Original Article

The Effect of Follicle-Stimulating Hormone Administration on the Day of Human Chorionic Gonadotropin Trigger on Assisted Reproductive Technique Outcomes in Patients Undergoing *In vitro* Fertilization-Embryo Transfer: A Retrospective Cohort Study

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Abstract

Aim: The aim is to study the effect of follicle-stimulating hormone (FSH) administration on the day of human chorionic gonadotropin (hCG) trigger on the assisted reproductive technique (ART) outcomes in *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles. **Settings and Design:** Retrospective cohort study was conducted in the ART center of our hospital. **Materials and Methods:** Two hundred and ninety IVF/ICSI cycles performed between September 2012 and August 2017 were included in the study. Patients who received 375 IU of FSH on the day of hCG trigger (149 cycles) were compared with those who did not receive FSH on the day of trigger (141 cycles). **Statistical Analysis Used:** Chi-square test and Student's *t*-test were used. **Results:** The FSH co-administered group had a significantly higher number of oocytes retrieved, mature oocytes, and fertilization rate compared to those who did not receive FSH on the day of trigger ($p < 0.001$). The total number of embryos, the number of grade 1 embryos and the number of embryos available for cryopreservation were also significantly higher in the FSH administered group ($p < 0.001$). Implantation rate, clinical pregnancy rate, and live birth rate were not significantly different between the two groups. **Conclusions:** This study has shown that FSH administration on the day of the trigger may be considered in IVF cycles receiving hCG trigger to improve the oocyte recovery and maturity if the patient is not at increased risk of ovarian hyperstimulation and serum estradiol on the day of the trigger is <4500 pg/ml. However, there is only an increase in the total number of oocytes retrieved and the number of mature oocytes but no significant change in the implantation, clinical pregnancy, and live birth rates.

Keywords: *Follicle-stimulating hormone administration with human chorionic gonadotropin trigger, in vitro fertilization, intracytoplasmic sperm injection, oocyte maturity, oocyte recovery*

Introduction

The decision for trigger is one of the most important steps in any *in vitro* fertilization (IVF) cycle. In the majority of the IVF cycles, exogenous human chorionic gonadotropin (hCG), which is a surrogate for endogenous luteinizing hormone (LH) is used to trigger the final oocyte maturation. In agonist cycles, the hCG trigger is the only available option as pituitary would not be responsive for an agonist trigger. However, one should not forget the fact that in the natural menstrual

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cycle, the mid-cycle surge of LH is accompanied by a follicle-stimulating hormone (FSH) surge also, even though of a lower amplitude as compared with the LH surge.[1,2] Whereas in assisted reproductive technique (ART), the FSH administration is usually stopped on the day before trigger. This deprives the follicles of FSH in the past 48–72 hours before oocyte retrieval, which is a very crucial time with regards to final oocyte maturation and competence. Furthermore, the gonadotropin-releasing hormone (GnRH) analogs which are used for the prevention of premature LH surge effects pituitary suppression limiting the availability of endogenous FSH. All these factors result in a significant fall in the serum FSH levels at a time when it is of utmost importance. FSH has a crucial role to play in improving the maturity and developmental competence of oocytes in the final stages.[3,4] Unfortunately, this important action of FSH is underestimated by many clinicians practicing ART. Regarding FSH administration on the day of trigger, the opinion and practice vary between physicians, and there is no definite consensus as of now. This merits further research as the number of good quality mature oocytes is the robust surrogate measure for success in any IVF cycle. Hence, we conducted this retrospective observational study to find whether FSH administration on the day of the trigger results in any improvement in the oocyte quality and ART outcomes.

Materials and Methods

The study was conducted in the Reproductive Medicine Unit of our hospital. Ethical clearance was obtained from the Institute Ethics Committee. A retrospective analysis of data of IVF cycles from September 2012 to August 2017 (5 years) was done. The various indications for IVF treatment were a tubal factor, endometriosis, male factor infertility, and unexplained infertility. Females aged between 21 and 39 years, who underwent a long agonist protocol were included in the study. Those having serum estradiol levels ≥ 4500 pg/ml on the day of trigger, all embryos are frozen, and cases that did not undergo ovum pick up (cycle canceled or converted to intrauterine insemination) were excluded from the study. A total of 290 patients who matched all the criteria were included in the study.

