# Cetrorelix protocol versus gonadotropin-releasing hormone analog suppression long protocol for superovulation in intracytoplasmic sperm injection patients older than 40

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**Objective:** To determine which protocols work better between cetrorelix and long protocols in older patients in a randomized controlled study.

**Design:** A controlled randomized study in a single private IVF center.

**Setting:** Infertile women referred to a private IVF center.

**Patient(s):** Five hundred sixty-four women 40 years or older undergoing IVF.

**Intervention(s):** At their first IVF cycle, the women were randomized into two study groups using a computer-generated number sequence: 281 cases were treated with the cetrorelix protocol, and 283 patients were treated with a long protocol for controlled ovarian hyperstimulation.

**Main Outcome Measure(s):** Days of stimulation, $E_2$ on the day of hCG administration, amount of FSH administered, number of oocytes yielded, number of embryos obtained, pregnancy rate, and implantation rate.

**Result(s):** Patients treated with the long protocol showed a significantly higher number of oocytes retrieved and a higher pregnancy rate for both the cycle and transfer with respect to the cetrorelix protocol patients. The other parameter evaluated did not show any statistically significant differences.

**Conclusion(s):** Our study showed that the long protocol performed better in older women than the cetrorelix protocol and that the GnRH antagonist may be detrimental in older women. (Fertil Steril® 2009;91:1842–7. ©2009 by American Society for Reproductive Medicine.)

**Key Words:** GnRH antagonist, cetrorelix, long protocol, GnRH analog, IVF outcome, controlled ovarian hyperstimulation

The introduction of pituitary suppression with GnRH analog during ovarian stimulation with hMG or FSH resulted in improved clinical pregnancy rates (1–5). Follicular recruitment is enhanced, and premature LH surges and follicular luteinization are avoided (6). It has been shown that without GnRH analog desensitization, about 20% of patients experienced premature LH surge during controlled ovarian hyperstimulation (COH) and that daily administration of 15 $\mu$g triptorelin is sufficient to prevent the LH surge (7).

The antagonists of GnRH, cetrorelix and ganerelix, have recently been introduced on the market for COH in IVF cycles (8–10). The GnRH antagonists have been proven to be effective and reliable in preventing the LH surge in cycles stimulated with gonadotropins for IVF; these substances do not show the so-called flare-up phenomenon. Instead, they induce the inhibition of pituitary secretion of FSH and LH immediately after injection (11).

Various stimulation protocols that incorporate the use of cetrorelix have been suggested. The multiple-dose regimen requires daily injections of the antagonist starting on day 5 or 6 of the stimulation period until the administration of hCG (12, 13): the dose of 0.25 mg per day, starting from day 7 of gonadotropin administration, is the minimal effective dose for a multiple-dose protocol to prevent the onset of premature rises of LH (10). On the other hand, the use of a single-dose protocol in the late follicular phase to prevent the LH surge has been proposed (14). Both protocols have shown good performance, even though the multiple dose gains more consensus from physicians because it also prevents LH rises during the entire stimulation, including the premature surge, with a lower LH tone for the entire follicular phase (12–14).

Several investigators have shown that the use of the antagonist reduces the dose of FSH needed to stimulate the ovary, especially when compared with the classical long protocol with GnRH analogs (16–20). Furthermore, it has been shown that the use of GnRH antagonists associated with

gonadotropin COH for IVF is at least as effective as the GnRH analog long protocol in patients with normal ovarian response, like women at the younger reproductive age (<35 years old). Furthermore, from the data published in the literature, it seems that cetrorelix decreases the risk of ovarian hyperstimulation syndrome (OHSS) with respect to the long protocol with GnRH analog (15–21).

However, with respect to poor responder patients or older women, the data in the literature are more conflicting. Early reports showed that the use of cetrorelix in these patients may improve the ovarian response and the number of oocytes that are yielded (22–25, 27). On the other hand, other data published did not confirm these findings (26, 28–30). In our previous report, we showed that the GnRH analog long protocol worked better than the short protocol in women 40 years or older, who in turn have a lower chance of pregnancy even for their reduced ovarian reserve (31). The aim of our study was to evaluate which protocol between cetrorelix and the GnRH analog long protocol is more effective in ovarian stimulation for IVF in women 40 years or older.

## MATERIALS AND METHODS
### Patient Selection
All patients who were at least 40 years old but younger than 45 who referred to the IVF program of Bioroma Paideia Hospital, Rome, Italy, to undergo their first IVF cycle from January 2003 to June 2007 were eligible for the study. The inclusion criteria were age 40 years or older and no previous IVF cycle, and the exclusion criteria were FSH >10 IU/mL, a previous IVF cycle, and age 45 years or older.

