Journal of Gynecology and Maternal Health
ISSN: 3068-3696
ScitechJournals
Open Access

Effect of Recombinant FSH versus Human Menopausal Gonadotropin on Embryo Profile in Assisted Reproduction Patients

Abstract

Introduction: The impact of different gonadotropins on the outcome of assisted reproduction has been widely debated. The differences in oocyte and embryo quality with the rFSH and hMG may also influence the clinical outcome.

Aims/ Objectives: To determine the effectiveness of rFSH and hMG on ovarian stimulation in terms of embryo profile (total number of oocytes, percentage of mature oocytes, fertilization rate, number of grade A embryos).

Methods: A retrospective observational study was conducted whereby the medical records of all the patients who underwent ovarian stimulation for assisted reproduction using rFSH or hMG from January 2022 to December 2023 were reviewed. All the required information in terms of dose and duration of treatment, and embryo profile (total oocytes/ mature oocytes, fertilization rate, and grade A embryos) were noted and analyzed.

Results: A total of 561 cases were eligible for enrollment, of which 329 received rFSH and 232 received hMG. The mean age and body mass index of the cases in the hMG group were significantly higher. The total number of oocytes, percentage of mature oocytes, total number of embryos, and number of grade A embryos were positively associated in the rFSH group on multivariate analysis.

Conclusion: As the embryo profile in the women receiving rFSH was significantly better than those receiving hMG, FSH alone is perhaps enough for ovarian stimulation in assisted reproduction cycles. However, further studies with participants of a comparable age group and endocrinological profile in both drug groups are recommended to overcome the possible effect of age and ovarian reserve as confounding factors in this study.

Research Article

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Received: 01 Dec, 2025; **Accepted:** 12 Dec, 2025; **Published:** 19 Dec, 2025.

Background and Rationale of Study

World Health Organization defines infertility as a disease of the male or female reproductive system characterized by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Treatment of infertility comprises various strategies including medical therapy, surgical therapy, and assisted reproductive technology (ART) [2].

ART is now a leading choice of treatment for infertility and in vitro fertilization/ intracytoplasmic sperm injection are well-acknowledged procedures in ART. IVF procedures consist of various steps of which one of the most important

steps is controlled ovarian hyperstimulation (COH) whereby the ovaries are stimulated using gonadotropins, such as recombinant Follicle Stimulating Hormone (rFSH) or human Menopausal Gonadotropin (hMG) [2].

The impact of these different gonadotropin preparations on the treatment outcome in women undergoing COH for assisted reproductive technologies has been widely debated [3]. In COH, the role of FSH is vital but the significance of luteinizing hormone (LH) supplementation is controversial [4].

Most of the studies by far have focused on the clinical outcomes associated with these different types of gonadotropins. However, the embryology aspect has been widely overlooked. It is of utmost need to understand if there are relevant differences in oocyte and embryo quality parameters with the different gonadotrophin preparations used for COH as these outcomes may influence the clinical outcome as well [5,6].

With this background, this study aimed to investigate and compare the embryo profile of patients receiving human menopausal gonadotropin and recombinant FSH for controlled ovarian hyperstimulation in ART cycles. Secondarily, the duration of treatment with gonadotropin was also compared and the outcomes of each group were further compared in younger and advanced reproductive-aged women.

Materials and Methods

Study Setting

Department of Obstetrics and Gynecology, Institute of Kidney Disease and Research Centre and Institute of Transplantation Sciences, Ahmedabad, Gujarat.

Study Design

A retrospective observational study was conducted to compare the embryo profile of patients undergoing ovarian stimulation using recombinant follicle-stimulating hormone and human menopausal gonadotropin.

Study Population

All the patients who underwent ovarian stimulation for assisted reproduction in the Department of Obstetrics and Gynecology, IKDRC-ITS from January 2022 to December 2023.

Selection Criteria

Inclusion criteria:

- All patients who received either human menopausal gonadotropin or recombinant follicle-stimulating hormone for ovarian stimulation from January 2022 to December 2023.

Exclusion criteria:

The following patients were excluded from the study:

- Any patient with incomplete or lost medical records.
- Any patient who received both human menopausal gonadotropin and recombinant FSH for ovarian stimulation.
- Any patient who underwent oocyte cryopreservation after COH.

