

## Selecting age-specific protocol for controlled ovarian hyperstimulation in IVF program with particular reference to advanced age group

Anjana Ray Chaudhuri and Siddhartha Chatterjee\*

Calcutta Fertility Mission, 21, Bondel Road, Kolkata-700019, West Bengal, India

**Abstract:** *Aim:* The present study was aimed at finding out the age specific best protocol for ovarian stimulation, particularly for advanced age group. *Materials and Methods:* This retrospective analysis was carried out from July 2005 to June 2009 in 617 cycles of In-vitro Fertilization (IVF) in different age group patients, performed at our centre. 617 patients having indications for female factor infertility were randomly divided into 3 age groups, as 26 to 30 years (younger age group); 31 to 35 years (middle age group) and 36 to 40 years (advanced age group). They were selected for IVF program, after routine investigations. 4 different stimulation protocols, e.g., Long Down Regulation (LDR), Ultra Long Down Regulation (ULDR), Soft Stimulation (SS) Protocol and Antagonist Protocol utilized were analyzed primarily on the basis of the amount of Gonadotrophin (Gn) ampoules required for ovarian stimulation, terminal estradiol (E<sub>2</sub>) value, oocyte recovery rate, clinical pregnancy rate (PR) and take home baby rate (THBR). *Results:* LDR was proved to be the protocol of choice for all the other groups except advanced age group where SS proved to be the best with antagonist protocol being the close follower. *Conclusion:* As least amount of ovarian stimulation is used in SS, it is cost effective and imposes minimum risk of ovarian hyperstimulation syndrome (OHSS) and offers maximum PR for advanced age group patients, and reasonable PR for other age groups.

**Keywords:** Controlled ovarian stimulation, gonadotrophin, letrozole, agonist, antagonist, estradiol, oocyte recovery rate, pregnancy outcome

### Introduction

The use of pituitary down-regulation for achieving controlled ovarian hyper-stimulation (COH) with Gn has revolutionized assisted reproductive techniques (ART), because more programmed cycles can be performed with the best possible instrumental and personnel back-up. Before the down-regulation era, the untimed and pre-mature LH surge was a nightmare to the reproductive physicians, as the planning of oocyte recovery was extremely difficult and uncertain. Introduction of Gonadotrophin Hormone Releasing Agonist (GnRH-a) to effect down regulation, causes an initial flare of pituitary hormonal secretion followed by pituitary exhaustion. This suppression remains for a longer period of time, even after stopping the medicine. This is in contrary to GnRH antagonist (GnRH-anta) that causes immediate down-regulation of the pituitary gland, and pituitary function returns to normal immediately after stopping the medicine. In COH, GnRH-a is used before follicular stimulation and GnRH-anta during stimulation.

Ovarian stimulation is usually effected by recombinant Follicle Stimulating Hormone (rFSH) and/or Human Menopausal Gonadotrophin (hMG). COH demands consumption of very high dose of Gn particularly in GnRH-a cycle, as endogenous Gn secretion is inhibited, and this may lead to one of the major complications like OHSS. The cost involved is also high as Gn is a very expensive drug. In order to reduce the cost as well as complications of COH, oral ovulation inducing agents were administered alongwith Gn, which reduced the overall Gn requirement per stimulation cycle. Many studies have used Clomiphene Citrate (CC) in combination with Gn for low cost / soft stimulation [1-5] in IVF program. The use of aromatase inhibitors (AI) like Letrozole has recently been tried, but the series is small and the studies are non-randomized. Letrozole does not antagonize estrogen receptors in the brain, but interferes significantly with overall production of estrogens, because of selective inhibition of aromatase. Consequently, withdrawal of the

negative feedback effects of estrogens may allow the pituitary to produce more endogenous FSH. Moreover, attenuated aromatization may secondarily lead to the accumulation of follicular androgens, which may increase the follicular sensitivity through amplification of FSH receptor gene expression [6-7], or stimulate insulin-like growth factor-1, which may act in synergy with FSH [8]. Letrozole, having a very short half life ( $\leq 45$  hours) is quickly cleared from the body (mainly by the liver), and therefore less likely to affect the endometrium and cervical mucus adversely [9-11]. With all these information, a study was undertaken in our clinic to find out a rational protocol of stimulation for COH in different age groups, with special attention to advanced age patients, with an idea to use the least amount of Gn.