The trial was designed according to Consolidated Standard of Reporting Trials guidelines. The study was reviewed and approved by the Institutional Review Board. Of the 585 eligible patients undergoing IVF during the study period, 570 agreed to participate. The patients were randomized by means of a computer-generated randomization number sequence at the time that their cycle was scheduled, with 285 patients for each arm of the study. All patients undergoing IVF and participating in the study gave their informed consent. All patients were nulliparous who underwent a standard infertility evaluation, and none of the patients eligible for the study showed FSH >10 IU/mL. Women with polycystic ovaries were excluded from the study because these women often respond unpredictably, with an increased risk of hyperstimulation or a low response and bad-quality oocytes. All basal FSH assays were done in the same laboratory using the same radioimmunoassay kit (OCFF07-FSH RIAgnost, CIS Bio-International, Milan, Italy).

Patients were randomly allocated into two study groups: the cetrorelix group (group A) in which ovarian stimulation comprised GnRH antagonist and recombinant FSH (recFSH) alone starting from cycle day 2, and the long protocol group in which GnRH analog, bBuserelin (group B), was given as a pretreatment; recFSH administration took place when pituitary desensitization was established. The patients allocated to group A started recFSH (Gonal-f, Serono, Italy) at a dosage of 300 IU daily from the second day of their menstrual cycle for 5 days until the first ovarian ultrasound on the sixth cycle day and plasma $E_2$ test; cetrorelix (Cetrotide, Serono, Italy) at a dosage of 0.25 mg/die was administered when the dominant follicle was of 14 mm in mean diameter or the $E_2$ plasma levels were 600 pg/mL, until the day of hCG administration. Patients in group B were administered buserelin (Suprefact, Hoechst, Milan, Italy) SC, 0.4 mg 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 third day. Suppression was confirmed when $E_2$ reached the level of <30 pg/mL and follicles with a dimension <15 mm in mean diameter were visible on ultrasound scan examination. When suppression was confirmed by $E_2$ and ultrasound examinations, recFSH was commenced at 300 IU of recFSH on the second day of the menstrual cycle in the long protocol.

From the sixth day 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 recFSH was adjusted depending on the individual response of each patient. The criteria used for triggering ovulation with 10,000 IU of IM hCG (Gonasi HP 5000, AMSA, Rome, Italy) were plasma $E_2$ between 800 and 3500 pg/mL and at least three follicles >16 mm in mean diameter (two perpendicular measurements). The cycle was cancelled in case of poor ovarian response, when less than three follicles were observed on the ninth day, or in case of OHSS with $E_2$ >3500 pg/mL.

Oocyte retrieval was performed under ultrasound guidance by the transvaginal route on day 0, 36 hours after the injection of hCG, and all patients underwent intracytoplasmic sperm injection (ICSI) according to published procedures (32) 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 embryos had six to eight or more equal and regular blastomeres without the presence of cytoplasm fragments; grade B embryos had less than six to eight unequal blastomeres with or without cytoplasmatic fragments; grade C embryos were fragmented embryos (more than 50%) (33).

Embryos were transferred about 72 hours after insemination using the Sydney Embryo Transfer Catheter (Cook Ireland Ltd., Limerick, Ireland). The policy of our clinic is to transfer no more than three embryos (preferably of the best quality). 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 12 days after ET and ultrasound demonstration of the gestation sac 4 weeks after the transfer. Biochemical pregnancies alone were not included.

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

### Statistical Analysis

The Mann-Whitney $U$-test, Student's $t$-test, $\chi^2$-test, and Fisher's exact test were used when appropriate to evaluate the differences of the variables in the cetrorelix and GnRH analog long protocols in all patients: clinical pregnancy rate per cycle started and per transfer were the primary outcomes, whereas secondary outcomes were considered days of stimulation, $E_2$ at the day of hCG, amount of FSH administered, number of oocytes yielded, number of embryos transferred, implantation rate, and abortion rate. All statistical analyses were performed using the SPSS statistical package.

The primary and secondary outcomes were chosen on the basis of our previous experience (31) and literature data (22–30) on these patients, for expected pregnancy rate, implantation rate, fertilization rate, number of oocytes harvested, and so on.

### RESULTS

There were 570 patients included in the study were; 564 patients started the treatment, 281 in the cetrorelix group and 283 in the long protocol with GnRH analog group. Four women in the cetrorelix group and two in the control group dropped out of the study. The two groups of patients were similar regarding the woman's age, parity, period of infertility, and causes of infertility: these data are shown in Table 1.

In the cetrorelix group, 274 women underwent oocyte retrieval; in the long protocol with GnRH analog group, 279 underwent oocyte retrieval. Of these patients, 257 of the cetrorelix group and 267 in the long protocol with GnRH analog group underwent ET (Table 2).

