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Original Research Article

A comparative study of rFSH, HMG, and FSH-HMG stimulation protocols on embryo quality and outcomes across first and second IVF-ICSI cycles

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ABSTRACT

Background: Optimization of ovarian stimulation is crucial for improving oocyte quality, embryo development, and implantation outcomes in IVF/ICSI cycles. This study compared clinical and embryological results of three stimulation regimens recombinant FSH (rFSH), human menopausal gonadotropin (HMG), and a combined rFSH-HMG protocol- across two consecutive IVF/ICSI cycles to evaluate potential improvements over time.
Methods: A retrospective analysis was conducted on 120 women who underwent two IVF/ICSI cycles between 2021 and 2024. Participants were grouped into rFSH (n=67), HMG (n=33), and rFSH-HMG (n=13). Stimulation characteristics and embryological outcomes, including oocyte retrieval, MII oocyte yield, fertilization, and blastocyst formation, were compared between cycles. Statistical analysis included t-tests, chi-square tests, Mann-Whitney U tests, and ROC curve analysis to determine predictive value for fertilization and blastocyst development.
Results: Baseline demographics and hormonal parameters showed no significant differences between groups. In the second cycle, the rFSH group demonstrated significantly higher oocyte retrieval, MII oocyte yield, fertilization, and blastocyst formation ($p<0.05$). ROC analysis showed rFSH-HMG had the strongest predictive value for blastocyst formation (AUC=0.963), followed by HMG (0.880) and rFSH (0.774).
Conclusions: Second-cycle IVF/ICSI outcomes improved across several embryological parameters, suggesting beneficial effects of prior stimulation or clinical adjustments. rFSH showed consistent improvement, while rFSH-HMG was the strongest predictor of blastocyst formation. Recognizing cycle-to-cycle dynamics may enhance individualized stimulation strategies and improve reproductive success.

Keywords: Human menopausal gonadotropin, Ovarian stimulation, Recombinant FSH

INTRODUCTION

Controlled ovarian stimulation (COS) is a basic building block of assisted reproductive technologies (ART), having a direct influence on the quantity of oocytes, fertilization rates, and, ultimately, the success of embryo growth and implantation.[1] Among the various gonadotropin regimens used in in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles, recombinant follicle-stimulating hormone (rFSH), human menopausal gonadotropin (HMG), and their combinations are most commonly used. Although popular, great controversy still exists regarding the relative effectiveness of these drugs, particularly the optimization of embryological success and the optimization of cumulative pregnancy rates.[2,3]

Previous literature has documented varied responses to stimulation regimens depending on the patient's ovarian reserve, age, and performance in the previous cycle.[4] Recombinant FSH, purity, and receptor affinity have also been credited with increased follicular recruitment and oocyte yield.[5] HMG, on the other hand, has FSH and LH activity and may offer a more physiological hormonal phase conducive to oocyte maturation and endometrium receptivity.[6] Combined rFSH-HMG protocol has been considered a balanced strategy, especially in suboptimal responders, but its advantages are yet to be an area of continued research.[7]

One of the leading areas of research interest in reproductive medicine is the effect of multiple IVF attempts on ovarian responsiveness and embryological performance. It has been suggested that the initial cycle might affect the intra-ovarian environment or influence clinical practice that can favorably modulate future attempts.[8,9] Few comparative data, however, have been available on cycle-to-cycle variation in outcome between stimulation protocols. This study aims to compare the clinical and embryological outcomes of three COS regimes, such as rFSH, HMG, and rFSH-HMG, in two subsequent IVF/ICSI cycles from the same group of patients. By evaluating cycles for differences in oocyte pickup, maturation, fertilization, and formation of the blastocyst between cycles, this study hopes to evaluate whether preceding stimulation influences ensuing cycle outcome, and whether there is protocol-dependent consistency that can direct personalized stimulation methods.

