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**Original Research Article**

# Efficacy of follitropin alfa versus follitropin beta in Indian women - impact on oocyte yield and pregnancy outcome: a retrospective study

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## ABSTRACT

**Background:** Recombinant follicle-stimulating hormone preparations, Follitropin alfa and Follitropin beta, are widely used for controlled ovarian stimulation in IVF/ICSI. Evidence comparing their reproductive outcomes in Indian women remains limited. This study aims to compare the efficacy of follitropin alfa and follitropin beta on oocyte yield and pregnancy outcomes among Indian women.
**Methods:** Women younger than 40 years who underwent their first ovarian stimulation cycle with either follitropin alfa or follitropin beta were included after applying the exclusion criteria. Their demographic data, follicular growth, gonadotropin dose used and duration of stimulation, oocyte yield, embryological and pregnancy outcome data are retrieved and compared.
**Results:** In this cohort of 177 patients 115 women underwent stimulation using follitropin alfa and 62 women with follitropin beta. Baseline demographic, clinical characteristics and ovarian indices were comparable. Follitropin alfa significantly produced higher oocyte (16.33±8.90 vs. 13.13±7.01) and top- intermediate quality embryo yield (8.92±5.46 vs. 7.19±4.04) than follitropin beta in a dose-independent manner, while clinical pregnancy rates (47.0% vs. 61.3%) and cumulative live birth rates (38.3% vs. 48.4%) were comparable between the groups in Indian women who underwent controlled ovarian stimulation.
**Conclusions:** The study provides encouraging evidence that in both the overall cohort and age-stratified analyses, follitropin alfa had a clear advantage in oocyte and embryo yield but no definitive superiority in final reproductive outcomes when compared to follitropin beta.

**Keywords:** Follitropin alfa and beta, Indian, ICSI, Oocyte, Pregnancy

## INTRODUCTION

Follitropin alfa and beta- the two exogenous follicle stimulating hormone (FSH) produced by recombinant DNA technology from Chinese hamster ovary cell lines, by nature of its different post translational glycosylation process and purification procedures, has variations in their biological activity, receptor binding and pharmacokinetics.[1] These differences may influence ovarian response, gonadotropin requirement, serum oestradiol dynamics, oocyte yield, embryo development, and pregnancy outcomes. Studies comparing the different gonadotropin preparations during invitro fertilisation (IVF) cycles have yielded conflicting results for oocyte yield as well as for pregnancy outcomes. Previous studies reported higher ovarian sensitivity to gonadotropins in black and Hispanic women compared with white and Asian women. However, live birth rates were highest in white women , followed by Asian women.[2] Similar ethnic study showed Indian women had lower implantation rate (IR) (30.1% versus 39.6%: p<0.001) and ongoing Pregnancy rate (OPR) (35.1% versus 41.7%: p<0.001) compared with white Caucasian women suggesting that

ethnicity, like age, is a major and an independent predictor of IVF outcome.[3] Very few studies have compared these two isoforms in Indian women, or incorporated male partner age into the analysis. This study aims to address these limitations by comparing the efficacy of follitropin alfa and follitropin beta on oocyte yield and pregnancy outcomes among Indian women. This study also captures real-world ovarian stimulation practice, wherein stimulation was initiated with recombinant FSH (r-FSH), followed by highly purified human menopausal gonadotropin in the late follicular phase, thereby underscoring the efficacy of recombinant FSH in follicular selection during controlled ovarian stimulation.

## METHODS

This is a retrospective study done between January 2020-January 2025 at the Institute of Reproductive Medicine, Madras Medical Mission, Chennai, Tamil Nadu, India.

### *Inclusion criteria*

Women who underwent their first controlled ovarian stimulation. Both partners <40 years of age. Endometrial thickness ≥8 mm on the day of embryo transfer. Cycles using donor sperms are also included.

### *Exclusion criteria*

Women with mullerian anomalies, uncontrolled diabetes, hypertension, thrombophilia, autoimmune diseases, grade III or IV endometriosis are excluded. Donor oocyte cycles, cycles with no embryo transfer done and severe male factor infertility using self-sperms are excluded.