### Material and Methods

The study was conducted at our center between a period of July 2005 to December 2009. Approval for carrying out the same was taken from the institute's ethics committee and informed consents from the patients were obtained. The study comprised of 617 cycles of IVF involving 617 patients, undergoing IVF for the first time. They were of different age groups, and enlisted for different stimulation protocols. The inclusion criteria for the patients were age between 26 to 40 years, BMI between 23-30, duration of infertility  $<10$  years, and the indications for IVF being tubal factor, unexplained infertility and endometriosis. The exclusion criteria being age  $>40$  years, BMI  $>30$ , duration of infertility  $>10$  years, all the seminal factors of infertility and Polycystic Ovarian Defect (PCOD). Initially, before inclusion to the IVF program, all the patients were screened endocrinologically for their basal hormonal status, especially for FSH, LH, Thyroid-stimulating Hormone (TSH), Prolactin (PRL) and Testosterone. They were also screened for tubercular bacillary infestation of endometrium, by TB-PCR test from the menstrual blood, collected on day 2 of the period (D2) [12]. The patients were also examined for HBsAg and HIV status. The husbands had semen analysis and culture. Transvaginal ultrasonography (TV-USG) before initiation of stimulation was mandatory, to exclude any ovarian cyst or pregnancy. When all these parameters were satisfactory, the patients were subjected to COH. This is to be mentioned that initially 635 patients undergo-ing first cycle

IVF were enlisted for the study, but 10 of them were excluded because they turned out to be very poor responders by D9, and another 8 developed multiple follicles of variable sizes by day8/9, presenting a threat of developing OHSS. In these cases, the stimulation was discontinued and patients were excluded from the study. The basic selection criteria for different stimulation protocols were based on the patient's age, D2 FSH value, and the desire and convenience of the patient couple. Patients of higher age group were promoted for lesser degree of down regulation (as in GnRH antagonist protocol), as well as soft / mild stimulation since the ovarian response becomes poorer as the FSH value increases. In elderly patients particularly nearing 40 years, even with low FSH value ( $< 9$  mIU/ml) shorter duration of stimulation protocol was method of choice. LDR was offered first to patients below 35 years of age with FSH value below 9. If the patient did not want to accept 2 consecutive cycles for IVF, long protocol was avoided. The stimulation protocols were selected following discussion with the patients. Particularly for lower age group, the first choice was the long protocol, as it produced better pregnancy rates, provided D2 FSH value was  $< 9.0$  mIU/ml. In diagnosed advanced endometriosis, ULDR was the protocol of choice.

The stimulation protocols were divided into 4 categories. Patients undergoing LDR received sub-cutaneous (sc) injections of GnRH-a (Leuprolide Acetate or LA: Sun Pharma) at a daily dose of 1.0mg started from day 21 of the previous cycle, and following the next period,  $17\beta$  estradiol ( $E_2$ ) and LH were estimated on D2, and ovarian stimulation with recombinant FSH (rFSH) and hMG started on D3. GnRh-a down regulation was maintained at  $\frac{1}{2}$  dose (i.e., 0.5 mg of LA) thereafter. In this study we used rFSH Gonal F (Serono International) for first 3 days (fixed dose) of stimulation followed by hMG (IVF-M: LG Life Sciences) for the rest period (variable dose). In Ultra-long down-regulation (ULDR), depot form of GnRh-a (3.75 mg of LA) was injected subcutaneously on the second half of the previous cycle, usually on day 21 and a second dose was injected 28 days later, and a fortnight following the

