Original Article

Effect of Pre-ovulatory Single Dose GnRH agonist Therapy on IVF Outcome in GnRH Antagonist Cycles; A Prospective Study

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Abstract

Background: The purpose of present study was to evaluate the role of pre-ovulatory GnRH agonist therapy on IVF outcomes in GnRH antagonist cycles.

Methods: In this prospective study we recruited 100 infertile women undergoing IVF cycles with GnRH antagonists. The patients were assigned to two groups: Group A (the study group, n=42) were assigned for receiving hCG+triptorelin for the final oocyte maturation and group B (the control group, n=58) were assigned for only hCG. The t-test, chi-square (χ^2), and Fisher's exact test were used for data analysis. A $p<0.05$ was taken as statistically significant. The results are presented by mean± SD, and in percents (%).

Results: LH levels significantly ($p<0.001$) increased in the study group on the day of oocyte retrieval. All embryological parameters including the number of mature oocytes, fertilization and cleavage rates, number of high quality embryos and number of cases whose embryos were frozen were non-significantly higher in the study group. There were small but non-significant improvements in the clinical pregnancy, ongoing pregnancy, live birth and implantation rates in the study group.

Conclusion: Administering a single dose of GnRH agonist before oocyte retrieval in antagonist cycles may be helpful in improving the pregnancy rate but the results need to be verified in a larger trials.

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Introduction

Ovarian stimulation is a key component of assisted reproductive technology. Ever since the start of IVF, there has been a lot of modifications and improvements in ovarian stimulation protocols, to retrieve the maximum number of fertilizable oocytes in a given cycle. The ability of GnRH analogue co-treatment to prevent premature LH surge during controlled ovarian hyperstimulation (COH) was a major breakthrough in the history of assisted reproduction. GnRH agonists and antagonists are the two analogues in use during COH (1).

GnRH antagonists have a relatively weaker binding affinity to GnRH receptors than that of GnRH agonists. GnRH antagonists bind immediately to the receptors and do not activate classic post-receptor events which leads to immediate pituitary down-regulation. This difference between agonists and antagonists in affinity to bind GnRH receptors has been favorably utilized in several studies that demonstrated the feasibility of triggering an endogenous LH surge by the administration of GnRH-agonist (as 0.2 *mg* triptorelin per patient) after ovarian stimulation with gonadotropins

and pituitary suppression with GnRH antagonists (2, 3). The GnRH agonist is capable of displacing the antagonist from the receptor and inducing an initial activation (flare-up) prior to down-regulation of the receptor, leading to a concomitant LH and FSH surge.

One of the major advantages of GnRH antagonist protocols is their use in hyper-responders and in patients at risk of ovarian hyperstimulation syndrome (OHSS). In these cases, the final oocyte maturation can be achieved with GnRH agonist to trigger endogenous LH surge, thereby decreasing the chances of OHSS (4).

During starting years of GnRH antagonist use, it was realized that pregnancy rates were lower in antagonist cycles as compared to standard GnRH long down-regulation protocol, though recent studies have shown comparable pregnancy rates in agonist and antagonist cycles (5, 6). The reason for decreased pregnancy rates in antagonist cycles may be due to detrimental effects of antagonists on oocyte or embryo quality or more likely due to their negative effect on endometrium (7, 8).

GnRH functions not only as a releasing hormone in the pituitary, but it is distributed widely in several extra-pituitary sites where it is believed to be involved in the regulation of cell growth and proliferation. Receptors for FSH and GnRH have been identified on the endometrium, therefore it is possible that GnRH or GnRH analogues may have a direct or indirect effect on the endometrium (9).

There are several reports in the literature indicating the deleterious effect of GnRH antagonist on endometrial receptivity. Moreover, it has been shown that GnRH agonists administered during the luteal phase or along with hCG, may displace antagonists from the GnRH receptors on endometrium, thus improving the pregnancy rate (10, 11). Keeping these observations in mind, the present study was undertaken to compare any beneficial effect of a single dose of GnRH agonist given on day of hCG administration on pregnancy rates in antagonist cycles.

Methods

This prospective study was conducted on 100 infertile women undergoing IVF with antagonist cycles at Bangalore Assisted Conception Centre, Bangalore, India during March 2009 to December 2010. The study was approved by the ethics committee of the institute and all patients consented to participate in the study.