### *Data collection*

Women who underwent ovarian stimulation using either follitropin alfa or follitropin Beta by flexible GnRH antagonist protocol were included. Daily r-FSH injections either follitropin alfa or beta were administered from day 2 of menstrual cycle in variable doses, depending on patient age, body mass index (BMI), anti mullerian hormone (AMH), antral follicle count (AFC), or ovarian responsiveness in previous cycles, and further adjusted according to serum $E_2$ levels and vaginal ultrasound measurements of follicular diameter obtained every 2 or 3 days. Serum oestradiol level was measured on the antagonist start day. On the day of antagonist start, highly purified human menopausal gonadotropins (HP-HMG) were added in addition to recombinant FSH and later switched over to HP-HMG by the end of stimulation. Depending on the response, dose of gonadotropins remained the same or increased as per clinician's discretion. When ≥3 follicle 18 mm is reached ovulation trigger injection given. Serum oestradiol level on trigger day measured. Data on follicular growth on antagonist start day and on trigger day was noted. Oocyte pick up done at 35 hours from trigger. Intracytoplasmic sperm injection (ICSI) done and embryos were cryopreserved. The first and the subsequent frozen embryo replacement (FER) of the embryos formed during that oocyte retrieval were studied. Frozen embryo transfers were done using top- intermediate quality embryos when ET- ≥8 mm and luteal phase support given. In each group, total dose of gonadotropin (both recombinant and HP-HMG) used, follicular growth, serum oestradiol rise, oocyte yield and maturity and the number of available embryos for cryopreservation assessed and pregnancy outcomes analysed.

### *Primary endpoint*

The total number of oocytes retrieved and the number of mature oocytes were recorded.

### *Secondary endpoint*

The follitropin dose required and the duration of ovarian stimulation were recorded. Implantation rate (IR) was defined as the visualization of a gestational sac on ultrasound approximately 5 weeks after embryo transfer. Clinical pregnancy (CP) rate per cycle was defined as the transvaginal ultrasound confirmation of fetal heart activity at 7 weeks in at least one frozen embryo replacement (FER) cycle. Cumulative clinical pregnancy was defined as the total number of clinical pregnancies achieved across all FER cycles using oocytes derived from a single stimulation cycle. Miscarriage rate (MR) was defined as pregnancy loss before 24 weeks of gestation in at least one FER cycle. Cumulative live birth rate (CLBR) per cycle was defined as the number of live births beyond 24 weeks of gestation achieved in at least one FER cycle.

### *Statistical analysis*

Data collected were entered in MS Excel and statistical analyses were performed using IBM SPSS Statistics version 16.0. Continuous variables are presented as mean±standard deviation and categorical variables as frequencies with percentages. Normality was assessed using the Shapiro-Wilk test. Normally distributed continuous variables were compared between groups using the independent samples t-test, with Levene's test used to verify equality of variances. Categorical outcomes were analysed using the Pearson chi-square test or Fisher's exact test as appropriate. Analysis of covariance (ANCOVA) was used to evaluate the independent effect of recombinant FSH type (follitropin alfa vs. beta). All tests were two-tailed, and a p value of <0.05 was considered statistically significant.

## RESULTS

A total of 177 women undergoing controlled ovarian stimulation (COS) followed by ICSI were enrolled. Of these, 115 received follitropin alfa (Fα) and 62 received follitropin beta (Fβ) as the primary recombinant FSH (r-FSH) agent. HP-HMG was added from antagonist start day.

**Table 1: Baseline demographic and clinical characteristics.**

| Characteristics | Follitropin alfa (n=115) | Follitropin beta (n=62) | P value |
|---|---|---|---|
| **Age (years)** | | | |
| Female | 30.10±3.39 | 30.74±3.49 | 0.233 |
| <35, N (%) | 101 (87.8) | 54 (87.1) | 0.888 |
| ≥35, N (%) | 14 (12.2) | 8 (12.9) | - |
| Male | 34.61±3.82 | 34.58±4.79 | 0.966 |
| **Anthropometric** | | | |
| BMI, kg/m² | 27.53±4.83 | 27.08±6.43 | 0.603 |
| **Infertility profile** | | | |
| Type primary, N (%) | 75 (65.2) | 48 (77.4) | 0.093 |
| Type secondary, N (%) | 40 (34.8) | 14 (22.6) | - |
| Duration (years) | 5.26±2.65 | 5.03±2.97 | 0.601 |
| **Pelvic pathology classification** | | | |
| PCOM, N (%) | 50 (43.5) | 32 (51.6) | 0.403 |
| Non-PCOM pelvic pathology, N (%) | 16 (13.9) | 10 (16.1) | - |
| No pelvic pathology, N (%) | 49 (42.6) | 20 (32.3) | - |
| **Baseline hormonal and ovarian reserve** | | | |
| Day 2 FSH, IU/l | 5.67±1.08 | 5.94±1.63 | 0.181 |
| Day 2 E2, pg/ml | 33.05±10.75 | 37.87±13.65 | 0.011* |
| Day 2 LH, IU/l | 4.67±2.58 | 5.16±2.72 | 0.247 |
| AMH, ng/ml | 4.25±2.41 (n=109) | 4.42±2.75 | 0.674 |
| AFC | 19.17±9.82 | 19.65±10.20 | 0.760 |

Note: Data are Mean±SD or N (%). Continuous variables compared by independent t-test; categorical variables by chi-square or Fisher's exact test. * p<0.05. Pelvic pathology chi-square p=0.403 (3-group).