All patients underwent standard pituitary down-regulation protocol with GnRH-a leuprolide (Zydus Cadila Healthcare Ltd.,) 0.5 mg subcutaneous starting from day 21 of the previous cycle. Fourteen days later, the complete pituitary desensitization was confirmed by the detection of serum estradiol concentrations < 50 pg/ml, LH < 3 IU/l, no follicle >10 mm in diameter, and endometrial thickness < 4 mm by the ultrasound examination. Gonadotropin (Recombinant FSH-Gonal F; Merck Serono, Mumbai, India) 150–300 IU/day was administered according to the age, body mass index (BMI), antral follicle count (AFC), and serum anti-Mullerian hormone (AMH). Serial follicle tracking was done to assess the ovarian response to stimulation and gonadotropin doses were adjusted accordingly. All patients were triggered with recombinant hCG (250 mcg, Ovitrel; Merck Serono, Mumbai, India) when there were at least 3 follicles ≥18 mm. Of the total 290 patients in the study, 149 patients received 375 IU of recombinant FSH on the day of hCG trigger, whereas the remaining 141 patients received only hCG on the day of trigger. In a previous study, the dose of FSH used was 450 IU, along with 10,000 IU of hCG.[5] However, in this study, the dose of 375 IU of FSH was chosen randomly with 250 mcg of recombinant hCG (equivalent to 6500 IU of hCG).

Transvaginal oocyte retrieval was performed 34–36 hours after the administration of the hCG trigger. Oocytes were fertilized either through conventional insemination in patients with endometriosis and tubal factor or by intracytoplasmic sperm injection (ICSI) in patients with unexplained infertility and male factor. Fertilization was assessed 16–18 hours after IVF or ICSI. Up to a maximum of two good quality embryos were transferred on day 3 or 5 under ultrasound guidance using a soft embryo transfer catheter (Cook's medical Sydney, Australia). Excess embryos were cryopreserved. Micronized Progesterone intramuscular injection 100 mg per day (Susten, Sun Pharma, India) was administered as luteal support from the day of oocyte retrieval. Pregnancy was confirmed by serum beta hCG estimation 16 days after the embryo transfer. Ultrasound was done 2 weeks after a positive beta hCG test to confirm viability.

The primary outcomes of the study were the number of oocytes retrieved and the number of mature oocytes. The secondary outcomes included the number of viable embryos, the number of grade 1 embryos, the number of embryos available for cryopreservation, fertilization rate, implantation rate, clinical pregnancy rate, and live birth rate. The fertilization rate was defined as the total number of fertilized oocytes by the total number of oocytes retrieved. Cleavage rate was defined as the total number of day 3 embryos by the total number of fertilized oocytes. Implantation rate was defined as the total number of gestational sacs visible on ultrasound by the total number of embryos transferred. The clinical pregnancy rate was defined as the presence of a

gestational sac with a fetal pole and cardiac activity on transvaginal ultrasound at 6 weeks. Live birth rate was defined as the percentage of all cycles that lead to live birth and is the pregnancy rate adjusted for miscarriages and stillbirths.

Statistical analysis

Data analysis was carried out using Stata software version 12.0 (StataCorp LP, College Station, Texas, USA) Normality assumptions of continuous variables were tested using Kolmogorov–Smirnov test. For variables that followed a normal distribution, descriptive statistics such as mean, and standard deviation were calculated. The comparison of mean values were tested using Student's *t*-independent test. Qualitative data were presented as frequency and percent values. The clinical pregnancy rate was presented with 95% confidence interval and relative risk with 95% confidence interval was calculated. The comparison of frequency data across categories was tested using Chi-square/Fischer's exact test. For all statistical tests, a two-sided probability of $p < 0.05$ was considered statistically significant.

Results

Data for a total of 290 patients who underwent IVF/ICSI cycles were available in the study (Group A: FSH on the day of trigger, $n = 149$; Group B: no FSH on the day of trigger, $n = 141$).

