In vitro fertilization cycles stimulated with follitropin delta result in similar embryo development and quality when compared with cycles stimulated with follitropin alfa or follitropin beta

Olga Haakman, M.D.,a Tina Liang, M.D.,a Kristen Murray, B.Sc.,b Angelos Vilos, M.D.,a George Vilos, M.D.,a Carlee Bates, Ph.D.,a Andrew J. Watson, Ph.D.,b Michael R. Miller, Ph.D.,c and Basim Abu-Rafea, M.D.a

a Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, and b Schulich School of Medicine and Dentistry, Western University, London Health Sciences Centre, London, Ontario

Objective: To study the impact of follitropin delta for ovarian stimulation on embryo development and quality compared with that of follitropin alfa or beta in in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles.

Design: Retrospective cohort study

Setting: University-affiliated, hospital-based fertility clinic

Patient(s): A total of 403 IVF/ICSI cycles were conducted from September 1, 2018 to December 31, 2019. Cycles were grouped on the basis of stimulation with follitropin delta vs. follitropin alfa or beta.

Intervention(s): None.

Main Outcome Measure(s): Embryo parameters and clinical pregnancy and implantation rates.

Result(s): Ovarian stimulation using follitropin delta resulted in no statistically significant difference in day 3 embryo quality between the control group and follitropin delta group (median 0.50 vs. 0.54 for good quality embryos and median 0.25 vs. 0.20 for intermediate quality embryos). Although on initial analysis there was a lower proportion of good quality blastocysts in the follitropin delta group than in the control group (0.11 vs. 0.22), this difference was no longer present when day 3 after fertilization vitrification and transfer cycles were excluded (0.26 vs. 0.33 follitropin delta vs. control). The clinical pregnancy rates and clinical implantation rates were similar in both groups in fresh transfer cycles.

Conclusion(s): Stimulation with follitropin delta in IVF/ICSI cycles resulted in similar embryo development and pregnancy rates compared with those of stimulation with follitropin alfa or beta. (Fertil Steril Rep® 2021;2:30–5. ©2020 by American Society for Reproductive Medicine.)

Key Words: Embryo quality, follitropin delta, intracytoplasmic sperm injection, in vitro fertilization, pregnancy rates

Discuss: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/xfre-d-20-00173

In recent years, the benefit of an individualized approach to ovarian stimulation in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) procedures has become evident. There is an increasing trend toward the selection of the starting dose of gonadotropin on the basis of the unique characteristics of each patient with the goal of improving oocyte yield while simultaneously minimizing the associated risks of excessive response and associated sequelae (1). Follitropin delta is a relatively new recombinant follicle stimulating hormone (FSH) expressed in a human fetal retinal cell line (1). It is administered according to a specific dosing algorithm, taking into account the patient’s body weight as well as anti-mullerian...
hormone (AMH) levels before treatment (1, 2). Follitropin delta was shown to be noninferior in terms of ongoing pregnancy and implantation rates when compared with conventional ovarian stimulation in the ESTHER-1 trial. Potentially improved safety was also noted, with more women responding within target, fewer poor responses, and fewer excessive responses (1). The differing glycosylation profile of this preparation has resulted in lower clearance and higher ovarian response in humans compared with other recombinant FSH preparations (1, 3, 4). However, this increase in oocyte yield may not have translated into an increased number of blastocysts (3). Follitropin alfa and follitropin beta have been available for use in clinical practice in North America since 2004. Although there are some differences between their pharmacokinetics, in clinical trials, these 2 medications showed similar safety and efficacy (5–9).

Since the introduction of follitropin delta into clinical practice, a variety of parameters, specifically related to stimulation response as well as the effect on the risk of ovarian hyperstimulation syndrome, have been assessed (10, 11). The resulting embryo quality has not, however, been reported to date. In this study, we aimed to assess the embryo quality

---

**Figure 1**

Flow chart of the cycles excluded from the initial sample to form the study and control cohorts. IVF/ICSI = in vitro fertilization/intracytoplasmic sperm injection; OPU = oocyte pick-up.

Haakman. Follitropin delta and embryo quality. Fertil Steril Rep 2020.
and development associated with the use of follitropin delta for stimulation in IVF/ICSI as compared with the use of follitropin alfa or beta. We anticipated that embryo and blastocyst quality after stimulation with follitropin delta would not differ significantly from that of cycles employing follitropin alfa or beta.