METHODS

The retrospective analysis was done on 120 infertile women who underwent IVF/ICSI Cycles between 2021 to 2024 in Nova IVF Fertility Center, Madurai, Tamil Nadu. The participants were divided into three groups based on the type of stimulation received: Group A, which received rFSH stimulation (n = 67); Group B, which received HMG stimulation (n = 33); and Group C, which received combined rFSH–HMG stimulation (n = 13). All patients followed an antagonist protocol for ovarian stimulation. Various parameters were analyzed, including hormonal profiles, stimulation protocols, and IVF/ICSI outcomes such as fertilization rates, embryo quality, and implantation rates across both the first and second cycles. Participants with incomplete clinical records, those who switched stimulation protocols mid-cycle, and cases involving male factor infertility were excluded from the study.

Statistical analysis was performed using IBM SPSS v26.0, with independent sample t-tests, chi-square tests, and Mann-Whitney U tests employed to evaluate the differences between groups. A significance level was set at $p<0.05$. Additionally, receiver operating characteristic (ROC) curve analysis was used to assess the predictive ability of fertilization and blastocyst formation outcomes for each stimulation protocol during the second cycle.

RESULTS

The demographic and clinical characteristics of the respondents across the three groups were compared (Table 1). The mean age, BMI, AMH, TSH, and prolactin levels were similar across the groups, with no statistically significant differences observed ($p>0.05$ for all variables). The mean age was approximately 31 years in Groups A and B, and slightly lower in Group C (30.5±3.3, p=0.829). BMI was consistent across groups, ranging from 27.6±5.8 to 28.2±3.8 (p=0.942). AMH levels were slightly higher in Group C (4.2±2.8) compared to Groups A and B, but the difference was not significant (p=0.450). Similarly, TSH and prolactin levels showed minor variations among the groups but did not reach statistical significance of p=0.475 and p=0.260, respectively. These findings suggest that baseline demographic and clinical variables were comparable across the groups, ensuring that differences in outcomes could be attributed to the stimulation protocols rather than underlying disparities in patient characteristics.

Table 1: Demographic and clinical information of the respondents.

Variables	Group A M±SD	Group B M±SD	Group C M±SD	P value
Age (yrs)	31.5±4.8	31.5±5.0	30.5±3.3	0.829
BMI	27.6±6.0	27.6±5.8	28.2±3.8	0.942
AMH	3.4±2.7	3.0±2.8	4.2±2.8	0.450
TSH	2.1±1.3	1.9±1.3	1.6±0.5	0.475
Prolactin	14.4±7.4	16.6±10.2	12.2±7.6	0.260

The embryological characteristics of the respondents were analyzed across the three groups for both the first and second IVF/ICSI cycles (Table 2). In the first cycle, there were no significant differences in baseline parameters, including AFC on the right and left ovaries of p>0.000, oocyte retrieval, MII oocytes, fertilization, and blastocyst formation of p>0.000, respectively. However, a significant difference was observed in the number of days of stimulation, with Group B requiring slightly more days compared to the other groups (p=0.021).

In the second cycle, significant differences were identified in oocyte retrieval, MII oocytes, fertilization, and blastocyst formation (p<0.000), indicating that Group A consistently performed better in these parameters compared to Groups B and C. Although AFC values on both the right and left ovaries remained similar across the groups (p=0.251 and p=0.286), the number of days of stimulation showed no significant difference (p=0.587). These findings suggest that Group A may provide better embryological outcomes, particularly in the second cycle, as reflected by improved oocyte quality, fertilization rates, and blastocyst formation.

The ROC curve analysis for fertilization and blastocyst formation during the second cycle of rFSH, HMG, and rFSH-HMG stimulation protocols demonstrates significant predictive ability across most groups (Table 3). For rFSH stimulation, the AUC values were 0.738 for fertilization and 0.774 for blastocyst formation (p<0.001 for both), indicating a good predictive ability for these outcomes. In the HMG stimulation group, fertilization had an AUC of 0.727 (p=0.002), while blastocyst formation demonstrated excellent predictive ability with an AUC of 0.880 (p<0.001). The rFSH-HMG group showed moderate predictive ability for fertilization (AUC=0.715, p=0.088) but excellent predictive ability for blastocyst formation (AUC=0.963, p<0.001).

Table 2: Embryological characteristics of the respondents.