Baseline demographic and clinical characteristics are presented in Table 1. The two groups were broadly comparable. Mean female age was 30.10±3.39 years (Fα) versus 30.74±3.49 years (Fβ); p=0.233), with over 87% of participants in both groups aged below 35 years.

Male partner age, BMI, duration of infertility, and type of infertility were similarly distributed (all p>0.05). For the purpose of pelvic-pathology stratification, participants were classified into three categories: Polycystic ovarian morphology (PCOM), non-PCOM pelvic pathology including endometriosis, adenomyosis, and leiomyoma combinations and no pelvic pathology.

The overall pelvic pathology distribution was comparable between groups (chi-square p=0.403). Baseline ovarian reserve indices- AMH, AFC, day 2 FSH and day 2 Luteinising hormone (LH)- were comparable between groups (p=0.674, 0.760, 0.181 and 0.247 respectively). Day 2 serum oestradiol (E2) was modestly higher in the Fβ group (37.87 vs. 33.05 pg/ml; p=0.011), though this was not considered clinically meaningful given equivalent reserve indices.

Ovarian stimulation characteristics are detailed in Table 2. The initial recombinant FSH given during the early follicular phase till the HP-HMG start day was significantly higher in the Fα group (1654.99±382.58 vs. 1494.36±410.80 IU; p=0.010), as was the total of r-FSH used (2000.44±581.05 vs. 1793.55±438.56 IU; p=0.015) and the total combined gonadotropin dose (r-FSH+HP-HMG) used during the entire stimulation period (3791.74±905.42 vs. 3464.52±868.93 IU; p=0.021).

These differences persisted after covariate adjustment in Analysis of Covariance (ANCOVA). Day 6 serum oestradiol was higher in the Fβ group numerically (979.13±835.54 vs. 806.22±638.00 pg/ml), though this did not reach significance (p=0.126).

Dose escalation was required in 48.7% of Fα patients versus 35.5% of Fβ patients (p=0.091); where escalation occurred, it happened earlier in the Fα arm (day 7.25±1.46 vs. 8.33±1.05; p=0.001). HP- HMG and antagonist doses used were comparable. Follicle counts on trigger day both at the >14 mm and 16-22 mm cut-offs and trigger-day serum E2 were comparable between groups. Duration of stimulation was identical (10.88±1.22 vs. 10.90±1.00 days; p=0.890).

Within-group Pearson correlation analysis demonstrated a significant positive association between patient age and total gonadotropin dose in both the Fα (r=0.296, p=0.001) and Fβ (r=0.379, p=0.002) groups, though age did not correlate significantly with stimulation duration in either group.

No patients in either group had ovarian hyperstimulation syndrome (OHSS). To isolate the independent contribution of the r-FSH preparation to differential gonadotropin

consumption, two ANCOVA models were applied with total r-FSH dose as the dependent variable (Table 3). In model 1 (Covariate: total HP-HMG dose), the gonadotropin treatment group effect remained significant after adjustment (f= 8.19; p=0.005; $r^2$=0.133). Model 2 (additional covariates: BMI, age, AMH, AFC) similarly confirmed an independent group effect (f=7.36; p=0.007; $r^2$=0.137). None of the ovarian reserve indices individually achieved significance, confirming that the follitropin type not patient characteristics is the primary determinant of differential r-FSH requirement. There was no statistically significant difference in the distribution of type of trigger used between the alfa and beta groups ($\chi^2$=3.211, DF=2, p=0.201) (Table 4).