Ethical Clearance

The study was conducted after the approval from the institutional ethical committee.

Sampling Technique

The sample collection was done using the total enumeration technique.

Methodology

The medical records of all the patients who met the inclusion criteria were reviewed. All the required information was filled into a pre-designed proforma and entered into Microsoft Excel. The data was divided into two groups, Group A and Group B, to compare the outcomes in both groups. Group A was the subjects who received recombinant FSH and Group B included those who received human menopausal gonadotropin for ovarian stimulation in ART.

Ovarian stimulation protocol:

The protocol for ovarian stimulation and dose of gonadotrophins was decided on a case-to-case basis considering the patient's age, body mass index, day 2 FSH, and AFC. The patients underwent GnRh agonist or GnRh antagonist protocol. The simulation was done either using recombinant FSH [Inj.GONAL F, Merck] or hMG [Inj Menotas, Intas]. Folliculometry was performed with transvaginal ultrasound. Ovulation was triggered using recombinant hCG [10,000 IU] when at least three or more pre-ovulatory follicles attained a size of ≥ 18mm in diameter. Ovum pick-up was done 36 hours after the trigger followed by IVF or ICSI of the retrieved oocytes. The culture was checked for status at the zygote, embryo and blastocyst stages. Fertilization rate was calculated as number of 2PN divided by

total number of mature oocytes. Embryo grading was done according to Gardner Grading System.

Outcomes

1. Comparison of embryo profile of patients receiving human menopausal gonadotropin and recombinant FSH:
- Total number of oocytes retrieved
- Number/ percentage of mature oocytes
- Fertilization rate
- Number of embryos
- Number of Grade A embryos
2. Comparison of total duration of treatment with gonadotropin.
3. Single controlled ovulation induction cycle per patient was taken in to consideration.

Statistical Analysis

The data entered in Microsoft Excel was converted to SPSS (Statistical Package for Social Sciences) 11.5 for further statistical analysis. The normality of distribution was assessed by the Kolmogorov-Smirnov test. Comparison of variables in normal distribution was done using independent t-test whereas that in non-normal distribution was done using Mann-Whitney test. At 95% confidence interval, p-value ≤0.05 was considered statistically significant.

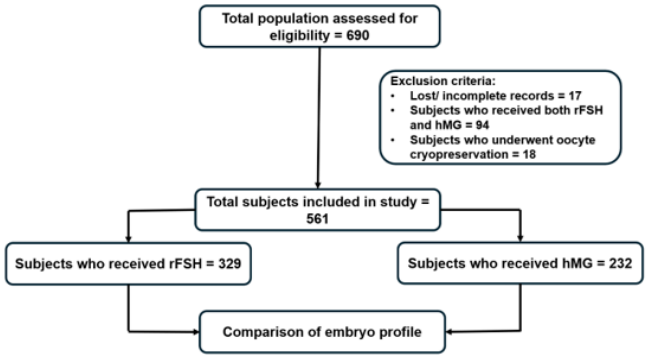

Figure: Flowchart of study design

Results

A total of 690 subjects had undergone controlled ovarian hyperstimulation (COH) during the study period of January 2022 to December 2023.

After excluding 17 subjects with lost/ incomplete medical records, 94 subjects who received both recombinant FSH and human menopausal gonadotropin for ovarian stimulation, and 18 subjects who underwent oocyte cryopreservation for various reasons, 561 subjects were finally enrolled in the study. Of the total subjects included in the study 58.6% received recombinant follicle-stimulating hormone (rFSH) while the rest 41.4% received human menopausal gonadotropin (hMG).

Groups	Gonadotropin used	Number of subjects (n = 561)	Percentage %
Group A	rFSH	329	58.6
Group B	hMG	232	41.4

Table 1: Distribution of subjects by the gonadotropin received

Baseline demographic characteristics of the subjects

Age

		Number of subjects (n = 561)	Percentage (%)
Age group (in years)	<25	27	4.8
	25 – 35	422	75.2
	≥36	112	20
Mean age (in years) 31.6 ± 4.47 (21 – 45)			

Table 2: Distribution of subjects by age group

As depicted in Table 2, most of the subjects were between 25 to 35 years of age. The ages of the subjects ranged from 21 to 45 years. The mean age of the subjects was 31.6 (±4.47) years.

