24/77 (31.5)	2.8 (2.0 to 3.9)	<i>P</i> <0.001 ^d
CPR/ per woman randomised ^b	46/174 (26.4)	24/87(27.5)	22/87 (25.2)	1.1(0.66 to 1.79)	0.86 ^c
CPR / cycle started ^b	46/159 (28.9)	24/82(29.2)	22/77(28.5)	1.0 (.6289 to 1.669)	1.0 ^d
Live birth rate/per woman randomised	41/174(23.6)	22/87(25.2)	19/87(21.8)	1.15 (0.6766 to 1.981)	0.72
Live birth rate/per cycle started	41/159 (25.7)	22/82(26.8)	19/77(24.6)	1.08 (0.64 to 1.84)	0.85

^a Mean ± SD^b *n* (%)^c *T*-test^d Fisher's exact test

In our study, we noted that most cases were obese (mean BMI 33.2±5.5) with BMI >25 in 85 % of cases (this estimate is not shown in results and is made directly from raw data). Insulin resistance was present in 41.4 % and clinical hyperandrogenism (hirsutism/acne) was present in 68 % of cases. It is to be noted that over 90 % of the study cases belonged to PCOS phenotypes A & B (both include anovulation plus hyperandrogenism) whereas phenotype D (anovulation plus PCO) accounted for less than 10 % of cases. Clomiphene citrate resistance is known to be associated with obesity, insulin resistance and hyperandrogenism [23]. It has been shown that despite the achievement of satisfactory ovulation and pregnancy rates using chronic low dose FSH regimens, the outcome was compromised in obese and insulin resistant PCOS cases which need more gonadotropin [24].

Clomiphene citrate co-treatment with low dose uFSH was used in this study in one arm to evaluate its impact on FSH requirement and on cycle outcome compared with FSH only. In our trial, we employed 37.5 IU starting dose because Balasch et al. proved in a randomized trial on CC-resistant PCOS that starting doses of 37.5 IU and 50 IU have comparable follicular response [7]. In our study we observed a lower ovulation rate in group II compared to the reported ovulation rate in other studies. This could be explained by the difference in the starting FSH dose as we adopted a minimal FSH starting dose (37 IU) compared to the mentioned study by Yarli et al. [25]. There were no cases of OHSS and only one set of twins in our series. We did not encounter any previous randomized trial comparing

CC-co administered with FSH for CC-resistant PCOS vs. any other protocol.

The use of adjuvant treatments to augment or minimize the need of exogenous FSH in ovulation induction in women with PCOS had been tried by many investigators. Many studies tried metformin co-treatment with FSH or CC in CC-resistant PCOS. Yarali et al. [25] in a randomized placebo controlled trial analyzed the impact of metformin on ovarian response in women with normal glucose tolerance when co-administered during a low-dose step-up protocol using rFSH in CC-resistant PCOS. The study employed a starting dose of 75 IU/day and an incremental dose of 37.5 IU plus metformin 850 mg twice/day. It was found that metformin pretreatment significantly restored ovulation compared with placebo. However, metformin co-administered with rFSH did not significantly improve ovulation or pregnancy rates. Also the total gonadotropin dose and duration of treatment were not significantly affected by metformin co-treatment.

Another randomized placebo controlled double-blind cross-over study [10] of metformin /placebo, followed by 3 months metformin/ placebo together with clomiphene for three cycles in CC-resistant PCOS found no impact of metformin on clinical (spontaneous menstruation ,ovulation, pregnancy) or biochemical (related to androgens and insulin) parameters. The study concluded that metformin is not always beneficial when given to clomiphene resistant infertile women with PCOS in clinical practice.

Dickey et al. [9] retrospectively compared per cycle fecundity and birth rates in 119 cycles of sequential clomiphene-HMG,

Fig. 1 Trial recruitment flow chart

524 cycles of clomiphene alone, 57 cycles of HMG alone, and 79 cycles of concurrent HMG and CC in patients receiving intra-uterine insemination who were free of endometriosis or tubal disease. The average number of ampoules of HMG required was decreased by 65 % when concomitant use of CC with HMG was employed. The study concluded that concomitant CC-and HMG use reduces the requirement for HMG. Although different from our study in many aspects, Dickey et al. [9] study showed, in agreement with our study, that stimulating endogenous gonadotropin production with the concomitant use of lower doses of exogenous FSH and CC is a feasible therapeutic approach.