**Table 2: Ovarian stimulation parameters.**

| Parameters | Follitropin alfa (n=115) | Follitropin beta (n=62) | P value |
|---|---|---|---|
| **Gonadotropin dosing** | | | |
| Early follicular phase r-FSH, IU | 1654.99±382.58 | 1494.36±410.80 | 0.010* |
| Total r-FSH dose, IU | 2000.44±581.05 | 1793.55±438.56 | 0.015* |
| Total HP-HMG dose, IU | 1791.30±1012.01 | 1670.97±669.26 | 0.401 |
| Total combined gonadotropin dose, IU | 3791.74±905.42 | 3464.52±868.93 | 0.021* |
| Total GNRH antagonist dose, mg | 1.58±0.47 | 1.67±0.54 | 0.246 |
| **Dose adjustment** | | | |
| Dose increase required, N (%) | 56 (48.7) | 22 (35.5) | 0.091 |
| Day of dose increase | 7.25±1.46 | 8.33±1.05 | 0.001* |
| **Stimulation duration and early follicular response** | | | |
| Duration of stimulation (days) | 10.88±1.22 | 10.90±1.00 | 0.890 |
| Day 6 serum E2, pg/ml | 806.22±638.00 | 979.13±835.54 | 0.126 |
| Follicles >10 mm on day 5/6 | 8.05±6.23 | 7.32±5.31 | 0.435 |
| Early FORT (day 5/6 follicles >10 mm/ AFC×100) | 44.67±32.04 | 38.00±23.56 | 0.152 |
| **Follicular profile at trigger day** | | | |
| Follicles of size >14 mm | 14.39±7.36 | 14.61±8.12 | 0.854 |
| Follicles of size 16-22 mm | 9.85±5.01 | 10.13±5.98 | 0.744 |
| Trigger day FORT (follicles 16-22 mm/ AFC×100) | 53.95±24.90 | 56.63±35.89 | 0.070 |
| Trigger day serum E2, pg/ml | 4354.66±2343.05 | 4515.95±2862.33 | 0.687 |
| **Age-dose correlation (Pearson R)** | | | |
| Age vs.total gonadotropin dose | R=0.296, p=0.001** | R=0.379, p=0.002** | Both significant |
| Age vs. duration of stimulation | R=0.165, p=0.079 | R=−0.120, p=0.355 | Not significant |

Note: Data are Mean±SD or N (%). * p<0.05. FORT=Follicular Output Rate. Day 6 E2 compared by equal variances not assumed (Levene's p=0.022).

**Table 3: ANCOVA: between-group differences in total r-FSH dose.**

| Source | Type III SS | DF | Mean square | F | P value |
|---|---|---|---|---|---|
| **Model 1 covariate: total HP-HMG dose** | | | | | |
| Total HP-HMG dose (covariate) | 5,207,962 | 1 | 5,207,962 | 20.13 | <0.001* |
| Treatment group | 2,118,021 | 1 | 2,118,021 | 8.19 | 0.005* |
| Error | 45,012,561 | 174 | 258,693 | — | — |
| $R^2$=0.133, adjusted $r^2$=0.123 | | | | | |
| **Model 2 covariates: BMI, age, AMH, AFC** | | | | | |
| BMI | 346,761 | 1 | 346,761 | 1.30 | 0.256 |
| Age | 825,891 | 1 | 825,891 | 3.09 | 0.080 |
| AMH | 169,645 | 1 | 169,645 | 0.64 | 0.426 |
| AFC | 880,078 | 1 | 880,078 | 3.30 | 0.071 |
| Treatment group | 1,964,995 | 1 | 1,964,995 | 7.36 | 0.007* |
| Error | 44,043,684 | 165 | 266,931 | — | — |
| $R^2$=0.137, adjusted $r^2$=0.111 | | | | | |

Note: Dependent variable=Total r-FSH dose (IU). * p<0.05. SS=Sum of Squares.

**Table 4: Distribution of ovulation trigger use in COS.**

| Group | Dual trigger | GNRH agonist | HCG | Total | P |
|---|---|---|---|---|---|
| **Alfa** | 93 (80.9%) | 6 (5.2%) | 16 (13.9%) | 115 | 0.201 |
| **Beta** | 52 (83.9%) | 6 (9.7%) | 4 (6.5%) | 62 | |
| **Total** | 145 (81.9%) | 12 (6.8%) | 20 (11.3%) | 177 | |

Chi-square test (2 DF): $\chi^2$=3.211, p=0.201. 1 cell had expected count < 5 (minimum=4.20) which slightly limits chi-square reliability.