Body Mass Index

		Number of subjects (n = 561)	Percentage (%)
Body mass index (BMI) (kg/m2)	<18.5	5	0.9
	18.5 – 24.9	270	48.1
	25 – 29.9	220	39.2
	≥30	66	11.8

Table 3: Distribution of subjects by body mass index (BMI)

Nearly half of the subjects included in the study were of normal body mass index (48.1%). 39.2% of the subjects were overweight and the remaining 11.8% and 0.9% of subjects were obese and underweight respectively (table 2).

Duration of Infertility

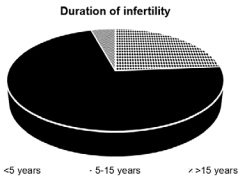

Figure 1: Distribution of subjects by the duration of infertility

The majority of the subjects, 72%, had infertility for as long as 5 – 15 years. The duration of infertility for 23.7% was less than 5 years whereas that for 4.3% was for more than 15 years (figure 1).

Cause of Infertility

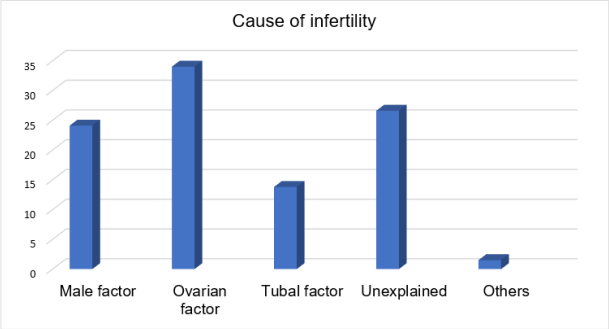

Figure 2: Distribution of subjects by cause of infertility

The most common cause of infertility among the subjects was ovarian factor (34%) which was followed by unexplained factor and male factor (26.6% and 24.1% respectively). The remaining 13.8% of subjects had tubal factor infertility and 1.5% had other factors that caused infertility.

Comparison of the subjects receiving rFSH and hMG

Baseline characteristics of subjects receiving rFSH and hMG for ovarian stimulation

The total number of subjects who underwent ovarian stimulation during the study period was grouped by the gonadotropin they received. rFSH was received by 329 subjects while 232 subjects received hMG. Baseline characteristics were compared between the two groups.

	rFSH (n = 329)	hMG (n = 232)	P-value
Age (years)	30.39±4.01	33.37±4.54	<0.001*
Body Mass Index (kg/m2)	25.01±3.47	25.92±3.50	0.003*
Duration of infertility (years)	6 (4-8)	7 (5-10)	<0.001**
Cause of infertility			
Male factor	82	53	0.392*
Ovarian factor	107	84	
Tubal factor	42	36	
Unexplained	94	55	
Others	4	4	
Ovarian stimulation protocol used			
Short protocol	178	133	<0.001*
Long protocol	86	16	
Antagonist protocol	65	83	
IVF/ICSI* **			
IVF	202	130	0.355*
ICSI	124	94	
Antral Follicle Count (AFC)	12 (8-16)	8 (6-10)	<0.001**

*Independent t-test
**Mann-Whitney U test
***No oocytes were retrieved in 3 subjects in the rFSH group and 8 subjects in the hMG group

Table 4: Comparison of the baseline characteristics of subjects in the rFSH and hMG group

The mean age of the subjects in the recombinant FSH group was 30.39±4.01 years and that in the hMG group was 33.37±4.54 years. The mean age of the subjects receiving hMG was found to be significantly higher (p-value <0.001).

The mean body mass index in the recombinant FSH and hMG group was 25.01±3.47 and 25.92±3.50 respectively which was significantly higher in the hMG group (p-value 0.003).

The duration of infertility was also found to be significantly higher in the hMG receiving group, 6 years in the rFSH group, and 7 years in the hMG group.

The causes of infertility classified as male factor, ovarian factor, tubal factor, unexplained factor, and other factors were comparable between the two groups (Table 4). Similarly, the number of subjects who underwent IVF and ICSI was also comparable in both groups (Table 4).