The inclusion criteria included women aged 20-40 years with a BMI of 18-30 kg/m^2, baseline FSH of 2-10 *IU/L* and >10 antral follicles on day two or three of the menstrual cycle using ultrasound scanning and a normal uterine cavity on hysteroscopy. Cases of pelvic tuberculosis, known corrected or uncorrected uterine malformations were excluded from the study. Other exclusion criteria were hydrosalpinx visible on USG, endometrioma >3 *cm*, adenomyosis, poor responders (<5 oocytes retrieved in previous IVF cycle with a conventional stimulation protocol), sub-optimal response in treatment cycle (<3 follicles with a size of ≥17 *mm* on day of hCG trigger), and surgically retrieved epididymal or testicular sperms.

Study Methodology: All included patients underwent transvaginal sonography on day two or three of the menstrual cycle to check for antral follicle count, endometrial thickness and to rule out the presence of ovarian cysts. Patients were assigned to two groups on day two of the cycle–Group A (Study group, n=42) were assigned for hCG+ triptorelin for final oocyte maturation and group B (Control group, n=58) were assigned for only hCG. Ovulation stimulation was started by recombinant FSH injection (Gonal-f, Merck Serono Specialities Pvt. Ltd., Italy) in appropriate dose on day two or three of menstrual cycle. Recombinant FSH was continued for the first four days. Subsequently, HMG (Ovugraf HP® VHB Life Sciences Ltd.,) was added according to physician's discretion. Follicular growth was monitored by serial ultrasonography. The doses of FSH and HMG were adjusted according to serum estradiol levels and dynamics of ovarian follicular growth. GnRH antagonist Orgalutran, 0.25 *mg* SC, (Organon Ireland Ltd.) was started when the lead follicle reached a diameter of 14 *mm* and/estradiol levels were >500 *pg/ml* and it was continued until atleast three follicles reached a diameter of ≥17 *mm*, when ovulation trigger was given.

Group A (study group n=42 cases) were given GnRH agonist triptorelin, 0.2 *mg* SC, (decapeptyl FERRING Pharmaceutical Pvt. Ltd, Mumbai, India) and hCG, 5000 *IU* IM (Ovumax-HP, VHB Life Sciences Ltd., Mumbai, India) as ovulation trigger. Group B (control group n=58 cases) were given only hCG, 5000 *IU* IM (Ovumax-HP, VHB Life Sciences Ltd., Mumbai, India) as ovulation trigger. Ovum retrieval was done 35 *hr* following hCG administration under transvaginal ultrasonography upon intravenous sedation. Oocyte as-

sessment was done by standard oocyte morphological criteria introduced by Lin et al. (12) and nuclear maturity assessment was done in cases subjected to ICSI. Conventional IVF or ICSI was performed depending upon semen parameters and previous fertilization history. We used culture media prepared and produced by, Vitrolife (Vitrolife, Sweden AB). Fertilization was defined as the presence of two pronuclei 16–18 *hr* post insemination/injection. Embryo assessment was done by standard morphological assessment according to Veeck's modified scoring system (13) Embryo transfer (ET) was done on day three or five following oocyte retrieval. All patients were advised to take 600 *mg* of micronized progesterone daily as luteal phase support for two weeks.

Serum estradiol, LH and progesterone levels were measured on day of oocyte retrieval and compared between the two groups. Levels of serum estradiol and progesterone were compared in the two groups seven days following embryo transfer. Measurements of estradiol, progesterone, LH, FSH and β-hCG were done by fully automated electro-chemiluminscence technology (Roche Cobas e411 analyser, HITACHI, Tokyo, Japan). β-hCG>50 *IU/L* two weeks after embryo transfer was considered as positive for pregnancy.

Outcome measures: Primary outcome measures included implantation and clinical pregnancy rates.

Implantation rate was calculated by dividing the number of gestational sacs seen on trans-vaginal sonography by the number of embryos transferred. Clinical pregnancy was defined as presence of one or more gestational sacs on trans-vaginal scan in the fifth week of gestation.

Secondary outcome included ongoing pregnancy, live birth and miscarriage rates, embryological details and hormonal profile on day of oocyte retrieval and seven days following embryo transfer.

Ongoing pregnancy was defined as continuing pregnancy with the presence of fetal cardiac activity beyond 12 weeks of gestation. Live birth rate was defined as number of deliveries that resulted in atleast one live born baby. Ectopic pregnancy was described as presence of extrauterine gestational sac and β-hCG>1000 *IU/L* in the absence of intrauterine gestational sac. Miscarriage was defined as discontinuation/failure to grow a pregnancy before the 12th week of gestation.

Embryological details included the mean number of mature oocytes retrieved per patient, fertilization and cleavage rates, mean number of grade one embryos available on day three, endometrial thickness on day of embryo transfer, number of cases whose embryos were frozen.

