Chronic Low-Dose Step-up Protocol in treating women with Unexplained Infertility: *(37.5 Units versus 75 Units of follitropin alpha as the Initial Dose)*

Ibrahim Polat[a], Gonca Yetkin Yildirim[a], Eser S Ozyurek[b], Vuslat Lale Bakir[c], Ali Ekiz[a], Deniz Acar[a], Merve Aldıkactioglu Talmac[a].

[(a)]: MD, Specialist, Istanbul Kanuni Sultan Suleyman Research and Training Hospital, Obgyn Department; Altınsehir Mah. Turgut Ozal Cad. No:1, Atakent, Kucukcekmece, Istanbul-Turkey

[(b)]: (Corresponding Author) MD, Specialist, Bagcilar Research and Training Hospital, Obgyn Department; Merkez Mahallesi Mimar Sinan Caddesi 6. Sokak, Bagcilar-Istanbul-Turkey
Email: eozyurek@yahoo.com;

[(c)]: Specialist, Haseki Research and Training Hospital, Obgyn Department
Millet Caddesi, Aksaray-Fatih Istanbul/Turkey
Email: vuslatlale@hotmail.com

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Abstract

Aims: To compare the treatment outcome of the 37.5 Units/day follitropin-alpha (Study Group) with 75 Units/day (Control Group) as the initial dose for chronic low-dose step-up ovulation induction for unexplained infertile, non-PCOS (polycystic ovarian syndrome) women.

Methodology: Retrospective study and comparison of the patient characteristics and treatment outcome of 2 patient groups of 100 patient-cycles (Study and Control groups: Low-dose step-up cycles with initial doses of 37.5 Units/day and 75 Units/day, respectively). 95 (Study group) and 98(Control group) ovulatory cycles were included in the final analysis.

Results: Cycle cancellations were less common in the Study Group (6.3% vs 15.3%; *P*=0.02); those in the control group being mostly due to excessive response. The conception rates were similar: 11.5% and 11.2% in the study and the control groups, respectively. Total and mean daily gonadotropin used were lower in the study group (*P*=0.02 and *P*=0.04). 1 mild OHSS (Ovarian hyperstimulation syndrome) was observed in each group. There were no multiple pregnancies in either group.

Conclusion: The initial daily dose of 37.5 Unit/day is more effective in achieving a unifollicular cycle while being as safe and effective as 75Units/day; requiring a lower amount of gonadotropin for the conventional treatment of unexplained infertility in non-PCOS women.

Keywords: *Unexplained infertility, Low-dose Step-up, ovulation induction, intrauterine insemination*

Abbreviations: OHSS, PCOS, FSH, IUI

Introduction:

The Chronic Low-dose Step-up protocol for ovulation induction in intrauterine insemination cycles was introduced for polycystic ovarian syndrome patients [1,2]. The goal for using this protocol is to provide three or less mature follicles in a cycle; preventing ovarian hyperstimulation, multiple gestations and cycle cancellations. The induction is started at a fixed initial dose and response to induction was checked at no shorter intervals than 7 days. [3]. When this interval is 5-7 days as it was initially introduced, it is called the 'conventional low-dose step-up protocol' which was not, as it turned out, as useful and safe as the Chronic Low-dose Step-up protocol. Studies analysing chronic low-dose step-up ovulation inductions have basically aimed to define any obtainable benefit by decreasing the starting dose [4,5,6], prolonging the initial dose adjustment interval before deciding for a step-up, lowering the incremental dose [7] and alternating the ovulation trigger medications. Recently, the Turkish Ministry of Health has announced a treatment regulation that 'in ovulation induction cycles, it is compulsory to abstain from administering ovulation triggering medications if mature follicle number is greater than 2.' Hence, in our practise, we lowered the starting dose to 37.5 Units/day of recombinant human FSH(follicle stimulating hormone)-follitropin alpha with 7 day dose adjustments and incremental doses of +37.5Units/day, if necessary to achieve a uni(bi-) follicular induction cycle with lower cancellations due to excessive response. Follitropin alpha is the human FSH molecule produced with recombinant technology; structurally identical to the natural FSH with only slight differences in the oligosaccharide component of the molecule [8]. In this study, we retrospectively compared the results of a starting dose of 37.5 Units/day recombinant human FSH (follitropin alpha) in the non-PCOS unexplained or subfertile male factor infertile patients with the routinely preferred (75 units/day) starting dose, which we had commonly used.

