# Short versus long gonadotropin-releasing hormone analogue suppression protocols for superovulation in patients ≥40 years old undergoing intracytoplasmic sperm injection

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**Objective:** To determine whether the short or long protocol for controlled ovarian hyperstimulation works better in older patients undergoing IVF.
**Design:** Controlled, randomized study.
**Setting:** A single private IVF center.
**Patient(s):** Two hundred twenty infertile women aged ≥40 years undergoing IVF.
**Intervention(s):** At their first IVF cycle, the women were randomized into two study groups according to a computer-generated number sequence: 110 patients were treated with a long protocol, and the other 110 were treated with a short protocol for controlled ovarian hyperstimulation.
**Main Outcome Measure(s):** Days of stimulation, $E_2$ level at the day of hCG administration, amount of FSH administered, number of oocytes collected, number of embryos obtained, pregnancy rate, implantation rate.
**Result(s):** Patients treated with a long protocol showed a significantly higher number of oocytes retrieved, a higher number of embryos obtained, and a higher pregnancy rate, both for cycle and transfer, compared with the short-protocol patients. The other parameters evaluated did not show any statistically significant differences.
**Conclusion(s):** Our study showed that the long protocol performed better than the short protocol in older women. Our findings demonstrated that flare-up in older women might be detrimental. (Fertil Steril® 2005;84:644–8. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** Short protocol, long protocol, GnRH analogue, IVF outcome, controlled ovarian hyperstimulation

The use of gonadotropins with GnRH agonist (GnRH-a), first reported for controlled ovarian hyperstimulation (COH) in IVF by Porter et al. (1) and Smitz et al. (2), has gained widespread popularity, and many IVF programs currently use this approach as the predominant, if not the only, method of ovarian stimulation (3, 4).

Pituitary suppression with GnRH-a before and/or during ovarian stimulation with hMG or purified FSH (pFSH) resulted in improved clinical pregnancy rates (5). Follicular recruitment is enhanced, and premature LH surges and follicular luteinization are avoided (6). Without GnRH analogue desensitization, approximately 20% of patients experienced premature LH surge during COH, and daily administration of 15 μg triptorelin is sufficient to prevent the LH surge (7).

There are two main ways to administer the GnRH-a treatment: the short protocol, which starts with menstruation and continues until hCG administration (8, 9), and the long protocol, which commonly starts in the middle of luteal phase of the previous cycle and continues until hCG administration (5, 10).

The short protocol allows the use of the flare-up, the acute secretion of gonadotropins by hypophysis when stimulated by GnRH-a with high recruitment of follicles; but higher levels of serum LH during pharmacologic stimulation with respect to long protocol–treated women have been reported. Both the flare-up effect and higher LH serum levels might be observed independently from the dosage and type of GnRH-a used, as several investigators have reported (11–14).

In patients aged ≥40 years, there is a reduced ovarian reserve, with a lower number of oocytes remaining in the ovary. In these patients, as evidenced also from the IVF registry data, the expectancy of pregnancy per cycle started is markedly lower than in younger women (15). Furthermore, the frequency of poor responder women, estimated to be 10% in the general population, is significantly higher in patients aged ≥40 years (16, 17).

In the past few years, several investigators have published data on poor responder patients, showing that COH protocols based on flare-up gave better chances of pregnancy in these patients, with either short (18–20), micro-flare (21), or low-

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flare (22) protocols. Furthermore, Tarlatzis et al. (23), in a recent review of the literature, concluded that "flare-up GnRH agonist protocols in all varieties produce better results than standard long luteal protocols."

In this study, we compared long and short protocols in the IVF population aged ≥40 years, to establish whether there was a difference between the two protocols for several parameters in women with reduced ovarian reserve.

## MATERIALS AND METHODS

### Patient Selection

All patients aged ≥40 years referred to the IVF program of the European Hospital, Rome, Italy, to undergo their first IVF cycle from January 1999 to July 2001 were eligible for the study. The trial was designed according to CONSORT guidelines.

The study was reviewed and approved by the European Hospital's Institutional Review Board. Of the 251 eligible patients undergoing IVF during the study period, 220 agreed to participate. The patients were randomized by means of a computer-generated randomization number sequence at the time that their cycle was scheduled. All patients undergoing IVF and participating in the study gave their informed consent. All patients underwent a standard infertility evaluation, and none of the patients eligible for the study showed FSH levels >10 IU/mL and $E_2$ levels >60 ng/mL on cycle day 3. All patients were nulliparous.