**Table 5: Oocyte yield, fertilisation, embryo quality, and implantation rate.**

| Parameters | Follitropin alpha (n=115) mean±SD | Follitropin beta (n=62) mean±SD | P (unadjusted) | P (ANCOVA adjusted) |
|---|---|---|---|---|
| **Oocyte yield** | | | | |
| Total oocytes retrieved | 16.33±8.90 | 13.13±7.01 | 0.015* | 0.001* |
| Mature (MII) oocytes | 11.71±6.69 | 8.55±4.18 | <0.001* | <0.001* |
| MII oocyte proportion (%) | 72.93±17.00 | 67.83±15.62 | 0.051 | 0.048* |
| **Fertilisation and early embryo development** | | | | |
| No. of 2PN zygotes | 10.06±5.82 | 7.74±4.17 | 0.006* | - |
| Fertilisation rate (%) | 86.69±14.35 | 89.57±13.47 | 0.195 | 0.210 |
| Day 2 cleaved embryos (4 cell stage) | 9.64±5.59 | 7.55±4.11 | 0.010* | - |
| Cleavage rate (day 2 cleaved embryos/2PN×100) (%) | 96.56±9.97 | 97.65±5.70 | 0.431 | - |
| **Embryo quality** | | | | |
| Top- intermediate quality embryos cryopreserved | 8.92±5.46 | 7.19±4.04 | 0.030* | 0.003* |
| **Implantation** | | | | |
| Implantation rate (sacs implanted per embryo transferred) | 27.49±33.33 | 37.20±37.57 | 0.079 | 0.128 |

Note: Data are Mean±SD. Unadjusted p:independent samples t-test (equal variances not assumed where Levene's p<0.05). ANCOVA-adjusted p:covariate=total combined gonadotropin dose (rFSH+HMG). *p<0.05. Fα=Follitropin alfa; Fβ=Follitropin beta. '—'=ANCOVA not applied (rate outcome or cohort-level statistic).

**Table 6: Overall pregnancy and live birth outcomes.**

| Outcome | Fα (n=115) n/N (%) | Fβ (n=62) n/N (%) | P value |
|---|---|---|---|
| **Clinical pregnancy** | | | |
| Clinical pregnancy (CP) per cycle | 54/115 (47.0) | 38/62 (61.3) | 0.069 |
| Singleton CP | 32/115 (27.8) | 34/62 (54.8) | <0.001* |
| Multiple CP | 24/115 (20.9) | 4/62 (6.5) | 0.016* |
| Cumulative CP | 58/115 (50.4) | 38/62 (61.3) | 0.167 |
| **Miscarriage** | | | |
| Miscarriage per CP | 14/54 (25.9) | 4/38 (10.5) | 0.108 |
| Miscarriage per sac implanted | 30/92 (32.6) | 10/50 (20.0) | 0.111 |
| **Live birth** | | | |
| Cumulative live birth rate (CLBR) | 44/115 (38.3) | 30/62 (48.4) | 0.193 |
| **ANCOVA-adjusted clinical outcomes (covariate: total gonadotropin dose)** | | | |
| Clinical pregnancy per cycle | 0.470±0.501 | 0.613±0.491 | 0.160[b] |
| Cumulative clinical pregnancy | 0.504±0.568 | 0.065±0.248 | <0.001*[b] |
| Cumulative live birth rate per cycle | 0.383±0.488 | 0.484±0.504 | 0.344[b] |

Note: n/N (%) reported for categorical outcomes; Mean±SD for ANCOVA proxies. Chi-square or Fisher's exact test for categorical comparisons. *p<0.05.[b] ANCOVA-adjusted p value, covariate=Total combined gonadotropin dose.

Oocyte and embryology data incorporating both unadjusted comparisons and ANCOVA-adjusted p values (covariate: total combined gonadotropin dose) are presented in Table 5. The Fα group yielded significantly more total oocytes (16.33±8.90 vs. 13.13±7.01; unadjusted p=0.015; ANCOVA-adj. p=0.001) and more MII oocytes (11.71±6.69 vs. 8.55±4.18; unadjusted p<0.001; adj. p<0.001), confirming that the superior oocyte yield in Fα is independent of the higher gonadotropin dose administered. The MII oocyte proportion was marginally non-significant on unadjusted testing (72.93% vs. 67.83%; p=0.051) but reached significance after ANCOVA

adjustment for total gonadotropin dose (p=0.048), indicating a subtle qualitative advantage in oocyte maturation with Fα when dose is controlled. The number of 2 pronucleus (PN) zygotes (10.06±5.82 vs. 7.74±4.17; p=0.006) and day 2 cleaved embryos (9.64±5.59 vs. 7.55±4.11; p=0.010) were significantly higher in the Fα group. However, fertilisation rate (86.69% vs. 89.57%; p=0.195) defined by proportion of inseminated mature oocytes fertilised at 17 hours post ICSI and cleavage rate (96.56% vs. 97.65%; p=0.431) defined by proportion of fertilised oocytes reaching 4 celled stages on day 2 post ICSI were comparable, indicating that the absolute numerical advantage in the Fα group reflects a larger input oocyte pool rather than superior per-oocyte fertilisation. Top- intermediate quality embryos (as per Istanbul Consensus update 2025) cryopreserved were significantly greater in Fα on both unadjusted (p=0.030) and ANCOVA-adjusted (p=0.003) analyses.[4]

