ORIGINAL ARTICLE

Effect of recombinant follicular stimulating hormone and human menopausal gonadotrophins on the oocytes number in patients undergoing assisted reproductive technique

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ABSTRACT

Background & Objective: The data regarding the effectiveness of various protocols used for controlled ovarian stimulation (COS) in assisted reproductive techniques (ART) in our own population is scant. This study compares recombinant follicular stimulating hormone (rFSH) and human menopausal gonadotrophins (HMG) in terms of follicular numbers and oocytes retrieved in Pakistani women undergoing ART.

Methods: A total of 300 patients were selected out of 1,950 patients who visited the hospital for *in vitro* fertilization/intra cytoplasmic sperm insemination (IVF/ICSI) from June 2018 to December 2020. These patients were further divided into two categories: first category (1) was given long protocol and the second category (2) was given short antagonist protocol. Each category was further sub-divided into two groups; group A who received HMG, and group B who received rFSH for COS.

Results: There was a significantly higher number of follicles and oocytes retrieved in category 1, with rFSH (20.01 ± 4.91, 15.19 ± 9.18) versus. HMG (16.07 ± 5.67, 11.10 ± 5.07) with a *p*-value (0.00, 0.004). On the other hand, in category 2, the number of follicles was insignificant (*p*-value = 0.319) in both groups. Contrary to that the number of oocytes retrieved was significantly higher with a *p*-value of ≤ 0.05 in both groups.

Conclusion: In COS in ART, long protocol with rFSH has much better results both in terms of follicular numbers and retrieved oocytes. While for the short protocol with the antagonist, rFSH has been demonstrated to be superior to HMG but that is limited to the number of oocytes.

Keywords: *In vitro* fertilization, assisted reproductive technology, controlled ovarian stimulation, human menopausal gonadotrophins, recombinant follicular stimulating hormone.

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Introduction

Subfertility is a common problem affecting about 10% of the world population.[1] Particularly, in developing countries where it bears a strong social, psychological, and financial impact. The prevalence of subfertility in Pakistan is 22% comprising 4% primary and 18% secondary subfertility.[2] This wide margin depicts that subfertility is not only a medical problem but also a victim of socio-cultural problem. In addition, religious belief equates the inability to conceive as a failure on a personal & social level.

According to the National Institute for Health Care Excellence, *in vitro* fertilization (IVF) is the unequivocal management for unresolved and pronounced subfertility especially when other treatments have deemed failed.[3] IVF/intra cytoplasmic sperm insemination (ICSI) is the most effective and advanced form of assisted reproductive techniques (ART) which includes complex series of procedures to achieve the best outcome with minimal chances of risk.[4] Controlled ovarian stimulation (COS) is the key step of IVF/

ICSI treatment; best stimulation gives the best results.[5] While choosing stimulation protocols and appropriate drugs and dosage, there are few factors that must be kept in mind like age, levels of follicular stimulating hormones (FSH), anti-Mullerian hormone (AMH), antral follicular count (AFC), and last but not the least patient's financial background.[6]

In COS, gonadotrophins are given to stimulate the ovaries in order to achieve a maximum number of mature oocytes in IVF/ICSI. Two types of gonadotrophins are used including human menopausal gonadotrophins (HMG), extracted from the urine of menopausal women, and recombinant follicular stimulating hormone (rFSH).[7] It is always a big debate which hormone either HMG or rFSH is better for COS. The results are variable while comparing stimulation with HMG or rFSH in patients undergoing IVF/ICSI.[8] However, there are obvious differences while considering the financial values for both hormones. Along with HMG & rFSH, there is a need to add gonadotrophin-releasing hormone agonist or antagonist to avoid premature luteinizing hormone (LH) surge.

After deciding the type and dosage of stimulation hormone, there is a need to focus on the type of stimulation protocol. The two most commonly used protocols in IVF/ICSI are long and short.[9] However, there is a need to decide the type of hormone and protocol according to the individualized patient requirement followed by a tailored stimulation protocol designed for every individual.[10]

The present study aims to compare effectiveness of various protocols used for COS in ART to add a significant data in a scant pool of local literature related to our own Pakistani population. To the authors' knowledge, this is the first study from Pakistan analyzing a variety of IVF patients with respect to of different COS techniques.