Baseline characteristics

The two groups were comparable with regards to baseline characteristics such as age, BMI, day 3 serum FSH, serum AMH, day 2 AFC, duration of gonadotropin stimulation, total gonadotropin requirement, serum estradiol on the day of trigger, number of preovulatory follicles on the day of trigger, serum progesterone on the day of trigger, and endometrial thickness on the day of trigger [Table 1].

Assisted reproductive technique outcomes

The ART outcomes of each group are shown in Table 2. The number of oocytes retrieved (9.4 vs. 8.5, $p < 0.001$), number of metaphase ii oocytes (7.3 vs. 5.2, $p < 0.001$) were significantly higher in Group A. The fertilization rate (0.71 vs. 0.54, $p < 0.001$), the total number of embryos (6.7 vs. 4.5, $p < 0.001$), the number of grade 1 embryos (3.7 vs. 3.2, $p < 0.001$) and the number of embryos available for cryopreservation (2.2 vs. 1.3, $p < 0.001$) were significantly higher in Group A. The cleavage rate and the percentage of cases that had blastocyst transfer were not significantly different between the two groups. There were 42 pregnancies in group A and 35 pregnancies in group B. The implantation rate, clinical pregnancy rate, miscarriage rate, and live birth rate were not significantly different between the two groups.

Discussion

This study showed significant improvement in the oocyte number and quality by FSH administration on the day of trigger. To the best of our knowledge, there has been only one randomized controlled trial to date, which studied the effect of FSH administration on the day of trigger but of a smaller sample size. Other studies have evaluated the role of FSH on final oocyte maturation through the use of GnRH agonist for the trigger, which causes a simultaneous release of endogenous FSH in addition to LH.

Studies have shown that FSH has an important role to play in improving the retrieval of the oocyte from the follicle. It stimulates the plasminogen activator activity within the granulosa cell, which consequently converts plasminogen to active plasmin, which in turn is involved in dissociating the oocyte from the follicular wall, weakening the wall, and facilitating the rupture

Table 1: Baseline characteristics

Baseline characteristics*	FSH on day of trigger (n=149)	No FSH on day of trigger (n=141)	p
Age (years)	29.2 ± 2.8	29.4 ± 2.7	0.59
BMI (kg/m^2)	23.5 ± 1.1	23.7 ± 0.9	0.06
Day 3 FSH (mIU/ml)	5.7 ± 1.0	5.89 ± 0.9	0.11
Serum AMH (ng/ml)	3.5 ± 1.0	3.5 ± 0.97	0.48
AFC	16.9 ± 2.4	17.0 ± 2.2	0.67
Duration of gonadotropin stimulation (days)	11.1 ± 0.7	11.1 ± 0.7	0.97
Total gonadotropin dose (IU)	2766.8 ± 626.8	2752.7 ± 619.5	0.85
Peak estradiol on day of trigger (pg/ml)	2696.6 ± 828	2731.2 ± 831.1	0.72
Serum P4 on day of trigger (ng/ml)	1.01 ± 0.2	0.98 ± 0.2	0.24
Endometrial thickness on the day of trigger (mm)	9.8 ± 1.0	10.1 ± 1.3	0.06
Number of preovulatory follicles on the day of trigger	11.8 ± 2.5	11.96 ± 2.4	0.52
Number of embryos transferred	2.1 ± 0.3	2.0 ± 0.3	0.09

*All values are presented as mean ± SD. SD=Standard deviation, AMH=Anti-Mullerian hormone, AFC=Antral follicle count, FSH=Follicle-stimulating hormone

Table 2: Assisted reproductive technique outcomes of patients receiving and not receiving follicle-stimulating hormone on the day of trigger in IVF/ICSI cycles