Patients and Methods:

We retrospectively analysed the clinical results of the chronic low-dose step-up ovulation induction protocol with an initial dosage of 37.5 Units follitropin alpha used in 100 cycles of the same number of patients (the Study Group) in comparison with a control group of 100 cycles with a 75 Units initial dose of the same number of women (the Control Group). The retrospective analysis of the patient data was approved by the hospital ethics committee and had been permitted for by the patients in the signed informed consent forms for ovulation induction with gonadotropins. The control group was formed by a random number generator *(www.random.org)* and age matching among similar unexplained infertile and subfertile male factor infertile couples treated with the chronic low-dose step-up ovulation induction protocol with a starting dose of 75 Units.

None of them were diagnosed as PCOS according to the Rotterdam Criteria [9]. A hysterosalpingography had been performed within the preceding 6 months of treatment in all of the patients. No patients with hyperprolactinemia, thyroid dysfunction, insulin resistance, diabetes mellitus, patients with BMI>30 or recurrent abortions were included in the analysis. No other infertility factors were defined in the 2 groups. 98 control and 95 study patients' ovulatory cycles were included in the analysis.

The cycles had been started having assured that blood estradiol levels were <50pg/ml; progesterone <0.5ng/ml; LH<5mIU/ml and no residual follicles >15mm were observed. Initial doses were 37.5 Units and 75 Units of follitropin alpha in the study and the control groups, respectively. The rest of the treatment was similar in the 2 groups. The initial daily doses were continued for 7 days. At the 7th day of treatment, follicular response was assesed sonographically and if confirmed (at least 1 follicle ≥10mm), the same doses were continued. If follicular respond was not confirmed, the daily doses were increased by 37.5 Units to be

continued and reassesed every 1-3 days in the following week. If 3 or more follicles were ≥10mm or if at the end of 20 days of induction (3 cycles of 7 days), no follicles were ≥10mm, the cycles were cancelled. Having obtained 1 or 2 follicles >16mm, rhCG 150μg sc. was administered to induce ovulation and intrauterine insemination performed 36 hours later following sperm preparation and with a soft Wallace catheter. 7 days following the ovulation trigger, blood progesterone was measured and a sonographic exam was performed. Progesterone blood levels higher than 3.5 ng/ml were considered indicative of ovulatory cycles [10]. 5 cycles in the study and 2 cycles in the control group were excluded because ovulations could not be confirmed. 15 days following the insemination, blood βhCG was measured to check for pregnancy. If βhCG measured was >10mIU/ml, a ***conception***; if fetal cardiac activity was present 2 weeks later, a ***pregnancy***; if the pregnancy was maintained until the 12th week of gestation, an ***ongoing pregnancy***; and if more than 1 intrauterine gestational sac was observed, a ***multiple gestation*** was defined.

The data analysis was made using the Microsoft Excel and the SPSS 17.0 packages. Student's t test was used for comparisons of parametric variables. Chi-square and Fisher's exact tests were used as required to compare distribution of categoriacl variables. *P* values less than 0.05 were considered to express significance. Parametric values were expressed as '*value±standard deviation*'.

Results:

A total of (98/100) control (75 Units of initial doses); and (95/100) study (37.5 Units of initial doses) ovulatory cycles were included in the analysis. Demographic characteristics were as in (**Table I)**. The patients had recieved previous infertility treatments and had not obtained a conception. Hysterosalpingography revealed minor abnormalities, including unilateral tubal blockage, uterus arcuatus or subseptus in 7 (7.5%) and 4 (4.1%) of the control and the study groups, respectively.

15 (15.3%) cases-cycles in the control and 6 (6.3%) cases-cycles in the study group were cancelled, the cancellation rate in the control group being significantly higher (P=0.02). In the control group, the dominant cause for cycle cancellation was excessive response 11 (73%), whereas in the study group, the cancellations were mostly due to the lack of response 4 (67%).

Durations of the induction cycles were similar: 10.4±2.8days and 10.6±3.3days for the control and study groups, respectively. The mean doses used in the 2 groups were significantly different: 87.5±11.9 Units/day and 46.1±19.9 Units/day for the control and the study groups, respectively (P=0.04).

There were 11 (11.2%) pregnancies in the control group and 11 (11.5%) in the study group, with no significant difference in the pregnancy rates (P=0.83). Interestingly, of the 11 conceptions in the control group and 11 in the study group; 6 (54.5%) and 9 (81.8%) were healthy ongoing gestations in the control and study groups, respectively; yet, this could not reach a significant difference (P=0.09). There was 1 (1%) mild ovarian hyperstimulation (OHSS) in the control group and 1 (1%) in the study group. There were no multiple gestations in either groups.

In summary. the ovulation induction performances were as in Table II. The unifollicular outcome rate was significantly higher in the study group (Study group:84 (88%) vs Control group:61 (62.2%); *P*=0.007); whereas, the uni(bi-) follicular rate was similar for the study and the control groups (Study group:89 (93.7%) vs Control group:86 (87.8%); *P*=0.09).

