

# Follitropin Alpha versus Follitropin Beta in IVF/ICSI Cycle: A Retrospective Cohort Study

Jing-Xian Cao<sup>1</sup>, Jing-Yan Song<sup>2,3</sup> 

<sup>1</sup>Gynecology, Binzhou Hospital of Traditional Chinese Medicine, Binzhou, People's Republic of China; <sup>2</sup>The First Clinical College, Shandong University of Traditional Chinese Medicine, Jinan, People's Republic of China; <sup>3</sup>Reproductive Center of Integrated Medicine, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, People's Republic of China

Correspondence: Jing-Yan Song, The First Clinical College, Shandong University of Traditional Chinese Medicine, Reproductive Center of Integrated Medicine, The Affiliated Hospital of Shandong University of Traditional Chinese Medicine, No. 42, Wen Hua Xi Road, Lixia District, Jinan, 250011, People's Republic of China, Tel +86-0531-68617921, Email hanlingjuzei91@126.com

**Purpose:** The purpose of this study was to compare the efficacy of Follitropin alpha (Gonal-F) and Follitropin beta (Puregon) on cumulative live birth rate (CLBR), defined as the percentage of the number of patients who delivered for the first time in a single ovarian stimulation cycle and the number of patients in all oocyte retrieval cycles.

**Methods:** A retrospective cohort study including 2864 infertile patients who underwent ovarian stimulation with Puregon (group A, n=1313) and Gonal-F (group B, n=1551) was conducted between July 2015 and June 2021 at a university-affiliated reproductive medicine center. Reduce potential confounding factors between groups, propensity scores and multivariable logistic regression analyses were estimated to obtain unbiased estimates of outcomes. The primary outcome was the difference in CLBR between the two groups.

**Results:** Each group identified 1160 individuals after propensity score matching (PSM). Baseline characteristics were similar between groups after PSM. The total gonadotrophin (Gn) dose (2400 vs 2325),  $p=0.038$  and cost of Gn usage (5327.9¥ vs 7547.2¥,  $p<0.001$ ) between the Puregon and Gonal-F groups were statistically significant. Nevertheless, the pregnancy outcomes between the two groups were comparable after fresh embryo transfer and subsequent frozen-thawed embryo transfer. Additionally, there was also no difference observed in the primary outcome of CLBR (52.8% vs 55.7%,  $p=0.169$ ). Multivariable regression analysis revealed that the type of Gn was not associated with CLBR ( $p = 0.912$ ).

**Conclusion:** Gonal-F may be a reasonable option for infertile patients who are hesitant to receive more Gn dosage injections. Furthermore, Puregon can eliminate unneeded anxiety and expenses while also administering more flexibility. Taken together, these findings could well be utilized in everyday clinical practice to better inform patients when deciding on an ovarian stimulation strategy.

**Keywords:** cumulative live birth rate, follitropin alpha, follitropin beta, in vitro fertilization, propensity score matching

## Introduction

The use of Gn has experienced human menopausal gonadotropins, urinary-derived gonadotropins, and recombinant human follicle-stimulating hormone (r-FSH), and is widely used in the process of in vitro fertilization and embryo transfer (IVF-ET).<sup>1</sup> In 1988, recombinant DNA technology was used to develop the gonadotropin for higher purity gonadotropin,<sup>2</sup> Gonal-F was successfully developed by transforming subunits of human Follitropin beta genes coding  $\alpha$  and  $\beta$  into expression vectors into Chinese hamster ovary cells,<sup>3</sup> licensed in Europe in 1996. In 1997, Puregon, another recombinant human FSH, was approved.<sup>4</sup> Advocation of recombinant DNA technology has promoted the development of high purity and high-specific r-FSH, according to the composition subunit, composed of two non-covalently connected, different glycoproteins, respectively designed as  $\alpha$  subunit and  $\beta$  base. These  $\alpha$  and  $\beta$  subunits were encoded by different genes, 92 and 111 amino acids, respectively. There are currently three r-FSH products on the market, FSH  $\alpha$ , FSH  $\beta$  and FSH  $\delta$ , and a fourth product, FSH  $\epsilon$ , is reported to be under development. In a systematic review and meta-analysis, 7 randomized controlled trials (RCTs) were included to compare the effects of personalized r-FSH  $\delta$  with traditional r-FSH

$\alpha/\beta$  in gonadotropin releasing hormone (GnRH) antagonist IVF/ICSI cycles. Neither group demonstrated significant differences in clinical pregnancy rates, ongoing pregnancy rates, or live birth rates.<sup>5</sup> r-FSH $\alpha$  (Gonal-F) and r-FSH $\beta$  (Puregon) in IVF/intracytoplasmic sperm injection (ICSI) are widely used, with many disclosed, safe and clinical evidence and experience.<sup>6,7</sup> The two preparations are similar to pituitary and urinary derived FSH, but show slight differences in carbohydrate side chain structure, containing more alkali and less acidic hormones than urinary derived gonadotrophin preparations.<sup>2</sup>

r-FSH $\alpha$  and r-FSH $\beta$  have been compared in previous studies, and although there are some differences between the two pharmacokinetics, they have shown similar safety and efficacy in clinical trials.<sup>8</sup> It has also been suggested that the differences in the isomer profiles of the two r-FSH preparations may affect their clinical efficacy.<sup>9</sup> In a recent study, the dose of r-FSH $\beta$  was lower than that of r-FSH $\alpha$ , and no significant difference was observed in oocyte production, but the clinical pregnancy rate was lower in the r-FSH $\beta$  group than that in the r-FSH $\alpha$  group. These discrepancies may be related to the individual characteristics of the subjects and the different procedures of ovulation induction.<sup>10</sup> In this retrospective cohort study, we aim to examine the ovarian responsiveness and cost of two different r-FSHs in Chinese women.