Although we noticed significantly higher number of medium sized follicles (12–15 mm) in the FSH arm, we did not encounter any instance of OHSS. We had 1 set of twins in the FSH arm (4.5 % of pregnant cases) but none in the CC-FSH

arm. In this respect our results agree with those reported by Homburg for CC-resistant PCOS treated by chronic low dose starting with 50 U/ day with small incremental doses of 25–37.5 IU/day [8]. Homburg reported multiple pregnancies in 5.7 %, and OHSS in 0.14 %. In a more recent study by Orviet and Homburg starting by 50 IU/day rFSH followed by an ultra-low incremental dose of 8.3 IU eliminated even mild OHSS and decreased multiple pregnancies to only one set of twins at a clinical cycle pregnancy rate of 30 % per started cycle [26]. These results give credence to our results showing good efficacy and excellent safety of CC-FSH combination using our starting and incremental doses of 37.5 IU/day HP uFSH. In another trial, Bedaiwy et al. showed that AIs co-treatment with rFSH reduced the total FSH consumption and cycle cost and was more cost-effective compared with rFSH used alone [10].

Table 3 Predictors of ovulation

	Ovulatory (<i>n</i> =96)	Anovulatory (<i>n</i> =63)	Difference between means & RR (95 % CI)	<i>p</i>
Age (year) ^a	24.9±4.8	24.5±2.7	0.40±0.66 (−1.7–0.9)	0.54 ^c
Menstrual pattern ^c				
Amenorrhea	3 (3.2)	7 (11.2)		
Oligomenorrhea	82 (85.4)	39 (61.9)		0.002 ^c
DUB	11 (11.4)	17 (26.9)		
Hyperandrogenism ^b	64(66.6)	40 (63.4)	1.15 (0.59 to 2.23)	0.73 ^d
BMI ^a	32.97±5.33	34.832±5.41	−1.86±0.86 (0.15 to 3.52)	0.033 ^c
AC (cm) ^a	104.7±12.7	105.22±13.75	−0.52±2.1(−3.2 to 4.6)	0.80 ^c
PCOS Phenotype				0.71 ^c
A	67(70.4)	42(66.6)		
B	15(15.6)	13(20.6)		
D	14(14.5)	8(12.8)		
Ov. volume (cm ³) ^a	14.3±5.7	12.807±5.4	1.49±0.91(−3.28 to 0.30)	0.105 ^c
LH/FSH ^a	1.110±0.7	1.260±0.43	−0.15±0.09 (−0.04 to 0.34)	0.13 ^c
Fasting insulin level (uU/mL) ^a	11.96±6.8	14.0±7.5	−2.03±1.14(−0.21 to 4.28)	0.09 ^c
Fasting blood glucose (mg/dL) ^a	101.23±18.23	96.07±22.05	5.16±3.0 (−11.19 to 0.86)	0.09 ^c
HDL (mg/dL) ^a	39.52±14.5	40.6±20.5	−1.10±2.74(−4.2 to 6.4)	0.68 ^c
Triglycerides (mg/dL) ^a	139.3±72.6	120.3±64.4	19.0±11.3(−41.1 to 3.1)	0.09 ^c
HOMA-IR ^a	3.5198±3.3	2.8108±1.58	0.70±0.44 (−1.5 to 0.16)	0.11 ^c
IR ^b	30 (31.25)	39 (61.9)	0.50 (0.35 to 0.71)	0.0002 ^d
MS ^b	49 (51.6)	34 (53.9)	0.94(0.70 to 1.27)	0.74 ^d
Protocol 1 ^b	53(55.7)	13(21)	2.67 (1.59 to 4.48)	<i>P</i> <0.001 ^d

^a Mean ± SD^b *n* (%)^c *T*-test^d Fisher's exact test^e χ^2 -test

In order to find the association between ovulation and specific clinical or laboratory features, we compared ovulatory and anovulatory cases in this study. The comparison, as shown in Table 3, showed that ovulatory, compared with non-ovulatory cases, had significantly higher percentage of oligomenorrhea (85.4 % vs. 61.9 %), lower percentage of amenorrhea, lower BMI, lower percentage of IR and significantly higher percentage of cases stimulated by CC-uFSH. Using the area under curve, only the type of protocol significantly predicted ovulation. Ovulation in response to stimulation by CC-uFSH or uFSH is not related to basal LH/FSH ratio, or to androgen level or the presence of metabolic syndrome.