Methods

It was a prospective study on couples coming for IVF/ICSI at the Lahore Institute of Fertility and Endocrinology, Pakistan. The data were collected from July 2019 to January 2021. This study was approved by the Ethical Review Committee (IRB/2020/025). The total number of patients was 1,950 and only 300 patients were selected (150 of agonist and 150 of antagonist protocol) who fulfilled the inclusion criteria. The patients who were less than 36 years of age, body mass index (BMI) of less than 30 kg/m^2, AMH > 2 pmol/l, FSH <10 IU/ml, having at least 4-10 AFC and with tubal factor but without any other female factor were included. Females showing any of the pathological conditions including polycystic ovarian disease, endometriosis, hydrosalpinx, poor ovarian reserve (FSH > 10 IU/ml, AFC count < 4, AMH < 2 pmol/l) were excluded from this study.

Preliminary assessment included hormonal profile (serum FSH, LH, prolactin, AMH, and thyroid profile) on cycle day (CD) 2-5, followed by the female transvaginal ultrasound (TVS) on CD 7-8 for the ovarian reserve (AFC), any uterine (fibroid, uterine septum, or adnexal pathology like ovarian cysts). It also included husband semen analysis with the abstinence of 3-7 days.

For stimulation protocol, an estimated dose was calculated according to the age, hormonal profile, and AFC count of the female. The long protocol was started from CD 21 of the previous cycle. Later, stimulation started with purified or recombinant FSH on CD 2-3. Serial TVS was performed on every 3-4 days to monitor the ovarian response. Once the follicles were mature (18 mm or above, seen by TVS), ovulation was triggered by human chorionic gonadotrophins (HCG) with the usual dose of 10,000 IU on the day of decision.

In short protocol, stimulation was started from CD 2-3, followed by the same protocol as in long protocol. Ultrasound oocyte pick-up was planned 34-36 hours after the trigger.

Statistical Analysis

Statistical Package for the Social Sciences 25.0 was used for data analysis; descriptive analysis was done i.e., frequencies and percentages for categorical variables whereas mean and standard deviation for numerical variables. Pearson's Chi-Square test was used to check the statistical significance. *p*-value ≤ 0.05 was considered as significant.

Results

The mean age of females was 29 + 4.5 years and the mean BMI was 25 $\pm$ 2.94 kg/m^2 in both categories. The demographic variables (age, infertility duration) were significant in both protocols except BMI in agonist and type of infertility in antagonist protocol (Table 1).

Regarding etiology, according to agonist protocol, in group A 18.7% (*n* = 14) females had a tubal factor, 20% (*n* = 15) had an unexplained subfertility, 53.3% (*n* = 40) had a male factor and 8% (*n* = 6) came for preimplantation genetic diagnosis (PGD). In group B, 17.3% (*n* = 13) females had tubal factor, 16% (*n* = 12) had unexplained factor, 53.3% (*n* = 40) had male factor and 13.3% (*n* = 10) came for PGD (*p*-value = 0.72).

On the other hand, according to antagonist protocol, in group A, 10.7% (*n* = 8) females had a tubal factor, 29.3% (*n* = 22) had an unexplained factor, 49.3% (*n* = 37) had a malefactor and 10.7% (*n* = 8) came for PGD. In group B, 12% (*n* = 9) females had tubal factor, 22.7% (*n* = 17) had unexplained subfertility, 45.3% (*n* = 34) had male factor and 20% (*n* = 15) came for PGD (*p*-value = 0.39).

According to baseline hormones, FSH was insignificant in category 1 and AMH was significant in both categories. The mean of decision day was 13 and showed significant results in category 1.

The primary efficacy criterion of the number of oocytes retrieved showed a significant difference in favor of rFSH in both agonist (category 1) and antagonist (category 2) protocols (*p*-value = 0.00, 0.04) respectively. The mean number of oocytes retrieved was 11.10 ± 5.07 for HMG and 15.19 ± 9.18 for rFSH in agonist protocol while in antagonist protocol the mean number of oocytes retrieved was 11.37 ± 6.11 in HMG and 13.51 ± 4.97 in rFSH.