Since they were authorized by the European Medicines Agency, there has been debate over the efficacy and safety of two r-FSH preparations.<sup>8,11–13</sup> Various clinical parameters of r-FSH $\alpha$  and r-FSH $\beta$  have been assessed, but few studies have included CLBR as the primary outcome. The CLBR has been paid increasingly attention, and has been used as the main pregnancy outcome of IVF/ICSI. The ultimate goal of assisted reproductive technology (ART) is to successfully conceive and deliver a live baby, and CLBR is of even greater clinical significance for the patient's physical, mental, and economic well-being, as well as for the clinician's treatment plan. Therefore, we conducted this retrospective study to analyze all cycles including fresh and frozen embryo transfer after one ovarian stimulation cycle of ovum pick-up, pregnancy outcomes including CLBR were compared between Gonal-F and Puregon groups to explore more optimized medication regimens for ovarian stimulation.

## Materials and Methods

### Study Design and Population

A retrospective cohort study of 2864 IVF/ICSI patients at the Affiliated Hospital of Shandong University of Traditional Chinese Medicine (SDUTCM) from July 2015 to June 2021 was conducted. Reproductive Ethics Committee of the Affiliated Hospital of SDUTCM approved this study (No. SDUTCM-RME2021-0212). Informed consent was obtained from all subjects.

### Eligibility Criteria

Inclusion criteria: (1) age 21–45 at oocyte retrieval; (2) ovarian stimulation with GnRH agonist (GnRH-a) long protocol and GnRH antagonist (GnRH-ant) long protocol; (3) Patients undergoing their first oocyte retrieval cycle; (5) The endometrial preparation protocol for all subsequent frozen-thawed embryo transfer cycles was artificial cycle.

Exclusion criteria: 1) A history of uterine abnormalities, either congenital or acquired; 2) patients undergoing blastocyst biopsy for preimplantation genetic testing (PGT); 3) failure to transfer all embryos following one egg retrieval cycle; 4) patients who underwent in vitro maturation (IVM); 5) embryos derived from previous ovarian stimulation cycles (ie, cryopreserved oocytes and/or donor oocytes); 6) incomplete case and follow-up data. All patients were divided into two groups according to the ovarian stimulation drugs. A total of 1313 patients who received Puregon injections (Group A) and 1551 patients who received Gonal-F injections (Group B) were included in the ovarian stimulation cycles.

### Ovarian Stimulation

For ovarian stimulation, all participants underwent either the pituitary down-regulated GnRH-a protocol or a GnRH-ant protocol comprising daily injections of r-FSH (Gonal-F or Puregon). The initial daily dose of r-FSH (112.5–300U) was determined by patients' age, anti-Müllerian hormone (AMH), basic antral follicle count (AFC), body mass index (BMI), and basic serum FSH. Follicle size and hormone levels were monitored during ovarian stimulation, which led to adjustments in the dose. Transvaginal ultrasound (TVUS) is essential for monitoring the ovarian response and serum level of steroid hormones,

which allows adjusting the dosage of Gn and promoting oocyte maturation. After hCG administration or GnRH-a combined hCG administration, cumulus oocyte complexes were collected by TVUS-guided needle aspiration 36–37 hours later depending on the size of follicles (at least two dominant follicles of  $\geq 18$ mm diameter) and serum estradiol levels (over 300 pg/ml). Following ovum collection, oocytes are fertilized with conventional insemination (standard IVF) or ICSI, as indicated.

## Embryo Culture and Embryo Transfer

Following insemination, the presence of two clearly distinct pronuclei containing nuclei was evaluated as a sign of normal fertilization 16–18 hours later. Under ultrasound supervision, embryo transfer was routine. Patient age, previous failed attempts, and embryo quality were considered when determining the number of embryos to be transferred. Cryopreservation of surplus embryos and blastocysts of high quality was performed using the fast freezing procedure described previously.<sup>14</sup> Embryos that reached at least the six-cell stage on Day 3 with less than 20% fragmentation were considered to be of good quality. AA, AB, BA or BB quality trophectoderm and inner cell mass scores were required for Day 5 blastocysts to be considered good quality. Regardless of the developmental stage or number of embryos transferred, our center consistently adheres to the principle of transferring one high-quality Day 3 embryo, two suboptimal Day 3 embryos, or one high-quality Day 5 blastocyst.<sup>15–17</sup> Until the 10th gestational week, intramuscular progesterone (20mg, Zhejiang Xianju Pharmaceutical Co., Ltd.) was administered at a daily dose of 40mg or 90mg of vaginal progesterone (8% Crinone; Merck-Serono), with oral progesterone (20mg, Dydrogesterone, Abbott Biologicals B.V.) twice daily. LPS was discontinued without pregnancy.

## Assessment of Pregnancy Outcomes

In this study, the primary outcome was the CLBR, which was calculated as a percentage of the number of patients who delivered for the first time at 24 weeks or more during an ovarian stimulation cycle (including fresh and frozen ETs) divided by the number of patients undergoing all oocyte retrieval cycles. Following fresh ETs, the secondary outcome measures included LBR, positive pregnancy rate, clinical pregnancy rate, ectopic pregnancy rate, and miscarriage rate. Serum  $\beta$ -HCG was measured 14 days after embryo transfer. By TVUS, clinical pregnancy is defined as an intrauterine gestational sac with a fetal heartbeat detected after 7 weeks; an ectopic pregnancy is defined as the development of the fertilized egg outside of the uterus; miscarriage is defined as termination of pregnancy at less than 28 weeks of gestation with a fetus weighing less than 1000g; a live birth occurs at a minimum of 24 weeks or 500 grams.