**Table 7: Age-stratified subgroup analysis: oocyte and embryological.**

| Parameters | Age < 35 years | | | Age ≥ 35 years | | |
|---|---|---|---|---|---|---|
| | Fα (n=101) | Fβ (n=54) | P value | Fα (n=14) | Fβ (n=8) | P value |
| **Total oocytes** | 16.71±8.90 | 14.30±6.67 | 0.082 | 13.57±8.73 | 5.25±3.15 | 0.018* |
| **MII** | 11.85±6.71 | 9.22±3.95 | 0.009* | 10.71±6.72 | 4.00±2.73 | 0.014* |
| **MII proportion (%)** | 72.15±17.65 | 67.32±15.98 | 0.096 | 78.60±9.95 | 71.25±13.30 | 0.155 |
| **Fertilisation rate (%)** | 87.15±14.59 | 90.34±12.82 | 0.179 | 83.38±12.39 | 84.38±17.36 | 0.877 |
| **Grade A/B embryos** | 9.27±5.59 | 7.82±3.93 | 0.092 | 6.43±3.67 | 3.00±1.51 | 0.021* |
| **Implantation rate (%)** | 29.32±34.17 | 37.16±36.95 | 0.188 | 14.29±23.44 | 37.50±44.32 | 0.201 |

Note: Data are Mean±SD. Independent samples t-test; equal variances not assumed where Levene's p<0.05. *p<0.05. Fα=Follitropin alfa; Fβ=Follitropin beta.

All embryos were transferred in frozen embryo replacement (FER) cycles. Follitropin alfa group had a higher embryos-transferred-per-patient than follitropin beta (3.43 vs. 2.48; z=3.60, p=0.000323). The implantation rate was numerically higher in Fβ (37.20% vs. 27.49%) but did not reach significance on either analysis (Table 5).

The clinical pregnancy rate per cycle was 47.0% (54/115) in the Fα group and 61.3% (38/62) in the Fβ group (p=0.069). Notably, the pattern of implantation differed significantly: singleton clinical pregnancies were substantially more common in Fβ (54.8% vs. 27.8%; p<0.001), while multiple (twin or higher) clinical pregnancies were significantly more frequent in Fα (20.9% vs. 6.5%; p=0.016). This difference in implantation that is more embryos implanting simultaneously per cycle in Fα likely reflects the larger cohort of available embryos transferred per FER cycle in that group rather than a higher per-embryo implantation rate. Cumulative clinical pregnancy rate did not differ significantly (50.4% vs. 61.3%; p=0.167) (Table 6).

The cumulative live birth rate (CLBR) was 38.3% (44/115) for Fα versus 48.4% (30/62) for Fβ (p=0.193). ANCOVA adjustment for total gonadotropin dose confirmed no significant group effect on either clinical pregnancy per cycle (ANCOVA p=0.160) or CLBR (ANCOVA p=0.344). However, the ANCOVA model did identify a significant group effect on cumulative clinical pregnancy as a continuous proxy (f=35.79; p<0.001), driven by the structural difference in how cumulative pregnancies accrued across successive FER cycles. Given the significant positive correlation between age and total gonadotropin dose observed within both groups, a pre-specified subgroup analysis was conducted stratifying participants by age (<35 years vs. ≥35 years) (Table 7). In women aged under 35 years comprising the large majority of the cohort (Fα 101, Fβ 54)- MII oocyte yield was significantly higher in the Fα group (11.85±6.71 vs. 9.22±3.95; p=0.009). Total oocyte yield showed a trend favouring Fα (16.71±8.90 vs. 14.30±6.67; p=0.082). Grade A/B embryos trended higher in Fα (9.27±5.59 vs. 7.82±3.93; p=0.092). Fertilisation rate, MII proportion, and implantation rate did not differ significantly in this subgroup.