Patients were randomly allocated into two study groups: the short protocol group (group A), in which ovarian stimulation comprised GnRH analogue and pFSH alone starting from cycle day 1; and the long protocol group (group B), in which GnRH-a was given as a pretreatment and pFSH administration took place when pituitary desensitization was established. The patients allocated to group A, treated with buserelin (0.4 mg SC daily) (Suprefact; Hoechst, Milan, Italy) and pFSH (300 IU daily) (Metrodin HP; Serono, Rome, Italy), underwent ovarian ultrasonography between cycle days 1 and 3 to exclude any ovarian cysts of >15 mm diameter. Patients in group B were administered buserelin (0.4 mg SC) daily on days 22–24 of their previous cycle. Ovarian suppression was assessed by daily hormonal profiles of $E_2$ and ultrasound scan of the ovaries every day. Suppression was confirmed when $E_2$ reached the level of <30 pg/mL and when no follicles with a dimension ≥12 mm mean diameter were visible on ultrasound examination. When suppression was confirmed by $E_2$ and ultrasound examinations, pFSH administration was commenced (300 IU) on the second day of the menstrual cycle in the long protocol.

From day 7 of stimulation in both groups, daily monitoring of follicle size by ultrasound was performed, and plasma levels of $E_2$ were measured. From this stage, the dose of pFSH was adjusted, depending on the individual response of each patient. The criteria used for triggering ovulation with hCG (10,000 IU IM) (Profasi HP 5000; Serono, Rome, Italy) were plasma $E_2$ level of 1,000–4,500 pg/mL and at least four follicles of >16 mm diameter. The cycle was canceled in cases of poor ovarian response observed from the beginning of monitoring in four patients in group A and four in group B.

Oocyte retrieval was performed under ultrasound control by the transvaginal route on day 0, 36 hours after the injection of hCG. All patients underwent intracytoplasmic sperm injection (ICSI), according to published procedures (24), to maximize chances of fertilization, especially considering the age of the women, and to avoid confounding factors due to different procedures of oocyte fertilization. Patients were aware of ICSI risks, and they agreed to undergo the procedure. Oocytes were observed 18 hours after ICSI for their pronuclei and 44 hours after insemination for embryo development.

The embryos obtained were categorized on day 3 into three categories, depending on their morphological appearance. Grade A had equal and regular blastomeres without the presence of cytoplasm fragments; grade B had unequal blastomeres with or without cytoplasmic fragments; grade C were embryos fragmented more than 50%, which were not transferred (25).

Embryos were transferred approximately 72 hours after insemination with an ET catheter (Wallace's catheter, Smith Medical Instrumentation, Ltd., Hythe, Kent, UK). All transfer procedures were performed by the same physician to avoid interoperator variability. All pregnancies were confirmed by a rising titer of serum $\beta$-hCG from 12 days after ET and ultrasound demonstration of the gestational sac 4 weeks after the transfer. Biochemical pregnancies alone have not been included.

The same luteal-phase support was used in both groups: daily P (50 mg IM) (Prontogest; AMSA, Rome, Italy) from the day of replacement.

### Statistical Analysis

All statistical analyses were performed with a commercial software package (SPSS, Chicago, IL). Clinical pregnancy rate per cycle started and per transfer were the primary outcomes; the secondary outcomes considered were days of stimulation, $E_2$ level at the day of hCG administration, amount of FSH administered, number of oocytes collected, number of embryos obtained, number of embryos transferred, and implantation rate. The Mann-Whitney *U* test and Student *t*-test were used for continuous variables (days of stimulation, $E_2$ level at the day of hCG administration, amount of FSH administered, number of oocytes collected, number of embryos obtained, number of embryos transferred); $\chi^2$ and Fisher exact tests were used for dichotomous variables (clinical pregnancy rate per cycle started and per transfer, implantation rate, abortion rate).

## RESULTS

Demographic characteristics of the patients are reported in Table 1. There were no statistically significant differences

**TABLE 1**

**Clinical characteristics of the cases included in the study.**

| Variable | Short protocol | Long protocol | *P* |
|---|---|---|---|
| No. of cycles | 110 | 110 | |
| Maternal age (y) | 41.6 ± 1.4 | 42.4 ± 1.5 | NS |
| Paternal age (y) | 44.1 ± 2.4 | 44.9 ± 2.0 | NS |
| Duration of infertility (y) | 3.5 ± 1.4 | 3.6 ± 1.5 | NS |
| Basal FSH levels (IU/L) | 6.5 ± 2.5 | 6.9 ± 2.4 | NS |
| Cause of infertility | | | |
| Tubal factor | 19 (23.1) | 13 (22.6) | NS |
| Male factor | 46 (41.0) | 49 (29.0) | NS |
| Endometriosis | 6 (12.8) | 4 (12.9) | NS |
| Endocrinological | 4 (10.2) | 5 (16.1) | NS |
| Unexplained | 35 (12.8) | 36 (19.3) | NS |

*Note:* Data are reported as mean ± SD or n (%). NS = nonsignificant.

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between the two groups for any of the parameters. Two women included in the short protocol group and 1 woman included in the long protocol group dropped out of the study. The study was conducted on 108 women for the short protocol group and 109 patients for the long protocol group. The cycle was canceled or did not arrive to transfer in 4 patients in each group.