ART outcome	FSH on day of trigger (*n*=149)	No FSH on day of trigger (*n*=141)	*p*	Difference between the means	95% CIs	
					Lower limit	Upper limit
Number of oocytes retrieved[a]	9.4 ± 1.9	8.5 ± 2.2	< 0.001	0.9	0.38	1.33
Number of metaphase ii oocytes[a]	7.3 ± 1.8	5.2 ± 1.4	< 0.001	2.12	1.7	2.47
Number of embryos[a]	6.7 ± 1.6	4.5 ± 1.9	< 0.001	2.16	1.8	2.46
Fertilization rate[a]	0.71 ± 0.1	0.54 ± 0.1	< 0.001	0.17	0.15	0.19
Cleavage rate[a]	0.96 ± 0.1	0.95 ± 0.1	0.19	0.01	−0.006	0.03
Number of grade 1 embryos[a]	3.7 ± 1.2	3.2 ± 1.1	0.001	0.46	0.20	0.73
Percentage of cases that had blastocyst transfer (%)[b]	38/149 (25.5)	34/141 (24.1)	0.78	1.39	−0.147	0.28
Number of embryos available for cryopreservation[a]	2.2 ± 1.5	1.3 ± 0.98	< 0.001	0.98	0.69	1.27
ART outcome	**FSH on day of trigger (*n*=149), *n* (%)**	**No FSH on day of trigger (*n*=141), *n* (%)**	***p***	**Relative risk**	**95% CIs**	
					Lower limit	**Upper limit**
Implantation rate[b]	42/304 (13.8)	35/282 (12.4)	0.62	1.11	0.73	1.69
Clinical pregnancy rate[b]	42/149 (28.2)	35/141 (24.8)	0.52	1.13	0.77	1.67
Miscarriage rate[b]	3/42 (7.1)	2/35 (5.7)	0.99	0.8	0.14	4.5
Live birth rate[b]	39/149 (26.2)	33/141 (23.4)	0.59	1.12	0.75	1.67

[a]Mean ± SD, [b]*n* (%). CI=Confidence interval, ART=Assisted reproductive technique, FSH=Follicle-stimulating hormone

process.[6-9] FSH administration on the day of trigger also increases the levels of FSH in the follicular fluid, and studies have shown improved oocyte recovery with high follicular fluid FSH levels.[10] These could be the reasons why we had a significantly higher number of oocytes retrieved in patients who received FSH on the day of trigger.

There have been studies suggesting the role of FSH in the final stages of oocyte maturation. FSH promotes the formation of the LH receptors in the preovulatory follicles.[11] It ensures that the full complement of LH receptors are in place before the LH surge ensues. FSH also promotes cumulus expansion by inducing the expression of hyaluronic acid that has the hydrophilic capacity to retain water.[4,8,12,13] FSH *in vitro* improves both nuclear and cytoplasmic maturation.[12] We should remember the fact that FSH is the major gonadotropin used to fulfill *in vitro* maturation of oocytes.[4,14,15] FSH also keeps the gap junctions open between the oocyte and cumulus cells and thus a crucial role in signaling pathways.[16] FSH induces the protein kinase C pathway and causes mobilization of intracellular calcium, which results in resumption of meiosis, oocyte maturation, and subsequent fertilization.[17-19] These facts could account for the significantly higher number of metaphase ii oocytes seen in this study.

Moreover, studies have shown a higher number of metaphase ii oocytes being retrieved following the GnRH agonist trigger as compared to the hCG trigger, which could be due to the FSH surge accompanying the LH surge similar to the natural cycle.[20] This also indicates the importance of FSH in the final stages of maturation.

The significant increase in the total number of embryos and the number of embryos available for cryopreservation noted in our study could be a reflection of better oocyte recovery rates, whereas the significant improvement in the fertilization rates could be the consequence of the significantly higher number of mature good quality oocytes. Even though the clinical pregnancy rates were not significantly different between the two groups, the significantly higher number of embryos available for cryopreservation in the FSH co-administered group might serve to give higher cumulative pregnancy rates.

This study was limited by its small sample size, retrospective nature. The dose of FSH as 375 IU was chosen randomly. Moreover, as no specific criteria were chosen for the randomization of patients to each group, this could result in a bias. Another drawback of the study was the lack of correlation with the intra-follicular FSH levels. Furthermore, we did not find the cumulative pregnancy rates. Further randomized controlled trials are recommended in this regard to consider the evidence more robust.

Conclusions

FSH administration on the day of the trigger may be considered in IVF cycles receiving hCG trigger to improve the oocyte recovery and maturity if the patient

is not at increased risk of ovarian hyperstimulation and serum estradiol on the day of the trigger is < 4500 pg/ml. However, there is only an increase in the total number of oocytes retrieved and the number of mature oocytes but no significant change in the implantation, clinical pregnancy, and live birth rates.

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Conflicts of interest

There are no conflicts of interest.

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