second injection, after evaluation of serum E<sub>2</sub> and LH levels, Gn stimulation started. The ULDR procedure was preferred in cases with endometriosis (78 patients). Of late, the antagonist protocol is becoming popular, due to its short duration of stimulation, and the completion of the treatment within the same cycle. We used antagonist in 127 cases, where gonadotrophin stimulation started on D3 and GnRH antagonist Cetorelix (Ovurelix: Sun Pharma) started at 14 mm diameter of the leading follicle. The antagonist was used at a daily dose of 0.25 mg (sc) injection for a minimum of 3 and maximum of 5 doses per cycle. A different protocol, which was otherwise called soft stimulation (SS), was used in 122 cases. This involved the use of oral ovulation-inducing agents along with gonadotrophin. In this series, we have used Letrozole (Letroz or Letoval: Sun Pharma) at a dose of 2.5 mg, twice daily, from D2 to D6 of the period, alongwith the addition of hMG from D3 and GnRH –anta was added for down regulation at 14 mm. follicle size. SS was preferred where the basal FSH was found to be on the higher side, but should be < 16 mIU/ml (the normal being 3 to 12 mIU/ml). The criteria for antagonist use were firstly, the preference of the patient and secondly, the high normal basal FSH, which might lead to a poor ovarian response, following down-regulation with agonist. The most preferred long protocol was used in maximum number of cases (269 cases), with normal basal FSH in different age groups, and as mentioned before that ULDR was the protocol of choice in the patients with endometriosis. In all the protocols with the leading follicle at ≥ 18mm., injection hCG 5000IU (im) (Fertigyn: Sun Pharma) was administered. The same morning all the patients underwent estimation of serum E<sub>2</sub> level. This hCG day E<sub>2</sub> was referred to as terminal E<sub>2</sub>.

Oocyte pick-up under US guidance was performed 34 to 35 hours following hCG injection. Embryo transfer (ET) was done 48 hours after insemination. Serum β-hCG was estimated on day14, post-transfer. USG detection of clinical pregnancy was performed 5 to 6 weeks after last menstrual period (LMP).

### Results

The primary outcome measure was the comparative evaluation of pregnancy as well as take-home baby rates, while the additional measures included the total number of Gn administered in relation to D2 FSH value, terminal E<sub>2</sub>, the number of oocytes retrieved and their patient to patient variations. The observations were numerically presented in the Table below.

It has been observed that inter & intra protocol variation of the total number of gonadotrophin ampoules required during COH was considerably wide. In all the protocols the hCG day E<sub>2</sub> value also varied, so also the number of oocytes retrieved. The terminal E<sub>2</sub> value depended on the number of follicles developed, which was quite variable from patient to patient. The D2 FSH did also vary between patients. This wide variation of the data was difficult to represent and interpret. Hence, they have been represented as the mean value with variation level which helped in finding out the protocol, where Gn requirement was less variable from patient to patient and the protocol that produced predictable number of oocytes. When the variability of E<sub>2</sub> was less, repeated E<sub>2</sub> estimation could be avoided, which would become more cost-effective. The pregnancy rates mentioned here referred exclusively to clinical pregnancies.

Protocols	Cases	Gn ampoules	E <sub>2</sub> (day hCG)	FSH (d <sub>2</sub> )	No. of oocytes	PR (Preg/No.)	THBR (Live birth/No.)
<i>26 - 30 years</i>							
SS	16	25.0 ± 5.0	1000 ± 200	9 ± 1.2	4 ± 2	12.50% (2/16)	6.25% (1/16)
Antagonist	55	27.0 ± 9.0	2000 ± 800	8.5 ± 3.5	10 ± 5	18.18% (10/55)	14.54% (8/55)
LDR	106	33.0 ± 12.0	2000 ± 500	6.9 ± 1.1	14.5 ± 2.5	46.23% (49/106)	39.62% (42/106)
ULDR	15	31.0 ± 7.0	2000 ± 500	7 ± 1.0	13 ± 2	33.33% (5/15)	33.33% (5/15)

Protocols	Cases	Gn ampoules	E <sub>2</sub> (day hCG)	FSH (d <sub>2</sub> )	No. of oocytes	PR (Preg/No.)	THBR (Live birth/No.)
<b>31 - 35 years</b>							
SS	71	24.0 ± 6.0	2000 ± 500	9.2 ± 1.2	6 ± 1	36.62% (26/71)	35.21% (25/71)
Antagonist	87	31.0 ± 13.0	2200 ± 800	8 ± 2.9	8.5 ± 4.5	22.98% (20/87)	18.39% (16/87)
LDR	120	30.0 ± 12.0	2400 ± 600	7 ± 2	14.5 ± 2.5	50.83% (61/120)	43.33% (52/120)
ULDR	47	46.0 ± 11.0	2500 ± 500	7.8 ± 1	9 ± 1	31.96% (15/47)	29.78% (14/47)
<b>36 - 40 years</b>							
SS	35	23.5 ± 10.5	1200 ± 400	9 ± 1.8	4 ± 2	28.57% (10/35)	25.71% (9/35)
Antagonist	36	31.0 ± 13.0	800 ± 400	9 ± 3.6	5 ± 3	27.77% (10/36)	22.22% (8/36)
LDR	43	34.0 ± 11.0	1400 ± 400	6.9 ± 1.7	7 ± 1	25.58% (11/43)	18.60% (8/43)
ULDR	16	53.0 ± 8.0	1500 ± 300	7 ± 1.2	5 ± 2	25.00% (4/16)	18.75% (3/16)