There were also significant differences between the two categories in several of the secondary efficacy parameters. The number of follicles 16-18 mm diameter on the day of HCG administration was significantly greater in rFSH treatment as compared to HMG treatment (20.01 ± 4.91 compared with 16.07 ± 5.67) (18.80 ± 4.77 compared with 16.01 ± 6.48) in both categories. On the other hand, the number of follicles was significant in category 1 and insignificant in category 2 (*p*-values = 0.00, 0.31).

Serum endocrine level on the day of HCG administration estradiol (E2) and progesterone (P4) measurements were carried out in most of the patients throughout treatment. According to the results, significant association was found between rFSH and HMG hormones in group A and group B with *p*-value 0.000, 0.002 versus 0.02, 0.01, respectively. The endometrial thickness on the decision day was significant (*p*-value = 0.001, 0.004) in both protocols (Table 1).

Discussion

Management option for subfertility in the form of ART depends on certain factors of the patient. The IVF/ICSI aims to individualize the treatment options according to the patient's selection. COS is the basic key step to a successful IVF cycle. The most important factor in COS is the selection criteria of the female partner in IVF. Because the success rate of IVF/ICSI is mainly dependent on COS. In the present study, different stimulation protocols of IVF/ICSI were compared with the aim to get adequate ovarian stimulation. Adequate ovarian stimulation means a way to have adequate number of ovarian follicles and eggs retrieved through Ovum pick up (OPU), with minimal possible side effects.

Table 1. *Characteristics of patients with respect to long and short stimulation protocols.*

	Long protocol (*n* = 150)			Short antagonist protocol (*n* = 150)		
	HMG (*n* = 75)	rFSH (*n* = 75)	*p*-value	HMG (*n* = 75)	rFSH (*n* = 75)	*p*-value
Age (years) (mean ± S.D)	29.53 ± 4.59	30.79 ± 3.24	0.003	29.80 ±3.27	29.48 ± 3.52	0.000
Infertility type [*n* (%)]						
Primary	58 (77.3)	46 (61.3)	0.000	50 (66.7)	56 (74.7)	0.552
Secondary	17 (22.7)	29 (38.7)		25 (33.3)	19 (25.3)	
Duration (mean ± S.D)	6.61 ± 4.67	5.88 ± 3.10	0.02	5.69 ± 3.20	5.73 ± 3.21	0.007
BMI kg/m^2(mean ± S.D)	25.60 ± 2.94	25.36 ± 2.77	0.32	24.32 ± 3.63	25.75 ± 2.88	0.002
Etiology [*n* (%)]						
Tubal	14 (18.7)	13 (17.3)	0.727	8 (10.7)	9 (12.0)	0.394
Unexplained	15 (20.0)	12 (16.0)		22 (29.3)	17 (22.7)	
PGD	6 (8.0)	10 (13.3)		8 (10.7)	15 (20.0)	
Male factor	40 (53.3)	40 (53.3)		37 (49.3)	34 (45.3)	
Hormonal profile (mean ± S.D)						
FSH IU/l	6.52 ± 1.86	6.30 ± 1.67	0.200	5.77 ± 2.29	6.34 ± 1.94	0.000
AMH ng/ml	3.40 ± 1.39	3.75 ± 2.34	0.031	4.32 ± 1.62	3.75 ± 1.15	0.000
CD (mean ± S.D)	13.03 ± 2.06	12.79 ± 1.45	0.000	13.10 ± 1.33	13.08 ± 2.03	0.70
Serum endocrine level on HCG decision day (mean ± S.D)						
E2 IU/ml	3,155.85 ± 1,326.86	3,346.65 ± 1,015.54	0.002	3,672.24 ± 1,010.36	3,229.88 ± 1,334.67	0.01
P4 IU/ml	6.79 ± 7.43	5.49 ± 2.40	0.000	5.94 ± 2.96	6.46 ± 2.57	0.02
Ovarian response						
No. of follicles	16.07 ± 5.67	20.01 ± 4.91	0.000	16.01 ± 6.48	18.80 ± 4.77	0.319
Oocytes	11.10 ± 5.07	15.19 ± 9.18	0.004	11.37 ± 6.11	13.51 ± 4.970	0.049
Endometrial thickness (mm)	9.79 ± 1. 35	9.99 ± 1.41	0.001	9.91 ± 1.35	10.13 ± 1.32	0.004

BMI = Body mass index, PGD= Preimplantation genetic diagnosis, FSH= Follicle stimulating hormone, AMH= Anti-mullerian hormone, E2= Estradiol, P4= Progesterone.