Hormonal profile included measurement of serum estradiol, LH and progesterone levels on day of ovum pick-up and that of serum estradiol and progesterone levels seven days following ET.

Statistical analysis: Descriptive statistical analyses were carried out in the present study. Results on continuous measurements are presented in Mean±SD and results on categorical measurements are presented in number (%). Student t-test (independent two-tailed) was used to find the significance of the study parameters on continuous scale between the two groups (Inter-group analysis) on metric parameters, Chi-square (χ^2) or Fisher Exact test were used to find the significance of the study parameters on categorical scale between two or more groups. A $p<0.05$ was taken as statistically significant. Two statistical software, SAS, version 9.2, and SPSS, version 15.0 were used for the statistical analysis.

Results

Of the 100 patients who met the inclusion criteria and gave consent for the study, 42 received 0.2 *mg* SC triptorelin in addition to hCG for the final ovulation maturation (cases) and 58 received only hCG (controls).

There were no significant differences in the baseline chacteristics in the two groups in age, BMI, or ovarian reserve assay (Table 1). Main indication for IVF were male factor infertility, unexplained infertility and tubal factor.

Both groups were comparable regarding the days of stimulation, dose of gonadotropins required and method of fertilization (Table 2).

There were no significant differences in hormonal profile on the day of hCG administration (Table 3), but LH levels were significantly ($p<0.001$) more in study group on the day of oocyte retrieval. Estradiol and progesterone levels were higher

Table 1. Comparison of baseline characteristics in the two groups

Hormone	Controls (n=58)	Cases (n=42)	p-value
Age (years)	30.10±3.91	29.76±3.52	0.654
BMI	24.83±3.21	24.01±2.85	0.190
FSH (*IU/L*)	6.98±1.60	6.76±1.63	0.644
LH (*IU/L*)	4.65±2.65	4.65±2.18	0.998
AFC	11.47±3.37	11.56±3.30	0.910
Primary infertility	38 (65.5%)	21 (50.00%)	0.119
Regular cycles	52 (89.7%)	35 (83.3%)	0.354

in study group on day of oocyte retrieval but the difference did not reach the level of significance (Table 3). Table 3 shows the hormonal profile seven days following embryo transfer.

All the embryological parameters including the number of mature oocytes, fertilization and cleavage rates, number of grade one embryos and number of cases whose embryos were frozen were non-significantly higher in the study group (Table 4).

Table 5 shows the pregnancy outcome in the two groups. In the study group, there was a small but non-significant improvement in the clinical pregnancy, live birth, ongoing pregnancy and implantation rates.

Discussion

The last 30 years have seen a remarkable development in the field of assisted reproductive technology (ART). With the advancement in culture systems and laboratory procedures, there was a need for parallel development in ovarian stimulation protocols so as to increase the yield of fertilizable oocytes. The aim of an ART protocol should be to retrieve a cohort of optimum number (8–12) of good quality oocytes leading to good quality embryos and simultaneously there should not be a compromise in endometrial receptivity.

With the introduction of GnRH antagonists into clinical use, it was realized that pregnancy rates are comparatively lower in antagonist protocols than the gold standard long agonist protocol (5),

Table 2. IVF cycle characteristics in the two groups

	Controls (n=58)	Cases (n=42)	p-value
Total dose of gonadotropins (*IU*)	2482.33±703.58	2485.71±647.82	0.980
Days of stimulation	9.89±0.99	9.88±1.09	0.941
Days of antag	4.58±0.70	4.88±0.83	**0.058**
No. of cases with IVF	25 (43%)	19 (45%)	0.832
No. of cases with ICSI	33 (57%)	23 (56%)	

Table 3. Comparison of hormonal profile in the two groups

Hormone	Controls (n=58)	Cases (n=42)	p-value
Day of hCG administration			
LH (*IU/L*)	**1.64±0.92**	**2.15±1.78**	**0.061**
E2 (*pg/ml*)	2379.37±966.14	2740.26±1222.31	0.103
Progesterone (*nM*)	2.96±0.91	3.19±1.54	0.346
Day of oocyte retrieval			
LH (*IU/L*)	0.80±1.03	2.26±1.17	**<0.001**
E2 (*pg/ml*)	1464.50±659.36	1722.06±879.12	0.147
Progesterone (*nM*)	22.14±13.25	27.03±17.88	0.280
Mid-cycle day (seven days following ET)			
E2 (*pg/ml*)	422.29±319.08	602.66±746.96	0.124
Progesterone (*nM*)	180.00±156.9	278.87±440.74	0.132