In the age group of 26 to 30 years, SS had the least success, i.e., 12.50% PR and 6.25% take home baby rate (THBR) along with significantly less oocyte recovery, inter patient variability of Gn consumption as well as terminal E<sub>2</sub> level, compared to the rest. Though D<sub>2</sub> FSH level was slightly higher in SS protocol total Gn requirement was less than the other three protocols. In the same age group, the antagonist protocol produced about 18.18% PR and 14.54% THBR, LDR produced 46.23% clinical pregnancy along with 39.62% live birth and for ULDR, the values were 33.33% for both the parameters. The hCG day E<sub>2</sub> level of patients varied to a large extent with antagonist protocol, but with the latter 2 protocols, it was less variable. The number of oocytes retrieved was much more with long or ultra-long agonists, and the variability of oocyte number was also less.

In the middle age group (31 to 35 years), the pregnancy rates were high with almost all the protocols. LDR offered maximum i.e., 50.83% PR and 43.33% THBR, ULDR produced 31.96% pregnancy followed by 29.78% term delivery, 36.62% PR and 35.21% THBR with SS, and the least success rate with antagonist (22.98% and 18.39% respectively). For this age group also the hCG day E<sub>2</sub> level varied maximum (2200 ± 800 pg/ml) with the antagonist protocol. The gonadotrophin requirement for individual patient in SS group was less variable (24 ± 6) than all the other protocols, in the aforesaid age group.

In advanced age group (36–40 yrs), all the 4 protocols produced comparable success rate between 25% to 28%. Gn requirements were less with SS, maximum with ULDR and comparable with LDR and antagonist. The average terminal E<sub>2</sub> value was less in antagonist, but comparable in all the other protocols. But the oocyte recovery rate was more variable among antagonist group than the other groups. So far as take-home baby rate is concerned, the question of miscarriage of clinical pregnancy comes. It has been observed that miscarriage is significantly higher in LDR in all age groups (about 77.8%). Lesser incidence was observed with SS protocol, the second in this list being antagonist. This indicates that down-regulation by GnRH-a may affect the endometrium, leading to miscarriages. High E<sub>2</sub> value may be responsible for the same. For statistical analysis, z score values were calculated and values of P less than 0.05 were considered statistically significant.

### Discussion

Efficacy of a stimulation protocol in any ART programme is judged primarily by its pregnancy outcome & secondarily by its cost-effectivity & patient compliance. Till date, there is paucity of literature regarding a clear consensus about an ideal stimulation regimen for advanced age group patients, who are usually poor responders.

During IVF whenever multiple good quality eggs are obtained in the initial attempt, more preservable embryos are available for subsequent Frozen Embryo Replacement (FER), thereby reducing the overall cost of the procedure and increasing PR per stimulation cycle. This is possible with agonist down-regulation, particularly in LDR protocol, but this involves the utilization of 2 consecutive cycles, which makes the treatment long lasting and leads to cycle wastage, particularly when it ends in failure. Antagonist down-regulation is able to combat the cycle wastage, but our study revealed that the oocyte recovery rate with conventional antagonist protocol is slightly less than what is found with the agonist. .

It has been observed that in the younger age group (26 to 30 years), the pregnancy rates in SS and antagonist protocols were much less, and often disappointing compared to the other age groups. But LDR in this group showed better result, so far as PR and take home baby rate is concerned, irrespective of the dose and duration of gonadotrophin treatment. In ULDR, the result was in between. This probably is because the indication for IVF for these younger age group patients was irreversible tubo-peritoneal pathologies or severe pelvic adhesions, which is known to have deleterious effects on the pregnancy outcome. LDR might have some favorable effects on the oocyte quality, thereby producing better success rate. In the middle age group (31 to 35 years) antagonist protocol produced poorer results even with more amount of Gn which is probably caused by inhibition of implantation by sudden anti-estrogenic factor of antagonist. In this age group also LDR offered maximum success SS protocol being the second best. ULDR produced success rate comparable to SS, with better number of oocyte recovery. In the advanced age group (36 to 40 years), SS and antagonist protocols produced comparable and better results as compared to their counterparts of LDR and ULDR though the average E<sub>2</sub> value was low and the oocyte recovery was not also very high in the former two groups.