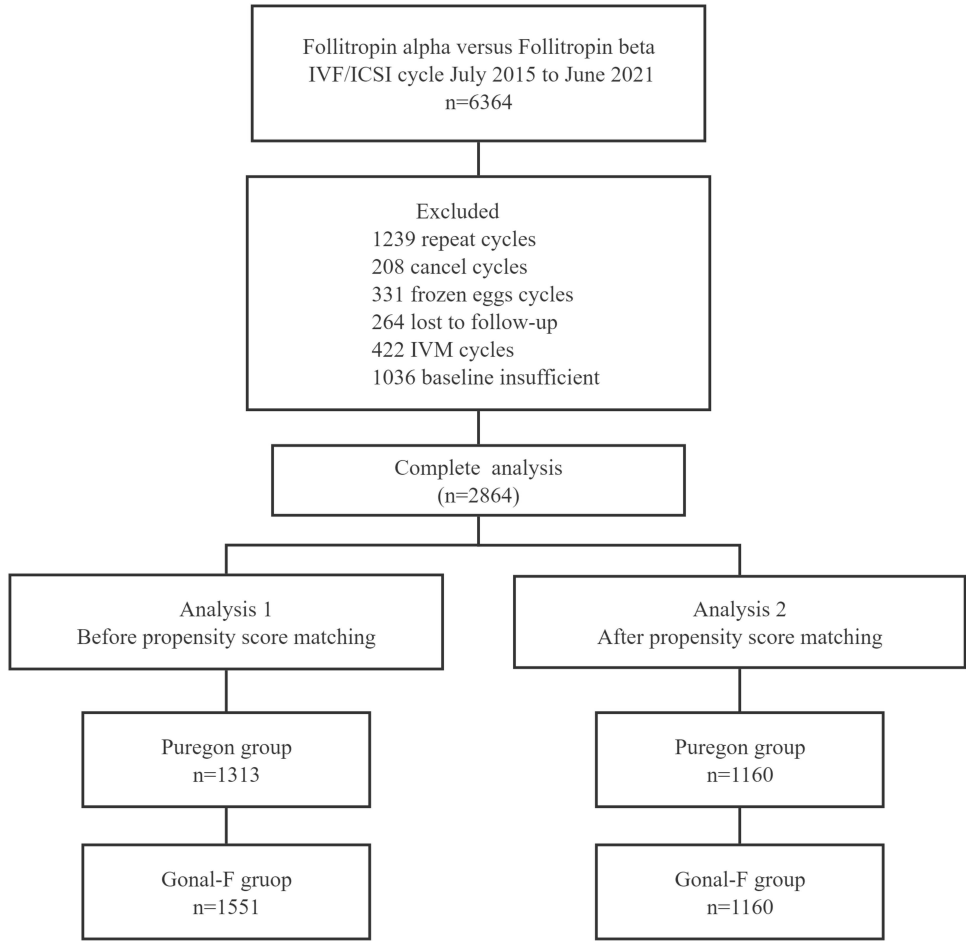
## Propensity Score Matching and Statistical Analysis

SPSS 26.0 (SPSS Inc., Chicago, IL, USA) was used to analyze all data. Data normality was assessed using the Shapiro-Wilk test. A student *t*-test or a Mann-Whitney U-test, as appropriate, was used to analyze the quantitative variables based on their normality. Frequency and percentage were used to express qualitative variables. The  $\chi^2$  or Fisher's exact test was performed to compare categorical variables. For all statistical tests, a two-tailed probability of 0.05 was applied. Moreover, PSM adjustments were made to account for potential confounding factors and selection biases. Using the Logit model, the personal propensity score (PS) was calculated. A nearest-neighbor random matching algorithm was used to pair the Puregon group with the Gonal-F group in a ratio of 1:1. In the PS, the caliper width was 0.01 standard deviation. With CLBR as the dependent variable, multivariable logistic regression analysis was conducted for ovarian stimulation medication type (Follitropin  $\alpha$  versus Follitropin  $\beta$ ) as the primary independent variable. In the analysis, female age, BMI, AFC, AMH, basic FSH, ovulation trigger drugs, and number of retrieved oocytes were considered as potential contributing factors. A logistic regression model was constructed simultaneously for all variables. An odds ratio (OR) with a 95% confidence interval is presented for CLBR following IVF/ICSI.

## Results

### Baseline Characteristics of the Study Population

The flow chart in [Figure 1](#) represents the study population. 2864 cycles out of a total of 6364 fulfilled the inclusion criteria. 1313 were identified as Puregon (Group A) and 1551 as Gonal-F (Group B). In [Table 1](#), the differences in duration of infertility



**Figure 1** The flow chart of study enrolment.

( $p = 0.255$ ), gravidity ( $p = 0.050$ ), infertility type ( $p = 0.229$ ), BMI ( $p = 0.465$ ) and basal  $E_2$  ( $p = 0.489$ ) were not statistically significant ( $p > 0.05$ ). There were significant differences in age ( $p < 0.001$ ), etiology of infertility ( $p < 0.001$ ), parity ( $p = 0.012$ ), AMH ( $p = 0.032$ ), basal FSH ( $p = 0.037$ ), basal luteinizing hormone ( $p = 0.001$ ), and AFC ( $p = 0.009$ ). Nevertheless, as shown in [Table 1](#), there was no longer a significant difference between the two groups after PSM. The distributions of PS and standardized difference were shown in [Figure 2](#).