Table 4. Comparison of embryological details in the two groups

Embryological data	Controls (n=58)	Cases (n=42)	p-value
Mean number of oocytes per patient	11.68±3.24	13.09±5.86	0.128
Mean no. of mature oocytes per patient	9.34±2.98	9.92±4.49	0.436
Fertilization rate (%)	79.3%	82.5%	0.886
Cleavage rate (%)	93.3%	94.8%	0.909
Mean number of embryos available per patient	4.84±2.09	5.31±2.88	0.352
Mean number of Grade 1 embryos per patient	4.33±2.36	4.81±3.19	0.395
Mean number of embryos transferred per patient	2.97±0.18	2.83±0.44	0.163
No. of cases with frozen embryos	15 (25.8%)	17 (40.47%)	0.122
Endometrial thickness on day of ET(*mm*)	10.03±0.97	10.42±0.88	0.574

Table 5. Pregnancy outcome analysis in the two groups

Outcome	Controls (n=58)	Cases (n=42)	p-value
Implantation Rate	15.5%	15.87%	0.819
Bio-chemical pregnancy	3 (5.2%)	3 (7.1%)	0.694
Ectopic Pregnancy	1 (1.7%)	1 (2.4%)	1.000
Clinical Pregnancy	20 (34.5%)	16 (38.09%)	0.899
Ongoing pregnancy	17 (29.3%)	14 (33.3%)	0.668
Live birth rate	17 (29.3%)	13 (30.9%)	0.860
Multiple Pregnancy Rate	5 (8.6%)	2 (4.8%)	0.455
Miscarriage rate	2 (3.4%)	1 (2.38%	1.000
OHSS	2 (3.4%)	1 (2.4%)	1.000

which may be related to their effect on oocyte/ embryo quality or endometrial receptivity. But there are many advantages of antagonist protocols over long agonist protocol, and the most important one are its being more patient friendly and its decreased chances of OHSS development. Therefore, there has been a continued effort to improve pregnancy rates in antagonist cycles. Some of the recent studies have shown comparable pregnancy rates in antagonist and agonist cycles (6).

In the present study, we administered hCG to both groups as it has been shown by various studies that pregnancy rates are lower if only GnRH agonist is given as ovulation trigger compared to hCG. Recent Cochrane review (14) on the topic recommends against routine use of only GnRH agonist therapy as the final oocyte trigger due to decreased live birth and ongoing pregnancy rates. Therefore, it was decided to administer hCG to both groups to eliminate any bias related to this factor.

Various studies have shown the beneficial effects of GnRH agonists if given during the luteal phase (10, 11). The present study was undertaken with a hypothesis that by giving pre-ovulatory GnRH agonists, it may be possible to displace the antagonists from the receptor site, thereby, leading to receptor activation (3) and avoidance of the deleterious effect of GnRH antagonists on embryo and endometrial receptivity, as it has been shown that GnRH antagonist is an inhibitor of cell cycle by decreasing the synthesis of growth factors (3, 15, 16). Our aim of giving triptorelin along with hCG rather than administering them separately in luteal phase was to decrease the number of hospital visits for the patient.

The studies comparing the impact of GnRH agonist and antagonist on endometrial receptivity have yielded variable results. To study the effect of GnRH antagonist on endometrial receptivity, Rackow et al. (17) studied the effect of GnRH antagonists on HOXA10, a well-characterized marker of endometrial receptivity which is up-regulated at the time of implantation. There was significantly less HOXA10 expression in endometrial stromal cells in the antagonist compared to agonist group and natural cycles.

In the present study, endometrial thickness on the day of embryo transfer was better in the study group compared to control group (10.42±0.88 *vs.* 10.03±0.97). LH levels on the day of oocyte retrieval were significantly higher in the study group compared to control group (2.26±1.17 *vs.* 0.80±1.03 *IU/L*, p<0.001). Serum estradiol levels were also higher in the study group (1722.06± 879.12 *vs.* 1464.50±659.36, p=0.147) but the difference did not reach the level of significance. So, therefore, we observed favourable effects of GnRH agonist on LH and estradiol levels. Similar study by Morey Schachter et al. showed significant improvement in the implantation and clinical pregnancy rates in the group receiving GnRH agonist. This was a prospective randomized study of 221 patients undergoing IVF with GnRH antagonist protocol. In this study, a total of 200 ET cycles were done. The control group received hCG (5000 *IU*) 34 *hr* before oocyte retrieval and the study group received triptorelin (0.2 *mg* SC) in addition to hCG. The Mean oocyte pick-up day FSH (11.26 *IU/L vs.* 6.27 *IU/L*, p=0.00015) and LH levels (5.19 *vs.* 3.28 *IU/L*, p=0.0001) were significantly better in the study group compared to control group (3).