As shown in Table 2, women aged ≥40 years treated with the long protocol had a significantly higher number of oocytes harvested ($P<.001$), higher number of embryos obtained ($P<.001$), higher implantation rate ($P<.05$), and higher pregnancy rate, both for cycle started and for transfer ($P<.01$), than patients treated with the short protocol, whereas the other parameters studied (number of days of stimulation, amount of FSH ampules used, $E_2$ levels at day of hCG administration, and number of embryos transferred) did not show statistically significant differences. The power of the study for pregnancy rate was >80% for $P<.05$ (1-$\beta$). All the statistical results were corrected for the number of comparisons performed.

## DISCUSSION

The use of short or long protocols in COH for IVF has been the subject of study and comparison for many years. A relevant number of articles with inconclusive data have been published. A meta-analysis of the data (26) has reported that the long protocol works better than the short protocol in terms of clinical pregnancy rates for cycles started, for oocyte retrieval, and for ET, even though most of the articles analyzed did not show any statistically significant differences between the two protocols. Moreover, the amount of gonadotropins used was lower in the short protocol, but the

**TABLE 2**

**Results of the trial between long and short protocols in women aged ≥40 years.**

| Variables | Short protocol | Long protocol | *P* |
|---|---|---|---|
| No. of cycles | 108 | 109 | |
| Days of stimulation | 12.5 ± 2.4 | 13.9 ± 1.7 | NS |
| Ampules of FSH | 51.3 ± 22.3 | 52.6 ± 21.9 | NS |
| $E_2$ levels at hCG day (pg/mL) | 1,952 ± 1,253 | 2,175 ± 763 | NS |
| No. of oocytes | 4.5 ± 3.1 | 8.4 ± 5.8 | .001 |
| No. of embryos obtained | 2.9 ± 1.8 | 4.9 ± 2.7 | .001 |
| No. of embryos transferred | 2.8 ± 1.8 | 3.1 ± 1.6 | NS |
| Implantation rate | 12/291 (4.1) | 26/326 (8.0) | .05 |
| Pregnancy rate per cycle | 12/110 (10.9) | 25/110 (22.7) | .01 |
| Pregnancy rate per transfer | 12/104 (11.5) | 25/105 (23.8) | .01 |

*Note:* Data expressed as mean ± SD or n (%). NS = nonsignificant.

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long protocol allowed a higher number of oocytes to be retrieved.

The studies considered for the meta-analysis included a heterogeneous group of patients who were not stratified for patient age or pathologies. There are no data in the literature regarding the response to short or long protocols according to the age of the women, though it is generally accepted that the short protocol works better in older patients or in poor responder women, as also reported in a recent review (23).

In our data, we compared patients aged ≥40 years, who are generally considered potentially poor responders. In these women, the long protocol worked better than the short protocol in terms of the pregnancy rate, implantation rate, number of oocytes retrieved, and number of embryos obtained. Several investigators have reported that in poor responder women, mostly aged >39 years, COH with "flare-up" might provide some advantages (18–22). Several types of short protocols have been proposed in the literature, in which the dosage, the type, and the way to administer the GnRH-a is modified; however, there is no evidence that the different protocols work in a different manner. The "flare-up" is the common mechanism of action of the different short protocols and the reason that they are chosen, to elicit at the beginning of the cycle the recruitment of follicles by the high levels of secreted FSH and LH (11–22).

Our results showed instead that the long protocol in women aged ≥40 years promotes a better follicle recruitment, with more oocytes harvested and a significantly higher pregnancy rate than the short protocol.

It has been shown that older women (>39 years old) have a shorter follicular phase, probably due to an earlier start of follicular growth during the previous luteal phase, defined as "advanced growth," than in younger patients (27–29). Therefore, in older patients it has been shown that the available cohort of antral follicles starting growth in each cycle is significantly smaller than in younger women (28).

Furthermore, it has been demonstrated that older women show lower levels of inihibin B in the early follicular phase than younger patients. This hormone, which selectively inhibits FSH secretion, is produced by developing early antral follicles, and its levels are correlated with cohort size of antral follicles (30). Lower levels of inhibin B might explain the elevated levels of FSH in the early follicular phase of older women and thus the advanced recruitment and selection of a dominant follicle in these women.

These data might explain why in older women ovarian stimulation with the short protocol gives worse results: the flare-up in the early follicular phase triggered by GnRH analogue arrives too late in older patients, because the cohort of growing follicles has already been recruited and selected, resulting in a lower number of developing follicles. Instead, the long protocol, starting in the mid-luteal phase, might allow an increase in size of the follicle cohort recruited for the cycle, and an increased length of stimulation might allow additional growing follicles to enter in the cohort of stimulated follicles.

It has been shown that higher doses of GnRH analogue used for desensitization in the long protocol gave better results in terms of number of oocytes and embryos in a normal responder (7). This might be due to the extended time when FSH is above threshold: a longer time of FSH stimulation might allow more follicles to enter the FSH-dependent growth stage and be recruited. This might be the case in our study for older patients treated with the long protocol.

Our data suggest that in older patients the short protocol might be detrimental, even though several articles have suggested new protocols to treat poor responders and older women based on flare-up (18–22). More studies are needed to confirm our findings, to evaluate the effectiveness of COH protocols in older women.

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