Variables	Group A, M±SD	Group B, M±SD	Group C, M±SD	P value
First cycle				
R AFC	5.8±5.3	4.9±3.2	4.8±2.3	0.201
L AFC	5.6±5.1	7.5±18.9	5.8±2.6	0.244
Follicles >14	-	8.2±4.3	9.7±3.9	-
Days of stimulation	8.9±1.6	9.3±1.9	9.0±0.7	0.021
Oocytes retrieval	9.7±5.9	7.0±5.5	6.6±3.5	0.066
MII oocytes	5.3±2.9	3.5±3.6	4.5±2.5	0.179
Fertilization	2.4±1.5	2.1±2.1	2.4±1.2	0.329
Blastocyst	0.9±0.4	0.5±0.5	0.8±0.4	0.519
Second cycle				
R AFC	5.3±4.4	3.9±2.7	5.2±2.3	0.251
L AFC	5.5±3.8	4.5±2.5	5.2±2.4	0.286
Follicles >14	-	7.6±3.9	9.8±4.2	-
Days of stimulation	8.7±1.6	9.5±2.3	8.8±1.3	0.587
Oocytes retrieval	10.1±5.4	7.7±4.6	5.8±3.2	0.026
MII oocytes	6.2±3.4	5.4±3.2	5.1±3.1	0.043
Fertilization	4.3±2.5	3.7±2.2	3.8±2.5	0.031
Blastocyst	2.1±1.4	2.0±1.1	2.2±0.8	0.029

Table 3: ROC curve analysis for fertilization and blastocyst formation during the second cycle of rFSH, HMG, rFSH-HMG stimulation period.

Groups	Variables	AUC	SE	P value	95% CL	
					LB	UB
rFSH	Fertilization	0.738	0.043	0.000	0.655	0.822
	Blast	0.774	0.042	0.000	0.691	0.856
HMG	Fertilization	0.727	0.063	0.002	0.603	0.851
	Blast	0.880	0.045	0.000	0.792	0.969
rFSH-HMG	Fertilization	0.715	0.111	0.088	0.497	0.933
	Blast	0.963	0.044	0.000	0.877	1.000
Total	Fertilization	0.732	0.033	0.000	0.667	0.798
	Blast	0.827	0.029	0.000	0.771	0.884

Overall, the combined analysis across all groups revealed strong predictive ability for fertilization (AUC=0.732, p<0.001) and blastocyst formation (AUC=0.827, p<0.001), with tighter confidence intervals for blastocyst formation (95% CI: 0.771-0.884). Notably, the rFSH-HMG protocol exhibited the highest predictive ability for blastocyst formation, emphasizing its potential effectiveness in achieving high-quality embryos. These results underscore the utility of ROC analysis in evaluating the predictive performance of stimulation protocols on key embryological outcomes.

The ROC curve in Figure 1 demonstrates the predictive ability of fertilization and blastocyst formation during the second cycle under the rFSH stimulation protocol. The AUC for fertilization was 0.738, reflecting good predictive accuracy, while the AUC for blastocyst formation was higher at 0.774, indicating a stronger predictive capacity. These results suggest that rFSH stimulation can reliably predict outcomes, particularly for blastocyst formation.

Figure 2 illustrates the ROC curve for fertilization and blastocyst formation during the HMG stimulation period. The AUC for fertilization was 0.727, indicating good predictive ability, while the AUC for blastocyst formation was 0.880, representing excellent predictive accuracy. This highlights the superior potential of HMG stimulation in predicting blastocyst development compared to fertilization outcomes.

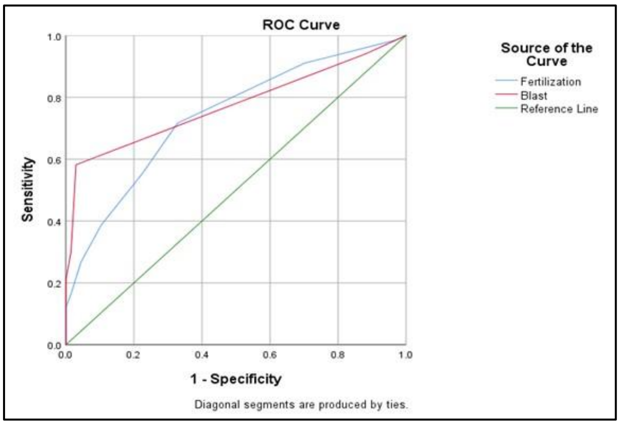

Figure 1: ROC curve for fertilization and blastocyst formation during the second cycle of rFSH stimulation period.