However, the ovarian stimulation protocol in both the groups, short protocol/ long protocol/ antagonist protocol, was significantly different in both the groups (Table 4).

The antral follicle count, suggestive of ovarian reserve, was found to be significantly higher in the group receiving rFSH, 12 vs 8 (p-value <0.001).

Endocrinological profile of patients receiving rFSH and hMG for ovarian stimulation

	rFSH	hMG	P-value
FSH (mIU/mL)	6.97 ± 2.19	8.36 ± 3.12	<0.001*
LH (mIU/mL)	7.33 (5.30 - 9.67)	5.75 (4.29 - 7.56)	<0.001**
E2 (pg/mL)	55.65 (38 - 76)	58.45 (38.87 - 82)	0.164**
TSH (µIU/mL)	1.97 (1.35 - 2.94)	2.02 (1.47 - 3.12)	0.146**

Prolactin (ng/mL)	13.85 (10.03 - 18.59)	13.14 (9.99 - 19.57)	0.881**
*Independent t-test **Mann-Whitney U test			

Table 5: Comparison of endocrinological profile of subjects receiving rFSH and hMG

Table 5 shows that the mean serum FSH level of the subjects in the hMG (8.36 ± 3.12) group was significantly higher than in the rFSH group (6.97 ± 2.19). However, the serum LH level of the subjects receiving rFSH [7.33 (5.30 - 9.67)] was significantly higher than those receiving hMG [5.75 (4.29 - 7.56)].

The serum estradiol level, serum prolactin level, and thyroid stimulating hormone levels were comparable between both groups (Table 5).

	rFSH	hMG	P-value
Total Number of Oocytes Retrieved	12 (7 - 18.5)	6 (4 - 10.75)	<0.001*
Number of Mature Oocytes	9 (5 - 15)	4 (2 - 8)	<0.001*
Percentage of Mature Oocytes	74.98 ± 20.38	70.11 ± 27.72	0.017**
Fertilization Rate	72.54 ± 26.39	71.54 ± 30.74	0.684**
Number of Embryos	6 (3 - 12)	3 (2 - 6)	<0.001*
Number of Grade A Embryos	5 (2 - 9)	2 (0 - 5)	<0.001*
*Independent t-test **Mann-Whitney U test			

Table 6: Comparison of Embryo Profile of Subjects in rFSH and hMG Group

Table 6 depicts the comparison of embryo profiles between the subjects receiving rFSH and hMG in terms of total number of oocytes, number of mature oocytes, fertilization rate, number of embryos, and number of grade A embryos.

The fertilization rate was comparable in both groups (p-value 0.684).

The number of embryos formed in the rFSH group was 6 (3–12) and that in the hMG group was 3 (2–6). The number of embryos formed in those receiving recombinant FSH was significantly higher (p-value <0.001). Similarly, the number of grade A embryos formed in the rFSH group [5 (2–9)] was also significantly more than that in the hMG group [2 (0–5)].

	rFSH	hMG	P-Value
Duration of Treatment with Gonadotropins (days)	8.57 ± 1.53	8.48 ± 1.83	0.087
Independent t-test			

Table 7: Comparison of Duration of Treatment with Gonadotropin in Subjects in rFSH and hMG Group

The duration in days was found to be statistically comparable in both groups (table 7).

The mean dose of recombinant FSH used was 1731.53 ± 344.05 IU and that of human menopausal gonadotropin in 2507.33 ± 533.62 IU.

	Young age (<35 years)			Advanced reproductive age (≥35 years)		
	rFSH	hMG	P-Value	rFSH	hMG	P-Value
Age (years)	29.24 ± 3.14	30.33 ± 3.09	0.001	36.69 ± 1.63	37.60 ± 2.27	0.012
Body Mass Index (kg/m2)	24.74 ± 3.52	24.73 ± 3.13	0.979	26.53 ± 2.75	27.58 ± 3.33	0.055
Duration of infertility (years)	6.04 ± 2.98	6.47 ± 2.55	0.143	9.86 ± 4.00	10.62 ± 4.71	0.331
Serum FSH level	6.86 ± 2.19	7.87 ± 2.52	<0.001	7.53 ± 2.13	9.04 ± 3.71	0.008
Serum LH level	7.88 ± 3.70	6.27 ± 4.39	<0.001	7.03 ± 2.90	6.27 ± 2.60	0.106
Antral Follicle Count	12 (9 - 16)	9 (7 - 12)	<0.001	10 (7 - 14)	7 (5 - 8)	<0.001

Table 8: Comparison of the baseline characteristics in young and advanced reproductive-aged subjects in rFSH and hMG group

Table 8 depicts the comparison of baseline characteristics of subjects below 35 years (young) and 35 years or above (advanced reproductive age) in both the rFSH and hMG groups.