As shown by the study of Ramalho et al.[11] for COS, the first and foremost important factor is the age of the female partner and we had found the same correlation between the two factors in the present study. Age of the female partner and ovarian reserves are directly related to each other. A study by Galey-Fontaine et al. [12] concluded that increasing age is associated with poor response in COS. Similar results were observed in the current study that more the age, the poor the response we get in COS. In the present study, the cutoff age of female partner is 35 years. The women of this age group have the best results in COS.[13]

In the present study, the other factor affecting ovarian COS is the BMI which is inversely related to the response of COS in IVF/ ICSI.[14] So that is the reason, we have only selected patients with a normal BMI and these findings are consistent with the study of Setti et al.[15] The age and BMI are initial best parameters to assess the outcome of IVF particularly in term of number of oocytes.

In the present study, primary subfertility was more prevalent as compared to secondary subfertility.[16] The reason for this trend might be financial constraints particularly in developing countries like Pakistan. After primary subfertility, male factor was the second most common (40%) factor of subfertility which is comparable to the results of the study by Kumar et al.[17] where male factor subfertility was found to be around 40%-50%.

COS results depend upon the baseline ovarian reserve. Baseline hormonal profiles, especially FSH and AMH can give the best guide about the results of stimulation. FSH and AMH are considered to be the best hormones to predict ovarian reserve in females undergoing IVF/ ICSI. [18,19]

The number of mature follicles on the day of the decision by TVS is actually the best guide about the number of eggs to be retrieved by OPU. In the present study, on average, the day of decision for the OPU was day 13 of stimulation with a cutoff value for the follicular size of 16-22 mm which is similar to the results of the study by Revelli et al.[20]

In this study, the number of follicles in both the long and short groups was compared with rFSH and HMG. The response of COS in both groups was compared in terms of oocytes retrieved. The overall response was better with rFSH in contrast to HMG in terms of retrieved oocytes similar to the results of study of Andersen et al.[21] Therefore, the response with rFSH is better as compared to that of HMG in COS.[22]

While comparing long and short protocol in COS, the results are better with short protocols, especially in terms of the number of visits and number of injections administered particularly in current COVID-19 situation. Similar results were found in the study of Duan et al.[23] However, when we compared the long and short protocol in the present study, the number of oocytes retrieved is better in long as compared to the short protocol which is comparable to the study of Ou et al.[24] Therefore. the outcome is better with rFSH in long protocol in terms of number of follicles and number of oocytes retrieved.[25]

Conclusion

In COS in ART, long protocol with rFSH has much better outcome in terms of follicular numbers and retrieved oocytes while for the short protocol with antagonist, rFSH has been demonstrated to be superior to HMG but that is limited to the number of oocytes.

Limitations of the study

To study the outcome of IVF and ICSI, a higher financial provision is required. In addition, while doing COS, the medical, financial, and social factors of the patients are some of the constraints that led us to work with a small sample size and a limited technique.

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List of Abbreviations

AFC	Antral follicle count
AMH	Anti-müllerian hormone
ART	Assisted reproductive techniques
BMI	Body mass index
COS	Controlled ovarian stimulation
HMG	Human menopausal gonadotrophin
IVF/ICSI	*In vitro* fertilization/intra cytoplasmic sperm insemination
OPU	Ovum pick up
PGD	Preimplantation genetic diagnosis
rFSH	Human recombinant follicle stimulating hormone

Conflicts of interests

None to declare.

Grant support and financial disclosure

None to disclose.

Ethical approval

The study is approved by the Institutional Ethical Review Board of Lahore Institute of Fertility and Endocrinology, Pakistan vide Letter No. IRB/2020/025.

Authors' contribution

RN: Study design, acquisition of data, and approval of final version of manuscript

YLK, HLK, AA: Concept of study and drafting of manuscript

NM, SS: Analysis of data and interpretation and revision of manuscript

RN: Data acquisition, analysis and interpretation and final drafting of manuscript

ALL AUTHORS: Approval of the final version of the manuscript to be published.

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