The amount of recFSH used in the women treated with cetrorelix was 2686 + 1994 IU, whereas in the long protocol group it was 3018 + 1989 IU. The difference was not statistically significant (Table 2). The $E_2$ levels on the day of hCG administration in the women treated with cetrorelix were 1125 + 1034 pg/mL, whereas in the long protocol group they were 1696 + 936 pg/mL. The difference was statistically significant ($P<.01$; Table 2). The number of days of stimulation in the women treated with cetrorelix was 11.3 + 1.8, whereas in the long protocol group it was 12.0 + 2.1. The difference was statistically significant ($P<.01$; Table 2). The number of oocytes collected in the women treated with cetrorelix was 3.7 + 2.5, whereas in the long protocol group it was 4.3 + 2.4. The difference was statistically significant ($P<.01$; Table 2). The number of embryos transferred per patient was 2.1 + 1.0 in the cetrorelix group and 2.1 + 1.1 in the

### TABLE 1

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

| Variable | Cetrorelix | GnRH long protocol |
|---|---|---|
| No. of women | 281 | 283 |
| Maternal age, years | 42.3 ± 1.4 | 42.1 ± 1.5 |
| Body mass index | 25.1 ± 2.6 | 24.8 ± 2.4 |
| Paternal age, years | 44.2 ± 2.4 | 44.5 ± 2.0 |
| Time of infertility, years | 3.3 ± 1.4 | 3.4 ± 1.5 |
| Basal FSH levels, IU/L | 7.0 ± 2.5 | 6.9 ± 2.4 |
| Basal $E_2$, ng/mL | 50.3 ± 12.1 | 48.9 ± 13.4 |
| Cause of infertility: | | |
|   Tubal factor (%) | 68 (24.2) | 74 (26.1) |
|   Male factor (%) | 119 (42.3) | 124 (43.8) |
|   Endometriosis (%) | 43 (15.3) | 46 (16.2) |
|   Endocrinological (%) | 23 (8.2) | 14 (4.9) |
|   Unexplained (%) | 28 (9.9) | 25 (8.8) |

*Note:* Data are reported as mean + SD. $P$ = NS for all rows.

*Sbracia. Cetrorelix or long protocol in older patients. Fertil Steril 2009.*

control group. The difference was not statistically significant (Table 2). No statistically significant differences were found between the two groups for the percentage of good-quality embryos (Table 2).

In the patients treated with cetrorelix, the pregnancy rate per cycle started was 9.2% (25 out of 274), whereas in the group treated with GnRH analog long protocol, the pregnancy rate per cycle started was 17.2% (48 out of 279). The differences were statistically significant ($P<.01$; Table 2). In the patients treated with cetrorelix, the pregnancy rate per transfer was 9.7% (25 out of 257), whereas in the group treated with GnRH analog long protocol the pregnancy rate per transfer was 17.9% (48 out of 267). The differences were statistically significant ($P<.01$; Table 2). The implantation rate was 4.8% in the cetrorelix group, whereas in the GnRH analog long protocol it was 8.7%. The difference was statistically significant ($P<.02$; Table 2). The abortion rate in the two groups was similar, without statistically significant differences (16.0% vs. 14.6% respectively; Table 2). The power of the study per pregnancy rate was >80% for $P<.05$ $(1 - \beta)$. All statistical results were corrected for the number of comparisons performed.

### DISCUSSION

Although GnRH antagonists have been used for more than 10 years, there is still no general agreement on the efficacy of these substances (cetrorelix and ganerelix) in COH for IVF

## TABLE 2

**Results of the trial between cetrorelix and long protocols in women age 40 years or more.**

| Variables | Cetrorelix (%) | GnRH long protocol (%) | P |
|---|---|---|---|
| Cycles started | 281 | 283 | |
| Cycles with oocyte retrieval (%) | 274 (97.5) | 279 (98.6) | |
| Cycles with transfer (%) | 257 (93.8) | 267 (95.7) | < .01 |
| Days of stimulation | 11.3 ± 1.8 | 12.0 ± 2.1 | |
| Amount of FSH used, IU | 2686 ± 1994 | 3018 ± 1989 | NS |
| $E_2$ levels at hCG day, pg/mL | 1125 ± 1034 | 1696 ± 936 | < .01 |
| Oocytes yielded | 3.7 ± 2.5 | 4.3 ± 2.4 | < .01 |
| Embryos transferred | 2.1 ± 1.0 | 2.1 ± 1.1 | NS |
| Embryo of type A, % | 56.7 | 47.7 | NS |
| Embryo of type B, % | 33.7 | 43.9 | NS |
| Embryo of type C, % | 9.5 | 8.3 | NS |
| Implantation rate (%) | 26/540 (4.8) | 49/561 (8.7) | < .02 |
| Pregnancy rate/cycle (%) | 25/274 (9.2) | 48/279 (17.2) | < .01 |
| Pregnancy rare/transfer (%) | 25/257 (9.7) | 48/267 (17.9) | < .01 |
| Abortion rate (%) | 4/25 (16.0) | 7/48 (14.6) | NS |

*Note:* Data are expressed as mean ± SD unless otherwise specified.