**Table 1** Baseline Characteristics

| Characteristic                                  | Before Propensity Score Matching |                      |                     | After Propensity Score Matching |                      |                    |
|-------------------------------------------------|----------------------------------|----------------------|---------------------|---------------------------------|----------------------|--------------------|
|                                                 | Puregon<br>(Group A)             | Gonal<br>F (Group B) | P-value             | Puregon<br>(Group A)            | Gonal<br>F (Group B) | P-value            |
| <b>Patients</b>                                 | <b>1313</b>                      | <b>1551</b>          |                     | <b>1160</b>                     | <b>1160</b>          |                    |
| Female age at oocyte retrieval (years; mean±SD) | 32.1±4.7                         | 31.3±4.4             | <0.001 <sup>a</sup> | 32.0±4.7                        | 31.9±4.5             | 0.593 <sup>a</sup> |
| Etiology of infertility                         |                                  |                      | <0.001 <sup>c</sup> |                                 |                      | 0.083 <sup>c</sup> |
| Tubal factor                                    | 1140 (86.8)                      | 1293 (83.4)          |                     | 1007 (86.8)                     | 990 (85.3)           |                    |
| Male factor                                     | 47 (3.6)                         | 52 (3.4)             |                     | 43 (3.7)                        | 41 (3.5)             |                    |
| PCOS                                            | 90 (6.9)                         | 177 (11.4)           |                     | 81 (7.0)                        | 110 (9.5)            |                    |
| Endometriosis                                   | 36 (2.7)                         | 29 (1.9)             |                     | 29 (2.5)                        | 19 (1.6)             |                    |

(Continued)

Table 1 (Continued).

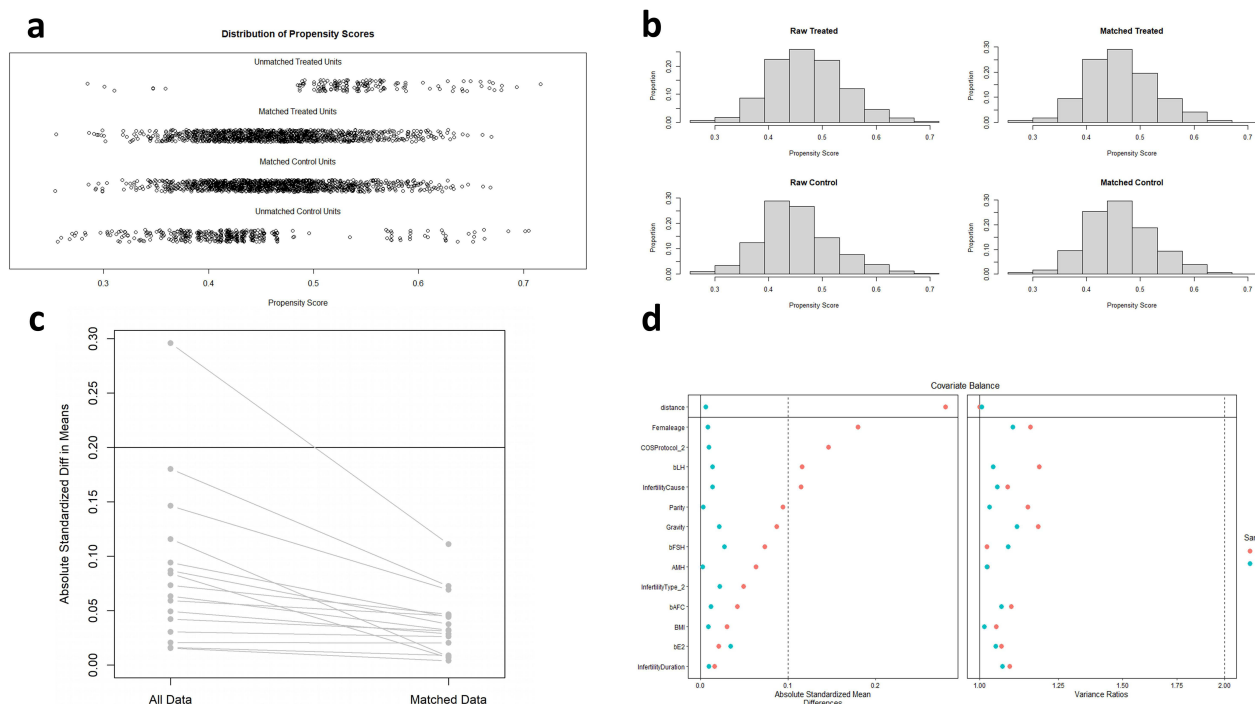
| Characteristic                                | Before Propensity Score Matching |                   |                    | After Propensity Score Matching |                   |                    |
|-----------------------------------------------|----------------------------------|-------------------|--------------------|---------------------------------|-------------------|--------------------|
|                                               | Puregon (Group A)                | Gonal F (Group B) | P-value            | Puregon (Group A)               | Gonal F (Group B) | P-value            |
| Duration of infertility (years; median (IQR)) | 3(2,4)                           | 3 (2,4)           | 0.255 <sup>b</sup> | 3 (2, 4)                        | 3 (2, 4)          | 0.571 <sup>b</sup> |
| Gravidity (median (IQR))                      | 1 (0,2)                          | 1 (0,2)           | 0.050 <sup>b</sup> | 1 (0, 2)                        | 1 (0, 2)          | 0.929 <sup>b</sup> |
| Parity (median (IQR))                         | 0 (0,1)                          | 0 (0,0)           | 0.012 <sup>b</sup> | 0 (0, 1)                        | 0 (0, 1)          | 0.507 <sup>b</sup> |
| Infertility type                              |                                  |                   | 0.229 <sup>c</sup> |                                 |                   | 0.739 <sup>c</sup> |
| Primary infertility                           | 607 (46.2)                       | 752 (48.5)        |                    | 540 (46.6)                      | 548 (47.2)        |                    |
| Secondary infertility                         | 706 (53.8)                       | 799 (51.5)        |                    | 620 (53.4)                      | 612 (52.8)        |                    |
| BMI (kg/m <sup>2</sup> ; mean (SD))           | 23.6±3.8                         | 23.5±3.9          | 0.465 <sup>a</sup> | 23.5±3.8                        | 23.5±3.9          | 0.650 <sup>a</sup> |
| AMH (ng/ml; median (IQR))                     | 2.6 (2.1, 3.5)                   | 2.7 (2.2, 3.5)    | 0.032 <sup>b</sup> | 2.7 (2.2, 3.5)                  | 2.6 (2.2, 3.4)    | 0.667 <sup>b</sup> |
| bFSH (mIU/ml; mean (SD))                      | 7.4±2.8                          | 7.2±2.7           | 0.037 <sup>a</sup> | 7.3±2.6                         | 7.3±2.8           | 0.745 <sup>a</sup> |
| bLH (mIU/ml; median (IQR))                    | 4.7 (3.3, 6.6)                   | 5.1 (3.7, 6.9)    | 0.001 <sup>b</sup> | 4.9 (3.4, 6.8)                  | 5.0 (3.5, 6.6)    | 0.710 <sup>b</sup> |
| bE <sub>2</sub> (pg/ml; mean±SD)              | 40.7±14.9                        | 40.4±14.5         | 0.489 <sup>a</sup> | 40.2±14.1                       | 40.5±14.9         | 0.680 <sup>a</sup> |
| Number of antral follicles (median (IQR))     | 12 (8, 19)                       | 13 (9, 18)        | 0.009 <sup>b</sup> | 13 (8, 19)                      | 13 (8, 19)        | 0.746 <sup>b</sup> |