The body mass index and duration of infertility were comparable in both young and advanced reproductive-aged subjects receiving rFSH and hMG (Table 8).

Upon comparing the endocrinological profile, it was seen that serum FSH was significantly higher in the hMG-receiving subjects while Serum LH was significantly higher in the rFSH-receiving subjects of the young age. Serum estradiol level, TSH level, and prolactin level were comparable in both age categories.

Antral follicle count, suggestive of ovarian reserve, was

significantly higher in the FSH-receiving group in both young and advanced reproductive age categories (Table 8).

Duration of treatment with gonadotropin was comparable in both the age categories of both gonadotropin receiving groups.

Embryo Profile Comparison (rFSH vs hMG)

Outcome	rFSH	hMG	P-value
Number of embryos	6 (3–12)	3 (2–6)	<0.001*
Number of Grade A embryos	5 (2–9)	2 (0–5)	<0.001*
Fertilization rate (%)	72.54 ± 26.39	71.54 ± 30.74	0.684**
Values shown as median (IQR) or mean ± SD, according to data distribution.			

Table 9: Embryo profile outcomes among women receiving rFSH versus hMG

Variable (Dependent outcome)	Effect of rFSH compared with hMG	Adjusted direction of effect	Statistical significance
Number of embryos	Higher in rFSH	Positive association	**Significant (P < 0.001)**
Number of Grade A embryos	Higher in rFSH	Positive association	**Significant (P < 0.001)**
Fertilization rate (%)	Similar between groups	No meaningful association	**Not significant (P = 0.684)**
Covariates considered: Age, BMI, duration of infertility, AFC, serum FSH/LH levels, oocyte yield, proportion of mature oocytes.			

Table 10: Multivariate comparison of embryo-quality outcomes between rFSH and hMG groups

Table 9 and 10 shows that the embryo profile in terms of the total number of retrieved oocytes, number of mature oocytes, total number of embryos formed, and number of grade A embryos formed were all significantly better in the rFSH receiving group. However, the fertilization rate was comparable in both the age categories of rFSH and hMG-receiving subjects.

Discussion

This study included 329 subjects who received rFSH and 232 subjects who received hMG.

The mean age of the subjects enrolled in this study was 31.6 years. Comparing intervention group-wise, the mean age was found to be significantly higher in the hMG group. In contrast to this finding, the study by Supriyadi et al did not find a significant age difference in the age of the subjects of the two intervention groups [2]. Such difference in findings could have been due to the retrospective study design with a total enumeration technique of sample collection.

However, many studies have commented on the adverse effects of increasing age on the adverse outcomes of assisted reproduction [2,7]. Therefore, the results of this study could be influenced by the significant difference in the age of the subjects in the two intervention groups.

The body mass index (BMI), although it appeared numerically similar, was statistically higher in the hMG group in this study. The impact of body mass index on the outcomes of assisted reproduction due to the effect on the quantity and quality of oocytes and embryos has been discussed in various articles [8,9]. The number of oocytes yielded, and available embryos were found to be significantly fewer in overweight women as compared to those with normal weight [9].

In this study, the cause of infertility indicating assisted reproduction was comparable in both groups. This finding was consistent with the findings of another study [2]. The indication of assisted reproduction does not seem to have any effect on the IVF outcome.

Similarly, the number of subjects undergoing conventional IVF and ICSI procedures was comparable in both groups. It has been discussed by another study that embryo quality is probably not influenced by the mode of fertilization (IVF or ICSI), rather it depends on intrinsic factors of the gametes involved [10].

The ovarian reserve of the subjects was evaluated in terms of antral follicle count (AFC) and serum follicle stimulating (FSH) hormone levels. AFC was significantly higher in the recombinant FSH group. Serum FSH was found to be significantly higher in the hMG group.