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cycles. The literature data seem to show that GnRH antagonists are as effective as the GnRH analog long protocol (8–21), in younger women with normal ovarian reserves, even though a recent meta-analysis reported that the antagonist showed a trend toward a lower pregnancy rate with respect to the GnRH analog long protocol, which became statistically significant when the data were pooled together (34). However, the antagonist shows the clinical advantage of a reduced risk of OHSS and lower amount of FSH needed for ovarian hyperstimulation (8–21). In poor responder or older women with a reduced ovarian reserve, the role of GnRH antagonists has not yet been well established (22–30).

Our study showed that the GnRH analog long protocol leads to a statistically significant higher pregnancy rate than the cetrorelix protocol. Furthermore, the long protocol yields more oocytes than in the cetrorelix group, even though in this group there were lower $E_2$ levels on the day of hCG administration and fewer days of stimulation than in the other group.

It is noteworthy to highlight that the amount of FSH administered is not statistically significant between the two groups. These data showed that in women 40 years or older cetrorelix may be detrimental in terms of pregnancy rate and number of oocytes yielded, even though it reduces the days of stimulation and the plasmatic $E_2$ levels and causes a slight reduction in the amount of FSH needed. Our data show a univocal trend toward the reduction of follicular recruitment by the cetrorelix protocol that leads to a decreased expectancy of pregnancy. Our data agree with the data reported by several investigators showing that cetrorelix did not benefit poor responders when compared with GnRH analog protocols (26, 27–30), even though earlier reports showed

that cetrorelix might be of benefit in these patients (22–25, 26). However, a recent meta-analysis showed that there are no differences between the cetrorelix and GnRH analog protocols in terms of pregnancy rates (35). These differences in the literature may be explained by the different selection of patients, the different protocols used in the ovarian stimulation, and the small number of women included in each study. Our study overcame at least the problem of the number of patients since it included a very large cohort of women to highlight even the small differences in pregnancy rate, which is expected to be low.

Our results may be explained by several physiological mechanisms; one of these may be the total suppression of LH secretion during the follicular phase. It has been reported that the absence of LH may be detrimental for follicular growth and that the supplementation with LH may reverse it (36, 37). However, other data showed that LH supplementation is not needed for follicular development and does not improve the pregnancy rate (38).

One other possible physiological mechanism may be due to the fact that older women (>39 years old) have a shorter follicular phase because of an earlier start of follicular growth during the previous luteal phase than in younger patients (39–41). Therefore, in older patients it has been shown that the available cohort of antral follicles starting the growth in each cycle is significantly smaller than in younger women (40). Older women show lower levels of inhibin 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 the cohort size of antral follicles (42). Lower levels of inhibin B

may 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 may explain why in older women ovarian stimulation with the GnRH antagonist gives worse results: cetrorelix, given in the mid follicular phase, has no effect on the number of follicles recruited in older patients, since the cohort of growing follicles has already been recruited and selected, with a consequent lower number of developing follicles. Instead, the long protocol, with the flare-up effect in the mid luteal phase, may increase the size of the follicle cohort recruited per cycle, and an increased length of stimulation may allow additional growing follicles to enter in the cohort of stimulated follicles.

It has been reported that the most relevant advantage of GnRH antagonists is the possibility of assessing ovarian reserve immediately before ovarian hyperstimulation to know the cohort amount of starting primordial follicles (43). However, in our study, all patients showed a good basal ovarian reserve. Furthermore, the patients were randomized before starting with ovarian stimulation. However, a consistent trend toward a lower pregnancy rate in patients treated with cetrorelix has been reported (43), as in our data; this is particularly detrimental in patients with a low chance of pregnancy such as in women 40 years or older and poor responders. Women at an advanced reproductive age also manifest a decline in oocyte quality, which leads to a decrease in pregnancy rate expectancy: our previous reports evidenced in these patients a pregnancy rate around 17% (31).

In conclusion, our results showed that the use of GnRH antagonists should be avoided in older women (40 years or older) for COH in IVF since it determines a lower pregnancy rate and a decrease in oocyte yield, whereas these compounds remain a useful tool in women with normal ovarian reserves or in younger women. Further studies are needed to evaluate which protocol or pharmacological association may improve the ovarian response to COH in these women.

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