**Notes:** Data are presented as numbers (%) unless otherwise noted. Data are presented as mean±SD, median (IQR) or n (%). <sup>a</sup>t-test for Equality of Means. <sup>b</sup>Independent-Samples Mann-Whitney U-Test. <sup>c</sup> X<sup>2</sup>-test.

**Abbreviation:** IQR, interquartile range; BMI, body mass index; AMH, anti-müllerian hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; GnRH-a, gonadotropin releasing hormone agonist; GnRH-ant, gonadotropin releasing hormone antagonist; 2PN, double pronuclear fertilization; Gn, gonadotropin; IU, international units.

## Ovarian Stimulation and Embryo Outcomes

In Table 2, after PSM, the total amount of Gn in Puregon group was significantly higher than that in Gonal-F group (2400 (1925, 3075) vs 2325 (1800, 3037.5),  $p = 0.038$ ), however, the total cost of Gn usage in Puregon group was significantly lower (5327.9¥ (4273.4, 6812.5) vs 7547.2¥ (5843.0, 9860.1),  $p < 0.001$ ). Differences in other parameters between groups were not statistically significant ( $p > 0.05$ ), as shown in Table 2.



**Figure 2** Distribution of propensity score (a and b) and standardized difference (c and d) before and after matching for Puregon and Gonal-F groups.