The effect of the need for a step-up was analysed. A step-up was required in 39 of the 193 cycles; the need for a step-up was 17 (17.3%) of the control group and 22 (23.1%) of the study

group, not representing a significant difference (*P*=0.45).

Discussion:

We have shown in this study that compared to the commonly used starting dose of 75Units/day, the low-dose step-up ovulation induction protocol with an initial dose of 37.5 Units/day used for non-PCOS unexplained infertile patients, (with a dose adjustment interval of 7 days and dose increments of +37.5Units/day) *provides a higher* ***unifollicular growth rate****, similar* ***ovulatory cycle rates****,* ***pregnancy rates****, while providing lower* ***cancellation rates due to hyperresponse****, lower* ***gonadotropin use*** *and lower* ***threshold doses for follicular growth****. The 37.5 Units/day presented similar rates of* ***OHSS****,* ***multiple pregnancy****, and* ***need for step-up*** *at dose adjustment.*

The chronic low-dose step-up protocol is a protocol introduced for its usefulness in PCOS patients, to reduce the complication rates resulting from multiple follicle development [1,2]. The basis of this modality in fact is the FSH threshold theory. FSH is known to be igniting and maintaining follicular growth at a dosage, slightly (+10-30%) above a level which produces no effect [11].

The main goal is to obtain a mono- or bifollicular cycle so as to prevent many complications including the ovarian hyperstimulation syndrome, cycle cancellations, multiple gestations while maintaining favorable pregnancy rates and pregnancy outcome.

To pursue this goal in low-dose step-up ovulation inductions, 4 points had been focused in studies: decreasing the starting dose, prolonging the duration of the starting dose to get the initial respond, reducing the incremental dose, and individualizing the type and dosage of the medication administered for ovulation triggering [3-7,8].

Brown et al. who first suggested the low-dose step-up concept, defined the initial dose as 75 units and recommended increasing the dosage at 5 day intervals and at +10-30%, each time [11]. This pioneer study reported an OHSS rate of 3% and multiple gestations of 26%. Supraphysiological concentrations of gonadotropins at the initial phase, inadvertently rescues those follicles which would be destined to go through atresia. This rescued growing follicle cohort is eventually the cause for ovarian hyperstimulation, cycle cancellations with a good enough conception rate [12].

Lower initial doses have been reported to increase the uni(bi-)follicular rates but not the pregnancy rates in PCOS patients by White et al. who attempted to lower it to 52.5 Units/day as the initial dose; and Alsina et al. who in the IO-50 study tried the 50Units/day as the initial dose [4,5]. Balasch et al. attempted to lower the initial dose down to 37.5 Units/day compared to 50 Units/day for PCOS patients [6]. Similar to our findings, this study reported that the pregnancy rates were not negatively affected; however, the induction times were longer and the thresholds were lower. Bruna-Catalan et al. reported a multicenter (30 centers) case series comprised of 217 (68%) PCOS patients (316 cycles) of 37.5 Units/day starting doses without a control group: at similar rates of 4.4% for *cycle cancellation*; 61.1% for *unifollicular development*; *the pregnancy rate* was 24.7%, probably due to the major cause of infertility in this study group being anovulation [13]. In a unique phase II dose-response study reported

by Taketani et al. comparing the 37.5Units/day, 75 Units/day and 150Units/day as the initial doses, the primary outcome measures of the study included *the unifollicular development rate* which were similar for the 37.5Units/day and 75 Units/day and higher than the 150Units/day (64.9%, 50.8% and 7.3% for the 3 groups, respectively); *the cycle cancellation rates* were similar for the 37.5Units/day and 75 Units/day (0% and 3.3%) and lower than that for the higher starting dose (38.2%). The biochemical pregnancy rates were 15.8%, 18% and 9.1% for the three groups, respectively. The authors concluded that the 75 Units/day starting dose was safe and effective for the WHO II or, the almost equivalent Anovulatory Infertility group of patients according to the the Japanese classification. The authors did not report the proportion of the typical PCOS cases in their study groups [14].

In our study, we questioned whether lowering the initial dose further down to 37.5 Units could provide us with the benefits of low-dose step-up while maintaining acceptable response and pregnancy rates in non-PCOS women.

We found that the unifollicular cycles were significantly more common in the study (37.5Units) group (86.4% vs 64.3%). This contrast did not translate into differences in the pregnancy (11.2% vs 11.5%), multiple pregnancy (null in both groups) or mild OHSS rates (1% vs 1%) among the 2 groups, but lower cancellation rates (6% vs 15%); most of which were due to hyporesponse in the 37.5 Units/day group, instead of the more common cause of hyperresponse in the 75 Units/day group.