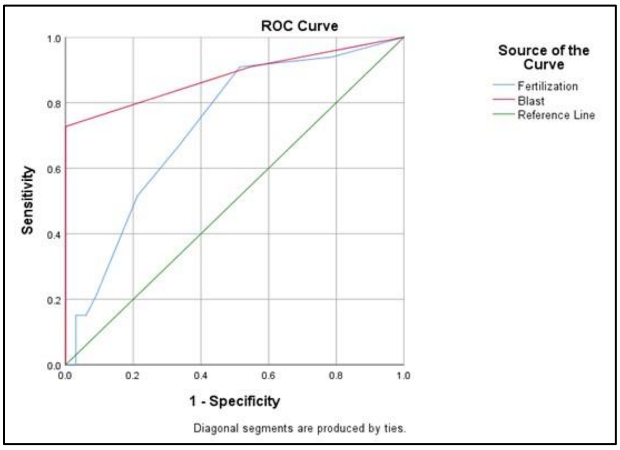

Figure 2: ROC curve for fertilization and blastocyst formation during the second cycle of HMG stimulation period.

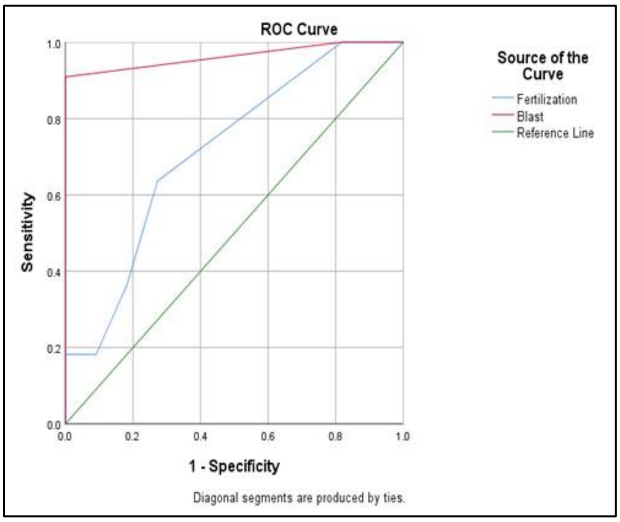

Figure 3: ROC curve for fertilization and blastocyst formation during the second cycle of rFSH-HMG stimulation period.

The ROC curve in Figure 3 shows the predictive performance of fertilization and blastocyst formation during the rFSH-HMG stimulation period. The AUC for fertilization was 0.715, reflecting moderate predictive ability, while the AUC for blastocyst formation was remarkably high at 0.963, suggesting outstanding predictive accuracy. This emphasizes the effectiveness of rFSH-HMG stimulation in achieving high-quality blastocyst outcomes.

Figure 4 combines the ROC curves for all stimulation protocols (rFSH, HMG, rFSH-HMG) during the second cycle. The AUC for fertilization was 0.732, representing good predictive ability, while the AUC for blastocyst formation was 0.827, indicating very good predictive accuracy. The combined analysis underscores the superior predictive value of blastocyst formation compared to fertilization across all stimulation protocols, with rFSH-HMG achieving the highest performance in this regard.

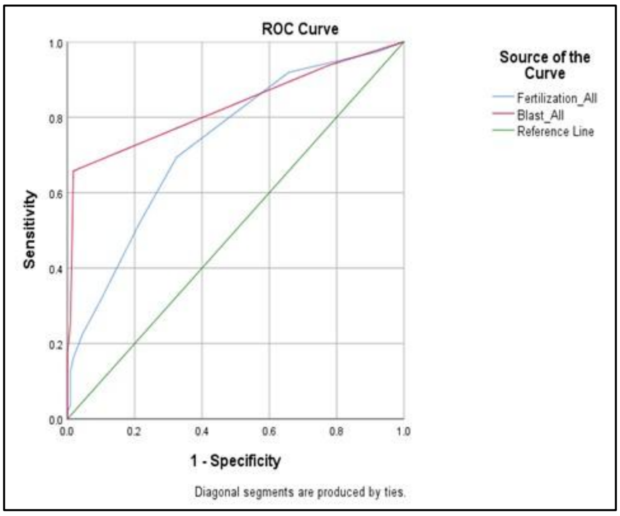

Figure 4: ROC curve for fertilization and blastocyst formation during the second cycle of rFSH, HMG, rFSH-HMG stimulation period.