**Table 2** Ovarian Stimulation Outcomes

| Characteristic                                          | Before Propensity Score Matching |                         |                     | After Propensity Score Matching |                         |                     |
|---------------------------------------------------------|----------------------------------|-------------------------|---------------------|---------------------------------|-------------------------|---------------------|
|                                                         | Puregon (Group A)                | Gonal F (Group B)       | P-value             | Puregon (Group A)               | Gonal F (Group B)       | P-value             |
| <b>Patients</b>                                         | <b>1313</b>                      | <b>1551</b>             |                     | <b>1160</b>                     | <b>1160</b>             |                     |
| Ovarian stimulation protocol                            |                                  |                         | <0.001 <sup>c</sup> |                                 |                         | 0.772 <sup>c</sup>  |
| GnRH-a long protocol                                    | 961 (73.2)                       | 1245 (80.3)             |                     | 874 (75.3)                      | 880 (75.9)              |                     |
| GnRH-ant protocol                                       | 352 (26.8)                       | 306 (19.7)              |                     | 286 (24.7)                      | 280 (24.1)              |                     |
| No of days of COS (mean±SD)                             | 8.4±2.8                          | 9.1±2.9                 | <0.001 <sup>a</sup> | 8.6±2.7                         | 8.6±2.7                 | 0.741 <sup>a</sup>  |
| Total Gn dose administered (IU; median (IQR))           | 2450 (1950, 3150)                | 2250 (1725, 2925)       | <0.001 <sup>b</sup> | 2400 (1925, 3075)               | 2325 (1800, 3037.5)     | 0.038 <sup>b</sup>  |
| Total cost of Gn usage (RMB; median (IQR))              | 5438.9 (4328.9, 6992.8)          | 7303.8 (5599.5, 9494.9) | <0.001 <sup>b</sup> | 5327.9 (4273.4, 6812.5)         | 7547.2 (5843.0, 9860.1) | <0.001 <sup>b</sup> |
| Serum LH on OPU (mIU/ml; median (IQR))                  | 1.4 (0.9,2.4)                    | 1.4 (0.9,2.3)           | 0.928 <sup>b</sup>  | 1.4 (0.9, 2.3)                  | 1.5 (0.9, 2.4)          | 0.609 <sup>b</sup>  |
| Serum E <sub>2</sub> on OPU (pg/ml; median (IQR))       | 3715.0 (2180.2, 4839.0)          | 3941.0 (2364.0, 4856.0) | 0.011 <sup>b</sup>  | 3773.0 (2304.0, 4839.0)         | 3840.5 (2279.5, 4856.0) | 0.351 <sup>b</sup>  |
| Serum progesterone on OPU (ng/ml; median (IQR))         | 1.2 (0.8, 1.7)                   | 1.1 (0.8, 1.5)          | 0.001 <sup>b</sup>  | 1.2 (0.8, 1.7)                  | 1.1 (0.8, 1.6)          | 0.162 <sup>b</sup>  |
| Ovulation trigger                                       |                                  |                         | 0.656 <sup>c</sup>  |                                 |                         | 0.590 <sup>c</sup>  |
| hCG                                                     | 1204 (91.7)                      | 1415 (91.2)             |                     | 1072 (92.4)                     | 1065 (91.8)             |                     |
| GnRH-a and hCG (dual trigger)                           | 109 (8.3)                        | 136 (8.8)               |                     | 88 (7.6)                        | 95 (8.2)                |                     |
| No of oocytes retrieved (median (IQR))                  | 13 (8,19)                        | 14 (9,20)               | 0.012 <sup>b</sup>  | 13 (9, 19)                      | 13 (8, 20)              | 0.946 <sup>b</sup>  |
| Grade I embryos on D3 (median (IQR))                    | 1 (0, 2)                         | 1 (0,2)                 | 0.006 <sup>b</sup>  | 1 (0, 2)                        | 1 (0, 2)                | 0.791 <sup>b</sup>  |
| Grade II embryos on D3 (median (IQR))                   | 2 (1,4)                          | 3 (1,4)                 | 0.002 <sup>b</sup>  | 3 (1, 4)                        | 2 (1, 4)                | 0.923 <sup>b</sup>  |
| No of blastocysts available for transfer (median (IQR)) | 2 (1, 3)                         | 2 (1, 3)                | 0.400 <sup>b</sup>  | 2 (1, 3)                        | 2 (1, 3)                | 0.919 <sup>b</sup>  |
| Total number of normally fertilized eggs (median (IQR)) | 8 (4, 11)                        | 8 (5, 12)               | 0.008 <sup>b</sup>  | 8 (5, 12)                       | 8 (4, 12)               | 0.754 <sup>b</sup>  |
| Day of transfer                                         |                                  |                         | 0.119 <sup>c</sup>  |                                 |                         | 0.580 <sup>c</sup>  |
| Day 3                                                   | 377 (84.2)                       | 394 (80.2)              |                     | 319 (83.7)                      | 324 (82.2)              |                     |
| Day 5                                                   | 71 (15.8)                        | 97 (19.8)               |                     | 62 (16.3)                       | 70 (17.8)               |                     |
| No. of embryos transferred                              |                                  |                         | 0.204 <sup>c</sup>  |                                 |                         | 0.148 <sup>c</sup>  |
| SET                                                     | 96 (21.4)                        | 89 (18.1)               |                     | 74 (19.4)                       | 61 (15.5)               |                     |
| DET                                                     | 352 (78.6)                       | 402 (81.9)              |                     | 307 (80.6)                      | 333 (84.5)              |                     |
| Ovarian stimulation cycle outcomes                      |                                  |                         | 0.162 <sup>c</sup>  |                                 |                         | 0.567 <sup>c</sup>  |
| Cycle with fresh embryo transfer                        | 448 (34.1)                       | 491 (31.7)              |                     | 381 (32.8)                      | 394 (34.0)              |                     |
| Cycle with freeze-all strategy                          | 865 (65.9)                       | 1060 (68.3)             |                     | 779 (67.2)                      | 766 (66.0)              |                     |
| OHSS                                                    | 58 (4.4%)                        | 79 (5.1%)               | 0.398               | 49 (4.2%)                       | 67 (5.3%)               | 0.086               |

**Notes:** Data are presented as mean±SD, median (IQR) or n (%). <sup>a</sup>t-test for Equality of Means. <sup>b</sup>Independent-Samples Mann-Whitney U-Test. <sup>c</sup> X<sup>2</sup>-test.

**Abbreviation:** IQR, interquartile range; LH, luteinizing hormone; GnRH-a, gonadotropin releasing hormone agonist; GnRH-ant, gonadotropin releasing hormone antagonist; Gn, gonadotropin; IU, international units; hCG, human chorionic gonadotropin; SET, single embryo transfer; DET, double embryo transfer; OHSS, ovarian hyperstimulation syndrome.

## Pregnancy Outcomes

As shown in Table 3, before and after PSM, the difference in pregnancy outcomes between the two groups of fresh embryo transfer was not detected. However, in frozen-thawed embryo transfer (FET) cycles, we found significantly reduced rates of positive pregnancy (64.3% vs 69.9%;  $P=0.007$ ), clinical pregnancy (60.6% vs 65.5%;  $P=0.020$ ), LBR (55.1% vs 60.4%;  $P=0.015$ ) and CLBR (52.5% vs 57.8%;  $P=0.005$ ) in the Puregon group compared with the Gonal-F group before matching. Nonetheless, the differences between the two groups, particularly for the primary outcome CLBR (52.8% vs 55.7%,  $P=0.169$ ), were not statistically significant after matching.