The influence of antral follicle count on the number of retrieved oocytes was discussed by Sun X et al whereby they concluded that the retrieved oocytes are positively correlated with serum AMH and AFC [11]. However, although AFC may be a good predictor of ovarian response, it cannot be used to predict oocyte/embryo quality or IVF outcome [12].

The association between serum FSH level and the embryo profile has been widely studied. It has been noted that high

FSH levels are either detrimental to or have no effect on the number of oocytes retrieved, fertilization rate, and the number and quality of embryos produced [13]. Likewise, another study by Abdalla H et al also concluded that higher levels of FSH were significantly associated with a lower number of oocytes collected, embryos available, and transferred embryos [14].

Comparing the embryo profile between the two groups in this study, it was noted that the total number of oocytes and the number/percentage of mature oocytes were significantly higher in the rFSH group. This could be due to the better ovarian reserve of the subjects in the rFSH group. The fertilization rate, however, was comparable in both groups. As the number of oocytes retrieved was higher in the rFSH group, the total number of embryos formed was also higher for them. Similarly, the number of grade A embryos formed was also significantly higher in the rFSH group.

The effect of predictive factors like age, BMI, and ovarian reserve on the results of this study showing better outcomes in the subjects of the rFSH-receiving group than those receiving hMG cannot be denied.

However, even in the study by Supriyadi et al where the baseline characteristics of the subjects in the two intervention groups, rFSH and hMG, were comparable, the embryo profile seemed to be better in the recombinant FSH receiving group [2].

The group of subjects receiving rFSH and hMG was further divided by age into young and advanced reproductive age for the comparison of embryo profile. The results were similar in both the subgroups.

A similar study was conducted by Tabata C et al where the embryo profile was compared in the young and advanced reproductive-aged subjects receiving recombinant FSH and human menopausal gonadotropin for ovarian stimulation. The baseline characteristics like age, BMI, and endocrine profile were comparable in both groups. Like the findings of this study, they too found a higher number of retrieved oocytes in the rFSH group below 35 years of age. The fertilization rate and number of good-quality embryos were comparable in both groups [4].

With this discussion, it appears safe to conclude that recombinant FSH is sufficient for ovarian stimulation in assisted reproduction in young women with a good ovarian reserve. The present analysis demonstrates that ovarian stimulation with recombinant FSH results in a markedly superior embryo profile compared with hMG. Even when accounting for age, ovarian reserve markers, and baseline differences in response, women receiving rFSH consistently produced more embryos and a higher number of top-quality (Grade A) embryos. The absence of a significant difference in fertilization rates between the two groups suggests that the advantage of rFSH is primarily driven by enhanced follicular recruitment and improved oocyte competence rather than by downstream laboratory processes. These findings align with the pharmacological characteristics of rFSH, which provides a more consistent and potent FSH activity than the mixed gonadotropin composition of hMG, leading to a more favorable biological response. Therefore, rFSH confers a clear advantage in embryo yield and quality, making it a compelling choice for ovarian stimulation in IVF cycles.

Limitation

This was a retrospective study that used a total enumeration technique to include all the subjects who received the gonadotropins, rFSH and hMG, for ovarian stimulation. The analysis resulted in significantly better prognostic factors, in terms of age, BMI, endocrine profile, and ovarian reserve, in the rFSH-receiving group. The outcome, embryo profile, was also found to be better in the rFSH-receiving group. It cannot be denied that these factors may directly affect the results of ovarian stimulation in assisted reproduction and may have been the confounding factors in this study.

Therefore, it is recommended that future studies be conducted in age and ovarian reserve matched individuals to avoid such confounding effects on the desired result.

Conclusion

As the embryo profile in the women receiving recombinant FSH was significantly better than those receiving hMG, FSH alone is perhaps enough for ovarian stimulation in assisted reproduction cycles, at least in the young population with a good ovarian reserve.

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Citation: Rohina Aggarwal, Deepa S, and Kunur S et al. “Effect of Recombinant FSH versus Human Menopausal Gonadotropin on Embryo Profile in Assisted Reproduction Patients.” J Gynecol Matern Health (2025): 123. DOI: 10.59462/3068-3696.3.2.123