In advanced age group, the comparatively new SS protocol has proved to be no inferior than the other established protocols as statistical analysis could not find any significant difference in the PR (p value 0.08) and THBR (p value 0.39)

among the protocols utilized. Soft protocol is gradually becoming the accepted procedure of stimulation [13-14]. In this protocol, use of Letrozol as adjuvant to Gn allowed consumption of lesser amount of the latter. This reduces the expenditure as well as chances of developing OHSS. Goswami et al. [15] reported the first RCT, to assess whether the incorporation of letrozole could be an effective low-cost IVF protocol for poor responders. Though cost-effective, the number of freezable embryo in this protocol may be less. There lie the advantages of LDR and ULDR which offer more surplus embryos to be cryopreserved for use in future thereby increasing the success rate in subsequent cycles following FER.

In the present study, LDR showed pioneering success rates in middle and younger age groups but failed to be the topper of the list in advanced age group, so far as clinical pregnancy rate or term delivery is concerned. But the cause of concern with this protocol is that in addition to imposing threat of OHSS, longer duration of stimulation and high E<sub>2</sub> value associated with LDR cause generic endometrial changes and poor corpus luteum development that might facilitate the rejection of implanted embryos [16-19]. Valbuena et al [20] showed that high E<sub>2</sub> levels are deleterious to embryo adhesion in vitro, mainly because they have a direct toxic effect on the embryo that may occur at the cleavage stage. This clearly explains the significantly higher miscarriage rate (77.8%) with LDR, in all the age groups. Lesser incidence of miscarriage was observed with SS protocol, the second in this list being antagonist. ULDR gives better success in advanced endometriosis provided the basal FSH is less than < 9 miu/ml.

The risk of OHSS in agonist COH cycle ranges from 6.6 - 8.4% in the literature. In this series, we have not mentioned about the incidence of OHSS. In LDR and ULDR, we had the threat of developing OHSS in less than 1.5% cases, and whenever there was a threat, the stimulation was withdrawn, because it was difficult to anticipate the cases which might proceed to moderate to severe OHSS, where the intensive care unit (ICU) facility would be essential and the cost of

treatment would be sky-high. In our series, the amount of gonadotrophin required for stimulation in antagonist and LDR was similar, though it has been mentioned in literature otherwise [21].

### Conclusion

Though the long protocol is considered the ‘gold standard’ in IVF cycles, in future, use of antagonist for pituitary suppression and agonist for ovulation trigger may make the ART protocols simpler and patient friendly. It will take some time before the antagonist replaces the agonist in COH. Some studies have shown antagonist to be better than agonist but these studies are small and more defined clinical trials are needed. It is true that the agonist protocol, either used in LDR or ULDR, produces more oocytes as well as embryos, there is always the risk of OHSS. LDR and ULDR require longer time for down-regulation and large number of Gn injections, which is a cause for concern to the patients, both physically and financially. The antagonists are better in this respect, but the PR is compromised as per our series, particularly in younger age group. The soft protocol, which utilizes antagonist as well for down-regulation, shows best success rate in the advanced age

group and reasonable success rate in middle age group. In soft protocol, the Gn requirement is also not very high. The inter-patient variability of Gn requirement is also low. Though oocyte recovery rate is low, which does not allow embryo cryopreservation most of the time, the PR is satisfactory, with least chance of OHSS. As the E<sub>2</sub> value on the hCG day is also not very high, the ill-effect of supra-physiological E<sub>2</sub> level on the endometrium and embryo is also less. Thus the present study analyses SS protocol in respect with cost effectivity, safety, success rate and patient acceptability compared to the other 3 conventional protocols in different age group patients. It, at the same time, conveys the message that in future SS protocol might be the choice of stimulation regimen particularly for advanced age group patients with relatively high basal FSH values. To serve the purpose a randomized controlled multi-centric trial including larger study group is necessary to arrive at a definite conclusion. Thus for ovarian stimulation, no consensus exists as yet on the end point measure, that defines success.

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\*All correspondences to: Dr. Siddhartha Chatterjee., FRCOG, Calcutta Fertility Mission, 21, Bondel Road, Kolkata-700019 West Bengal, India. E-mail ID: sidchat54@gmail.com