## Multivariable Regression Analysis of CLBR

As shown in Table 4, multivariable logistic regression analysis, allowing adjustment for relevant confounders, revealed that female age, AMH, and number of oocytes retrieved were the only variables significantly associated with CLBR (OR 0.74,

**Table 3** Pregnancy Outcomes

| Characteristic                    | Before Propensity Score Matching |                   |              | After Propensity Score Matching |                   |              |
|-----------------------------------|----------------------------------|-------------------|--------------|---------------------------------|-------------------|--------------|
|                                   | Puregon (Group A)                | Gonal F (Group B) | P-value      | Puregon (Group A)               | Gonal F (Group B) | P-value      |
| <b>Fresh ET</b>                   | <b>448</b>                       | <b>491</b>        |              | <b>381</b>                      | <b>394</b>        |              |
| Positive pregnancy per ET         | 221 (49.3)                       | 252 (51.3)        | 0.542        | 193 (50.7)                      | 196 (49.7)        | 0.800        |
| Clinical pregnancy rate per ET    | 207 (46.2)                       | 235 (47.9)        | 0.612        | 178 (46.7)                      | 182 (46.2)        | 0.883        |
| Miscarriage rate per ET           | 43 (19.5)                        | 39 (15.5)         | 0.254        | 40 (20.7)                       | 32 (16.6)         | 0.296        |
| Ectopic pregnancy rate per ET     | 8 (3.6)                          | 13 (5.2)          | 0.418        | 8 (4.1)                         | 10 (5.1)          | 0.653        |
| Live birth rate per ET            | 170 (37.9)                       | 200 (40.7)        | 0.383        | 145 (38.1)                      | 154 (39.1)        | 0.769        |
| <b>FET</b>                        | <b>942</b>                       | <b>1146</b>       |              | <b>848</b>                      | <b>833</b>        |              |
| Positive pregnancy rate per ET    | 606 (64.3)                       | 801 (69.9)        | 0.007        | 543 (64.0)                      | 565 (67.8)        | 0.101        |
| Clinical pregnancy rate per ET    | 571 (60.6)                       | 751 (65.5)        | 0.020        | 507 (59.8)                      | 524 (62.9)        | 0.189        |
| Miscarriage rate per ET           | 79 (13.0)                        | 90 (11.2)         | 0.304        | 71 (13.1)                       | 83 (14.7)         | 0.437        |
| Ectopic pregnancy rate per ET     | 8 (1.3)                          | 15 (1.9)          | 0.418        | 6 (1.1)                         | 11 (1.9)          | 0.254        |
| Live birth rate per ET            | 519 (55.1)                       | 696 (60.4)        | 0.015        | 468 (55.2)                      | 482 (57.9)        | 0.269        |
| <b>Cumulative live birth rate</b> | <b>689 (52.5)</b>                | <b>896 (57.8)</b> | <b>0.005</b> | <b>613 (52.8)</b>               | <b>646 (55.7)</b> | <b>0.169</b> |

**Notes:** Data are presented as numbers (%) unless otherwise noted.

**Abbreviations:** ET, embryo transfer; FET; frozen embryo transfer.

**Table 4** Multivariable Logistic Regression for Clbr

|                                           | Crude Model <sup>a</sup> |           | Adjusted Model <sup>b</sup> |           |
|-------------------------------------------|--------------------------|-----------|-----------------------------|-----------|
|                                           | OR (95% CI)              | P-value   | OR (95% CI)                 | P-value   |
| <b>Type of gonadotrophin</b>              |                          |           |                             |           |
| Gonal F (Follitropin $\beta$ )            | Reference                | Reference | Reference                   | Reference |
| Puregon (Follitropin $\alpha$ )           | 0.98 (0.84–1.13)         | 0.745     | 1.01 (0.87–1.17)            | 0.912     |
| <b>Female age</b> (< 37 yrs., ref)        | 0.70 (0.58–0.85)         | <0.001    | 0.74 (0.61–0.89)            | 0.002     |
| <b>BMI</b> (< 28 kg/m <sup>2</sup> , ref) | 0.82 (0.66–1.02)         | 0.079     | 0.84 (0.67–1.04)            | 0.115     |
| <b>AMH</b> (<1.2ng/ml, ref)               | 2.09 (1.50–2.93)         | <0.001    | 1.60 (1.08–2.37)            | 0.019     |
| <b>Antral follicle count (AFC)</b>        | 1.01 (1.00–01.02)        | 0.022     | 0.99 (0.98–1.01)            | 0.376     |
| <b>Basic FSH</b>                          | 0.96 (0.94–0.99)         | 0.005     | 0.99 (0.96–1.02)            | 0.504     |
| <b>Etiology of infertility</b>            |                          |           |                             |           |
| Tubal factor                              | Reference                | Reference | Reference                   | Reference |
| Male factor                               | 1.37 (0.93–2.04)         | 0.116     | 0.98 (0.62–1.57)            | 0.946     |
| PCOS                                      | 1.38 (1.09–1.77)         | 0.013     | 0.74 (0.50–1.11)            | 0.146     |
| Endometriosis                             | 0.81 (0.50–1.31)         | 0.389     | 0.62 (0.33–1.17)            | 0.139     |

(Continued)

**Table 4** (Continued).

|                                                              | Crude Model <sup>a</sup> |         | Adjusted Model <sup>b</sup> |         |
|--------------------------------------------------------------|--------------------------|---------|-----------------------------|---------|
|                                                              | OR (95% CI)              | P-value | OR (95% CI)                 | P-value |
| <b>Ovarian stimulation protocol</b> (GnRH-ant protocol, ref) | 1.08 (0.91–1.29)         | 0.353   | 1.01 (0.85–1.22)            | 0.886   |
| <b>Ovulation trigger drugs</b> (hCG, ref)                    | 1.10 (0.84–1.43)         | 0.485   | 1.09 (0.96–1.22)            | 0.174   |
| <b>No of oocytes retrieved</b> (< 5, ref)                    | 1.80 (1.41–2.30)         | <0.001  | 1.47 (1.10–1.96)            | 0.008   |

**Notes:** <sup>a</sup>No adjustments for other covariates. <sup>b</sup>Adjusted for female age (<37 yrs., ≥37 yrs.), BMI (<28 kg/m<sup>2</sup>, ≥28 kg/m<sup>2</sup>), AFC, AMH (<1.2ng/ml, ≥1.2ng/ml), basic FSH, etiology of infertility, ovarian stimulation protocol (GnRH-a long protocol, GnRH-ant protocol), ovulation trigger drugs (hCG, dual trigger), and number of oocytes retrieved (<5, ≥5).