In women aged 35 years or older (Fα 14, Fβ 8) the between-group differences were more pronounced. Fα yielded significantly more total oocytes (13.57±8.73 vs. 5.25±3.15; p=0.018), MII oocytes (10.71±6.72 vs. 4.00±2.73; p=0.014), and Grade A/B embryos (6.43±3.67 vs. 3.00±1.51; p=0.021). Fertilisation rate, MII proportion, and implantation rate did not differ significantly. These findings suggest that Fα confers a consistent and, in older patients, amplified advantage in absolute oocyte and embryo yield but the result should be interpreted with caution due to small sample size.

## DISCUSSION

In this retrospective cohort of 177 women undergoing antagonist-based COS followed by ICSI, the use of Follitropin alfa was associated with a clear advantage in oocyte and embryo yield compared with follitropin beta, while cumulative live birth outcomes remained broadly comparable between the two preparations. Baseline characteristics, including female and male age, BMI, infertility type and duration, pelvic pathology distribution, and ovarian reserve markers (AMH, AFC, day 2 FSH and LH), were well balanced, supporting internal validity of the comparison. The modestly higher day 2 oestradiol level in the follitropin beta group was statistically significant but small in magnitude and unlikely to drive the

substantial differences observed in stimulation and embryological parameters.

In the present cohort, women treated with follitropin alfa received significantly higher early follicular r-FSH doses, higher total r-FSH, and a higher combined gonadotropin dose (r-FSH + HP-HMG) than those receiving follitropin beta. Dose increases were more frequent and instituted earlier in the alfa arm. ANCOVA models adjusting for total HP-HMG exposure and then additionally for age, BMI, AMH, and AFC confirmed that treatment group remained an independent determinant of total r-FSH dose, whereas ovarian reserve indices did not significantly influence dose in the multivariable framework. This pattern suggests a genuine preparation-level effect on dose requirement or clinician titration behaviour rather than confounding by baseline ovarian potential.

Despite the higher gonadotropin burden, follitropin alfa delivered a superior quantitative ovarian response. Total oocyte yield and MII oocyte yield were significantly higher in the alfa group on both unadjusted and adjusted analyses. The proportion of MII oocytes was marginally non-significant in crude comparisons but achieved significance after controlling for total gonadotropin dose, indicating a modest qualitative advantage in oocyte maturation once dose is accounted for. The downstream effect on embryology was consistent: the alfa group yielded more 2PN zygotes, more Day 2 cleaved embryos, and more top–intermediate quality embryos available for cryopreservation, while fertilisation and cleavage rates per oocyte were comparable. Thus, follitropin alfa did not improve per-oocyte competence but produced a larger cohort of mature oocytes and embryos.

The superior yield observed with follitropin alfa is biologically plausible in light of known structural and glycosylation differences between recombinant FSH preparations. Changes in glycosylation alter receptor affinity and bioactivity of FSH variants, supporting the concept that follitropin alfa and beta are not pharmacologically identical despite both being standardized in international units. In clinical practice, follitropin beta has often been perceived as "more potent," and was originally marketed at somewhat lower recommended doses, reflecting this impression.[5]

However, clinical comparative data have been heterogeneous, with some studies reporting similar ovarian responses at different doses and others suggesting subtle differences in dose requirement and oestradiol production.[6,7] Earlier comparative IVF series reported broadly similar outcomes between alfa and beta, with no consistent differences in oocyte numbers, oestradiol levels, or pregnancy rates, although some suggested a trend towards lower pregnancy rates or higher estradiol levels at trigger day with beta at lower gonadotropin doses.[8,9]

More recent large retrospective cohorts have also found that, although dose requirements may differ, embryological and pregnancy outcomes are often comparable; for example, a Chinese cohort of 2,864 patients reported that follitropin beta required a higher dose than alfa but showed similar pregnancy and cumulative live birth rates.[7] A Belgian single-centre study comparing biosimilar follitropin alfa with follitropin beta found only a longer stimulation duration with alfa, with other outcomes similar.[10]

In this present cohort, the implantation rate in Fβ (37.20% vs. 27.49%), clinical pregnancy rate per cycle (61.3% vs. 47.0 %) was numerically higher than Fα but did not reach significance. The miscarriage rate per clinical pregnancy (25.9% vs. 10.5%) and miscarriage per sac implanted (32.6% vs. 20.0%) was numerically higher in the Fα group, though neither reached statistical significance (p=0.108 and p=0.111, respectively). The cumulative live birth rate (CLBR) was 38.3% (44/115) for Fα versus 48.4% (30/62) for Fβ (p=0.193) were comparable.