In the present study all parameters including post-embryo transfer hormone levels (E2, 602.66± 746.96 *vs.* 422.29±319.08, and progesterone 278.87 ±440.74 *vs.* 180.00±156.9), number of mature oocytes (9.92±4.49 *vs.* 9.34±2.98), fertilization rate (82.5% *vs.* 79.3%), mean number of grade A embryos per patient (4.81±3.19 *vs.* 4.33±2.36) were

better in triptorelin group although the levels did not reach statistical significance. This may be attributable to the small sample size in the present study. Clinical pregnancy rate (38.09% *vs.* 34.5%), live birth rate (29.3% *vs.* 30.9%), implantation rate (15.87% *vs.* 15.50 %) and ongoing pregnancy rate (33.33% *vs.* 29.3%) were higher in the study group but none of levels were statistically significant. This favourable effect on both embryo and hormonal profile may be responsible for improved pregnancy rates in the study group.

Study by Schachter et al. (3) showed higher implantation, clinical pregnancy and ongoing pregnancy rates in the triptorelin group but the improvement in pregnancy rate per started cycle did not reach statistical significance (40.9% *vs.* 28.3%).

In a placebo-controlled trial Tesarik et al. (18) evaluated the effect of a single dose of triptorelin given six days following ICSI. Oocytes from each donor were shared by two receipients, one of whom was given a single dose of triptorelin, 0.1 *mg*, six days following ICSI and the other received placebo at the same time. Oocyte recepients treated with GnRH agonist had higher implantation (36.9%, *vs.* 25.1%), twin pregnancy (16.7% *vs.* 3.6%), twin delivery (13.8% *vs.* 2.2%) and birth rates (31.1% *vs.* 21.5%). It was postulated that improved implantation rate could be attributed mainly to the beneficial effect of GnRH agonist on embryo development. The above observation has been supported by various other studies where luteal phase administration of Gn RH was shown to improve pregnancy rates (10, 11, 19).

Tesarik et al. (11) evaluated the effect of GnRH agonist administration in the luteal phase on ICSI outcome in both agonist and antagonist cycles. Six-hundred women (300 in the agonist and 300 in the antagonist group) were randomly assigned to receive 0.1 *mg* of triptorelin or placebo six days after ICSI. Administration of triptorelin led to a significant improvement in the implantation and live birth rates in both agonist and antagonist cycles.

The beneficial effects of luteal phase GnRH agonist administration may be due to drug action across multiple levels. GnRH agonist may support the corpus luteum by stimulating the secretion of LH by pituitary gonadotroph cells, direct effect on the embryo or by acting directly on the endometrium through locally expressed GnRH receptors.

Another study by Isik et al. (19) showed higher implantation and clinical pregnancy rates with a single dose of GnRH administration in a study of 164 patients. To the contrary, Ata and Urman (20) evaluated the role of luteal phase GnRH agonist administration in antagonist cycles but they failed to find any beneficial effect of such intervention on pregnancy rates. They carried out the meta-analysis of three available studies regarding clinical pregnancy rates, which failed to show any beneficial effect of single-dose GnRH administration on pregnancy rates.

Therefore, administration of a single-dose GnRH agonist (0.2 *mg* triptorelin) on the day of hCG trigger in antagonist cycles lead to a small but non-significant improvement in clinical pregnancy and live birth rates. There was significant improvement in the LH levels on the day of oocyte retrieval in the study group indicating the role of triptorelin in inducing the endogenous LH surge to supplement the exogenous LH surrogate, *ie.* hCG. Serum estradiol and progesterone levels on day of oocyte retrieval and seven days following embryo transfer also showed a favourable trend in triptorelin group though the results did not reach the significance level.

Conclusion

So, giving a single dose of GnRH agonist before oocyte retrieval in antagonist cycles may be helpful to improve the pregnancy rate but the results need to be verified in a larger trial.

Acknowledgement

No financial support was received for the study. Limitations of Study: Number of participants is small, so most of results showed a favourable trend but couldn't reach significance level.

Implication for future studies: There can be future studies analyzing the effect of antagonist and agonist cycles on endometrium at molecular levels.

Conflict of Interest

Authors declare no conflict of interest.

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