DISCUSSION

The findings of this comparative study suggest that several physiological and pharmacological interactions that were induced during the first stimulation cycle may have an impact on the results obtained from the second IVF/ICSI cycle, particularly in higher rates of oocyte retrieval, fertilization, and blastocyst formation. Although the cause-and-effect relationship cannot be firmly determined, one possible explanation is that the initial cycle could have prepared the ovarian environment, thereby potentially improving responsiveness in the second stimulation.[10,11] Recurrent ovarian stimulation can induce increased follicular synchrony or increased ovarian blood flow, which in turn can induce a more consistent microenvironment for folliculogenesis during the next cycle. Another possibility is that prior gonadotropin exposure modifies the ovarian receptor sensitivity or the endocrine environment to facilitate more effective follicle recruitment and development in later cycles. Pharmacological "conditioning," with the first stimulation as a sensitizer, although not perfectly understood, is an intriguing hypothesis to investigate.

The second reason may be the individualized adjustment of the stimulation protocol based on clinical experience gained during the initial cycle. Physicians also like to modify gonadotropin doses or triggering procedures after assessing the first response, possibly resulting in improved ovarian performance in the second attempt.[12,13] This adaptive approach may be applied to account for the enhanced efficacy of certain protocols like rFSH, particularly in the second cycle. The prognostic value of blastocyst formation is also supported by the ROC curve analysis of all groups of the second cycle, with particular reference to the efficacy of the rFSH-HMG combination.[14] While the AUC values reflect a statistical advantage, the biological determinants are multifactorial and complicated. Patient-to-patient variability in ovarian reserve, oocyte quality, and intrinsic ovarian responsiveness should be considered, in addition to the efficacy of the treatment regimen.

Furthermore, while Group A (rFSH) showed a notable improvement in embryological parameters at the second cycle, one needs to be careful in the interpretation of these results. The observed improvement may not be entirely due to the stimulating agent and may be a function of an indirect effect of cumulative hormonal exposition, the interval between cycles, or even better laboratory handling and clinical expertise at the second attempt.[15] In summary, while the data reveal statistically significant changes in several of the reproductive parameters in the second cycle, with rFSH stimulation, these results are best assessed from a multi-faceted viewpoint. The interplay between patient physiology, drug pharmacodynamics, and clinical approaches unique to the cycle most likely is responsible for these changes. Further prospective studies with controlled interventions are required to determine the precise mechanisms by which events during the first cycle affect outcome in later cycles.

CONCLUSION

This research focuses on the differential effects of rFSH, HMG, and rFSH-HMG stimulation regimens on embryological results between two consecutive IVF/ICSI cycles. Remarkably, the second cycle revealed better performance in major reproductive parameters, specifically in the recovery of oocytes, fertilization, and the formation of blastocysts, most notably under rFSH stimulation. The results imply that previous cycle exposure, either through pharmacological conditioning or physiological acclimatization, could maximize ovarian responsiveness during subsequent treatment cycles.

These increases should not be interpreted as direct reactions to the stimulation regimens in and of themselves, even though they are statistically significant. Rather, they most likely result from a confluence of enhanced cycle planning, cumulative gonadotropin exposure, and physician modifications tailored to the first cycle. The increased predictive value of second-cycle blastocyst formation, especially with the rFSH-HMG regimen, also bears testimony to the merit of customizing stimulation approaches by response pattern in patients. Finally, the next IVF/ICSI cycle is a potentially useful opportunity to maximize outcomes, most likely taking advantage of the physiological basis laid down on the first attempt. Additionally, future randomized trials are required to determine the pathophysiological basis of these gains and to validate individualized stimulation protocols that optimize cumulative live birth outcomes.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Nova IVF Fertility Centre, Madurai, Tamil Nadu, India

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