Broader literature has often treated follitropin alfa and beta as interchangeable under the umbrella of "recombinant FSH." In two landmark meta-analyses comparing HMG with recombinant FSH in agonist long protocols, HMG was associated with higher live birth rates than recombinant FSH overall, yet alfa and beta were grouped into a single r-FSH arm and considered "similar."[11,12] Against this mixed background, the present data contribute by demonstrating that in Indian women, under a uniform antagonist protocol with systematic HP-HMG supplementation, follitropin alfa can deliver a reproducibly higher oocyte and embryo yield, especially when adjusted for dose, even if live birth rates remain similar to follitropin beta.

The age-stratified analysis is particularly informative. In women under 35 years, follitropin alfa produced significantly more MII oocytes and showed trends towards higher total oocytes and better-quality embryos, while fertilisation indices and implantation rates were comparable. In women aged 35 years and above, the advantage became more pronounced: alfa yielded significantly more total oocytes, more MII oocytes, and more top-quality embryos than beta, again without differences in per-oocyte fertilisation or implantation.

Large registry-based analyses have shown a strong association between the number of oocytes retrieved and cumulative live birth, with a plateau occurring at different thresholds depending on age. Older women typically require larger oocyte cohorts to achieve live birth probabilities comparable to younger women (≤ 35 years).[13] Within this conceptual framework, a preparation that consistently increases oocyte and embryo yield in older patients is clinically attractive, even if a single-centre study is underpowered to detect modest differences in cumulative live birth. However, the present data illustrates that higher yield does not automatically translate into greater live birth efficiency. All transfers were performed in frozen cycles, and women in the alfa group had more

embryos transferred per patient yet the cumulative live birth rate was numerically higher with beta but not statistically significant, even after adjustment for total gonadotropin dose. Per-embryo implantation rates were similar. Moreover, the ANCOVA model demonstrating a significant group effect for "cumulative clinical pregnancy" as a continuous proxy likely reflects structural differences in repeated FER cycle accrual one group having more opportunities for repeated transfers rather than fundamental differences in embryo implantation potential. Taken together, these findings suggest that the larger embryo cohort in the alfa group was "used" by transferring more embryos per cycle, contributing to higher multiple implantations rather than clearly improving the probability of live birth per cycle or per embryo.

Clinically, Follitropin alfa may be preferable when maximizing oocyte and embryo numbers from each cycle is a priority, such as in women of advanced reproductive age or those with limited opportunity for repeated stimulation, provided embryo transfer policies are tailored to avoid unnecessary multiple gestations. Conversely, in settings where dose efficiency, cost, and strict single embryo transfer are central, the higher gonadotropin requirement associated with alfa need to be weighed against its yield advantage.

Differences in FSH isoforms and LH supplementation timing have been linked to variations in fertilisation, blastocyst development, and implantation in antagonist cycles.[14] These data underscore that stimulation outcomes reflect not only the nominal FSH dose but also isoform acidity, LH activity, and protocol design. In that context, the present finding of higher yield with follitropin alfa, under a protocol that includes mid-follicular HP-HMG, fits with the broader concept that combining distinct gonadotropin properties can shape oocyte and embryo output without necessarily altering per-embryo viability.

### *Limitations*

The retrospective, non-randomized design introduces the possibility of selection bias and residual confounding, despite well-matched baseline characteristics and multivariable adjustment. The relatively small sample size and unequal patient distribution in groups limits statistical power; therefore, the findings should be interpreted as preliminary and hypothesis-generating rather than definitive.

Embryo transfer practices were not protocol-standardized, and higher embryos-per-transfer in the alfa group likely contributed to the higher multiple pregnancy rate. The results reflect experience from a single centre using specific proprietary preparations and stimulation strategies and may not be generalizable to other biosimilars, dosing schemes, or centres with strict single embryo transfer policies.

### *Future prospects*

Although the study is limited by sample size, it provides preliminary evidence from a clinically relevant population and may serve as a basis for larger future studies. Future randomized, adequately powered trials directly comparing follitropin alfa and beta under standardized antagonist protocols ideally with consistent use of HP-HMG, with dosing protocol in place, strict single embryo transfer, and cumulative live birth as the primary outcome are needed to confirm these findings and to assess which patient subgroups are most likely to derive a meaningful benefit from the higher oocyte and embryo yield associated with follitropin alfa.

## CONCLUSION

This study demonstrates a consistent and dose-independent superiority of follitropin alfa over follitropin beta in oocyte and embryo yield but with comparable clinical pregnancy and cumulative live birth rate in Indian ethnic women undergoing COS-ICSI- a finding robust across the overall cohort and the age-stratified analysis thus suggesting a yield advantage without clear superiority in ultimate reproductive efficacy.

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