The published studies report different predictors of cycle outcome in chronic low dose FSH stimulation in PCOS depending on the type of PCOS cases, CC-resistant or not, FSH used, uFSH or rFSH, and sample size and study design. Dale et al. compared insulin resistant and non insulin resistant CC-resistant PCOS women who were treated by low dose FSH. They found that insulin resistant PCOS women required more gonadotropins and a longer time to achieve follicular maturation response [27]. In our study less than one third of cases were IR among ovulatory compared with nearly two thirds of the cases among non-ovulatory. Our results agreed with those from Yarali et al. who found that pre-treatment serum

luteinizing hormone and testosterone levels predicted neither the rate nor the outcome of pregnancy [25]. The only factor that influenced outcome significantly was body mass index. In our study, anovulatory women had significantly higher mean BMI compared with ovulatory women. Wely et al. analysed clinical ultrasonographic and biochemical features of 272 treatment cycles in 85 CC-resistant cases who underwent ovarian stimulation utilising chronic low rFSH dose and found that responsive cases, in partial agreement with us, had more history of oligomenorrhea than amenorrhea, with shorter duration of infertility and lower free androgen index [28]. Andersen et al. in a univariate and multivariate logistic regression of prestimulation predictors of live birth in 335 first cycles of CC-resistant PCOS cases stimulated by chronic low FSH doses found that higher FSH [within the normal range] before the start of stimulation have negative influences on the likelihood of achieving a live birth which conflicts with our findings in this respect [29]. On the other hand, oligomenorrhea was more significantly associated with live birth compared with amenorrhea and DUB giving support to our findings in this respect. The same study reported that baseline concentrations of luteinizing hormone, androgens, glucose and insulin, as well as body mass index, were not predictors of live birth also in agreement with our data. It

should be mentioned that our study addressed predictors of ovulation as end point whereas the last study addressed live-birth. Meanwhile, women in our study were only included should they have failed to ovulate for three cycles using clomiphene citrate alone.

One of the limitations of this study is that it was open label but this limitation was compensated by adherence to strict inclusion and exclusion criteria and by fact that the measured cycle clinical data were objective with clear cut point thus minimizing bias resulting from non-blinding. Meanwhile, there was a higher rate of drop outs in group II compared to group I.

In our study, the mean age of participants was around 25 years old which may be significantly lower the mean demographically reported age in other studies. This could contribute to the higher rates of live births observed in our study and may be explained by different trends in societies for seeking fertility care.

Another theoretical limitation of the study is that it addressed ovulation rate rather than pregnancy rate or live birth rate as a primary outcome measure. We calculated the sample size needed to conduct a trial that assesses live birth per woman and we found that for a study postulating 15 % difference in live birth rate (assuming that urinary FSH would result in 25 % live birth rate) and 80 % power, with 5 % level of significance, we need to randomise more than 9,000 women. This is practically impossible for a single centre. It remains to be seen through another study if reducing the dose of CC to only 50 mg/day will minimize this discrepancy between ovulation and pregnancy rates in CC-FSH arm. We are currently undergoing an ongoing clinical trial comparing CC plus low dose uFSH versus AIs plus low dose uFSH in an attempt to decrease the gap between ovulation and pregnancy rates in treatment of CC-resistant PCOS.

We conclude that CC co-administered during exogenous low dose HP uFSH step-up protocol for CC resistant PCOS is comparable to low dose step-up HP uFSH in terms of endometrial thickness, number of large pre-ovulatory follicles and pregnancy rates. CC plus FSH has significantly higher ovulation rate and shorter stimulation duration when used for ovulation induction in women with CC-resistant PCOS. Based on our findings, CC/FSH may be an appropriate interval step in CC resistant PCOS prior to considering laparoscopic ovarian drilling in this group of patients. Clomiphene citrate co-administration with HP uFSH is an independent predictor of ovarian response regardless of the clinical or biochemical features of CC-resistant PCOS cases.

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Contributors

MG was the principal investigator, designed the trial, wrote the protocol, managed the trial, recruited participants, interpreted the data and results, and wrote the first draft of the paper. LA advised on the study design and data analysis and helped to draft the paper. AG helped in the design and performing the statistical analysis, interpreted the data and results, and helped to draft the paper. MH and MES performed the biochemical assays. MH, AH and IB and YM recruited patients and entered data.