The main cause in WHO II (PCOS) patients predicating the Chronic Low-dose Step-up protocol is not necessarily the high FSH responsiveness of the follicles, but the excessive number of FSH responsive follicles [15]. This, however may not be the actual the case for women with non-PCOS women with unexplained infertility. In these patients, follicles are normally responsive to FSH, and the FSH responsive follicles are relatively lower in number. The need for a step-up at dose adjustment with a starting dose of 50Units/day in PCOS

patients is reported to be up to 50% [16]. Our step-up rate was 23.1% in the study group, which must be due to a closer to normal initial sensitivity of non-PCOS ovaries to gonadotropins.

When compared to the outcome of previous low-dose step-up studies, our fecundity rates were lower 11.5%, because our couples had various unfavorable baseline prognostic characteristics, especially having previously gone through unconcieved intrauterine insemination cycles [17]. In contrast to previous reported studies, severe OHSS was not observed in our study group.

The net effects of still ***lower unifollicular development rates***, ***the similar cancellation rates*** (mostly due to hyporesponse), ***similar pregnancy rates***, and ***similar ovulation rate of 98% with 37.5 Units/day*** are mostly due to the exclusion of the PCOS cases from our study and control groups, in contrast to the previous studies on 37.5 Units/day conducted with patient groups including PCOS cases.

Whether the first line treatment approach to unexplained infertility/male subfertility should be expectant management or ovulation induction with (clomiphene citrate / gonadotropin)±IUI (intrauterine insemination) is a common subject of discussion. It is evident that expectant management for a certain length of time, is at least as effective as empirical clomiphene citrate or unstimulated intrauterine inseminations [18]. After a certain interval, ovulation induction with gonadotropins and intrauterine insemination has a better outcome and proves also to be more cost-effective than ovulation induction with clomiphene citrate, when continued for up to 3 cycles [19]. In these cases, ovulation induction with a standard initial dose of 50-150 Units/day of gonadotropins is the standard protocol. Our approach to non-PCOS unexplained infertile cases may render the primary gonadotropin induction and IUI more cost effective and safer as a first line treatment option, with limited (less than 2) number of follicles at the time of trigger, hence with lower cancellation rates in unexplained infertility

patients. It may be interesting to see if the improved pregnancy outcome impression in our findings would be observed in cumulative pregnancy rates of patients in larger series.

Conclusions:

The initial daily dose of 37.5 Unit/day in chronic low dose step-up cycles is more effective in achieving a unifollicular cycle while being as safe and effective as 75Units/day; requiring a lower amount of gonadotropin for the conventional treatment of unexplained infertility in non-PCOS women.

'Authors have declared that no competing interests exit.'

Authors' Contribution Section:

The 1st and 2nd authors have designed the study and contributed to the data collection procedure. The 3rd and 4th authors have prepared the manuscript and performed the data analysis. The 5th, 6th and the 7th authors have as well contributed to the data collection procedure and done the final proof reading of the manuscript.

Consent Section:

All authors declare that a written informed consent was obtained from each patient for publication of clinical data. A copy of the written consent form may be provided for during the review process by the editorial office of this journal.

Ethical Approval:

All authors hereby declare that all experiments have been examined and approved by the apppropriate ethics committee and therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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Table I: Demographic characteristics.

Demographic Characteristics:

	Groups	Mean±Std.Deviation
Age*	Control group	30.1±4.8
	Study group	30±3.8
Time of infertility (Months)*	Control group	42.4±27.3
	Study group	50.5±33.7
BMI (kg/m^2)*	Control group	24.8±3.7
	Study group	24.5±3.8
Day 3 FSH (mIU/ml)*	Control group	6.4±2.3
	Study group	7.1±1.8
Day 3 Estradiol (pg/ml)*	Control group	56.3±32.8
	Study group	52.6±18.8
Day 3 antral follicle count*	Control group	9.4±4.8
	Study group	10.4±5.4
Infertility: Primary n (%); Secondary n (%)*	Control group	80 (83);18 (17)
	Study group	76 (80);19 (20)

*: No statistically significant difference observed

Table II: Results of induction:

Results of Treatment:		
Total gonadotropin dosage used (Units)*	Control group	677.1±223.6
	Study group	365.3±206.7
Number of follicles >16mm at the day of trigger[β]	Control group	1.5±0.9
	Study group	1.2±0.7
Endometrial thickness at the day of insemination (mm) [β]	Control group	9.6±2.3
	Study group	8.9±2.3
Induction time (Days) [β]	Control group	8.5±5.3
	Study group	9.6±4.7
Conception (+)[β]	Control group %	11.2
	Study group %	11.5
Ongoing pregnancy (ongoing pregnancy/cycles) [β]	Control group %	6.4
	Study group %	9.2

* Showing a significant difference (P=0.02)
[β]: No significant difference among the 2 groups.