**Abbreviations:** BMI, body mass index; FSH, follicle stimulating hormone; PCOS, polycystic ovary syndrome; OR, odds ratio; CI, confidence interval.

95% CI 0.61–0.89, P=0.002; OR 1.60, 95% CI 1.08–2.37, P=0.019 and OR 1.47, 95% CI 1.10–1.96, P=0.008, respectively), while the type of Gn was not significantly associated with CLBR (OR 1.01; 95% CI 0.87–1.17; P = 0.912).

# Discussion

To the best of our knowledge, only a few studies have compared the CLBR of r-FSH in infertile patients undergoing IVF/ICSI.<sup>18–20</sup> We expanded the sample size to include more patients and compared CLBR, pregnancy outcomes, ovarian stimulation and embryo laboratory outcomes of Puregon and Gonal-F groups in IVF/ICSI patients. The results showed that there was no significant difference in the CLBR between the two groups. It is nevertheless interesting to note that despite similar outcomes, fewer amounts of Gn were used in the Gonal-F group, and reduced funds were spent on Gn in the Puregon group.

The main goal of ART is to achieve healthy live births. Although various research have indicated the CLBR as a major endpoint,<sup>18,21,22</sup> both studies on the two groups of recombinant human FSH mentioned above were implemented prior to 2014.<sup>23,24</sup> Statistics on the cumulative probability of pregnancy and live birth are extremely beneficial to patients, however cumulative data on the likelihood of live birth are insufficient at present.<sup>25</sup> The objective of ovarian stimulation with r-FSH preparations is to produce numerous oocytes to permit in vitro fertilization and, eventually, a live baby. As a result, we opted to adopt the CLBR as the main endpoint in this trial, therefore providing additional data support for future studies.

In our study, there was no significant differences between the two groups in CLBR. In a retrospective study, lien et al evaluated CLBR after ovarian stimulation with Ovaleap (Follitropin α) and Puregon (Follitropin β), respectively, in ICSI patients undergoing a GnRH-ant protocol. The results of this study did not detect a statistically significant difference in CLBR between the two groups, which is consistent with our findings.<sup>18</sup> Several randomized controlled trials (RCTs) have been conducted to evaluate the impact of various r-FSH dosages on IVF/ICSI treatment outcomes, and researchers have identified a dose-effect relationship between the total dose of Follitropin β and the number of oocytes retrieved.<sup>26</sup> Raoul Orvieto and colleagues found that r-FSH β and r-FSH α resulted in comparable number of oocytes retrieved after ovarian stimulation, but r-FSH β tended to require less dosage, apparently with greater recruitment of follicle capacity.<sup>27</sup> This conclusion seems to contradict our findings. In this large retrospective study, we found no statistically significant difference in the number of oocytes retrieved between the two groups, but the Gonal-F group used less total dosage than the Puregon group. Therefore, Gonal-F may be a friendlier choice for patients who are afraid of receiving injections.

With the development of ART, treatment selection is not only based on drug efficacy and safety, but also cost considerations, which are determined in part by drug dosage.<sup>28</sup> Economic benefits have always been an important measure for patients and clinicians. A pharmacoeconomic evaluation of IVF-ET based on the Dutch healthcare system found that Follitropin α may be a cost-effective option compared to u-FSH and HP-HMG.<sup>29</sup> Because of differences in cost, the decision to use Follitropin α or Follitropin β to stimulate the ovaries in low income families may have a significant impact on economic stress and treatment discontinuation rates.<sup>30</sup> In our present study, Gn days between two groups after PSM was no statistically significant difference, while the total dose of Gonal-F was less than that of Puregon. However, the cost of live birth based on Gonal-F therapy was found to be higher than that of Puregon (7547.2

RMB and 5327.9 RMB, respectively). Thus, Puregon has better cost-effectiveness to some extent, more meaningful to relieve economic pressure of patients, especially those with low income.

Compared with the previous retrospective study, our advantage lies in expanding the sample size, increasing the reliability and persuasiveness of the results of this study. And considering the effects of confounding factors and selection bias, we established a 1:1 PS matching to limit bias and obtain relatively reliable data. Furthermore, the economic and psychological burden of economic cost-effectiveness on patients was considered, as was the detailed information of patients missing from previous studies, such as duration of infertility, gravidity, parity, basal serum reproductive endocrine hormones, AFC, AMH, and so on. This will act as a guidance for IVF-ET clinical medication selection. Despite efforts to control selection bias, there are limitations of retrospective studies. This study did not assess the usage or handling faults of both pen injectors, nor did it provide additional practical information on self-injection. An easy-to-use pen-based device can reduce patient stress, as handling errors can lead to overdose or underdose, as well as adverse events or delays in treatment.

## Conclusion

In conclusion, the Gonal-F and Puregon employed in ovarian stimulation protocols results in similar CLBRs for infertile patients undergoing IVF/ICSI. Patients should be informed that Gonal-F is a suitable option for patients who wish to decrease Gn dosage of injections in daily clinical practice. Puregon can allow patients and IVF clinics to minimize unneeded stress and expenses while also providing them more flexibility. Larger studies, as well as an economic assessment of the costs involved, are needed to validate the current findings.

## Data Sharing Statement

The data generated and analyzed from the current study will be availed by the corresponding author upon request.

## Ethics Approval and Consent to Participate

This study was conducted in accordance with the Helsinki Declaration. The study was approved by the Reproductive Ethics Committee of the Affiliated Hospital of Shandong University of TCM (No. SDUTCM-RME2021-0212). Written informed consent was obtained from all participants.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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## Disclosure

No potential conflict of interest was reported by the authors.

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