MATERIALS AND METHODS
A retrospective cohort study was performed at The Fertility Clinic in London, Ontario, Canada, a hospital-based, university-affiliated fertility clinic. The study included all IVF/ICSI cycles from September 1, 2018 to December 31, 2019. Ethics approval was provided by the Western University Health Sciences Research Ethics board under project ID number 115800. All IVF/ICSI cycles in which follitropin delta (Rekrolle, FE 999049; Ferring Pharmaceuticals, St. Prex, Switzerland) was used for ovarian stimulation were identified as the exposure cohort (Delta-1). The start date of follitropin delta use in our center was September 1, 2018; all remaining IVF/ICSI cycles from September 1, 2018 to December 31, 2019 were then identified as the control cohort (Control-1), including cycles stimulated with follitropin alfa (Gonal-F; Merck KgaA, Darmstadt, Germany) and follitropin beta (Puregon; MSD, Darmstadt, Germany). Cycles that did not result in embryos were excluded (Fig. 1).

All cycles involved controlled ovarian stimulation using recombinant FSH with gonadotropin-releasing hormone (GnRH) antagonist, long GnRH agonist, or flare GnRH agonist protocols. Growth hormone 3.33 mg daily for 9 days was used during stimulation as an adjunct treatment for patients with a history of prior inadequate ovarian response. The follitropin preparation used in the cycle was based on health care provider preference. Menotropin was added in certain cycles on the basis of patient history, recombinant FSH used, and practitioner preference. Follitropin alfa and beta were dosed taking into account the patient’s age, weight, baseline FSH level, and prior history. Follitropin delta was dosed using the patient’s weight in kilograms and the AMH level. Ultrasound monitoring was started on day 4 or 5 of stimulation until the lead follicles reached 17–18 mm in diameter. Final oocyte maturation was triggered with recombinant human chorionic gonadotropin or GnRH agonist, and oocyte retrieval was performed 36–37 hours later. Conventional IVF or ICSI were performed according to standard protocols. The method of oocyte fertilization used was based on practitioner preference and previous patient history, with only a small number of cycles using insemination through standard IVF (4 in the Delta-1 group and 15 in the Control-1 group). The criteria for the extended culture of embryos consisted of the presence of ≥4 good quality embryos on day 3. Embryo transfers were performed on day 3 or day 5 under ultrasound guidance. Generally, only embryos that reached the blastocyst stage by day 5 or 6 were cryopreserved.

The demographic data were collected from paper-based patient treatment records. For the purposes of analysis, the patient’s body mass index was categorized as underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²), and obese (≥30.0 kg/m²). An ovarian reserve category was assigned on the basis of the total number of antral follicles measured by ultrasound on day 2 or 3 of the cycle as follows: low (0–8 antral follicles), medium (9–19 antral follicles), and high (≥20 antral follicles).

The outcome data were collected from paper-based embryology laboratory records. The primary outcome was embryo quality. The quality of embryos on day 3 after fertilization and of blastocysts on days 5 and 6 were categorized according to the system used in our clinic, largely on the basis of the Istanbul consensus recommendations (12), taking into account the cell number and grade of each embryo on days 2 and 3 after fertilization and the Gardner grade (13) of each blastocyst on day 5 and 6 (Table 1). Secondary outcomes included the clinical pregnancy rates and clinical implantation rates. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound on luteal day 40. The clinical pregnancy rate was calculated per fresh transfer on day 3 or day 5 after fertilization. The clinical implantation rate was defined as the number of clinical pregnancies per total number of fresh embryos transferred on either day 3 or day 5. Live birth rates were not a part of the study objective.

Continuous variables were summarized using medians (interquartile ranges [IQRs]), and group comparisons were examined using Mann-Whitney U tests. Categorical variables were summarized using frequencies (%), and group comparisons were examined using chi-square tests (or exact chi-square tests, when appropriate). Analyses of covariance and logistic regression models were conducted to examine group differences for continuous and dichotomous outcomes, respectively, while also controlling for potential confounding variables, including etiology of infertility.

### Table 1

**Ranking of the embryo quality on day 3 after fertilization and ranking of the blastocyst quality on day 5/6 after fertilization.**

| Day 3 embryo quality ranking | Good | Intermediate | Poor |
|------------------------------|------|--------------|------|
| Day 2 cell number            | 3–5  | 2; >5        | Non-division |
| Day 3 cell number            | 6–10 | 6–10, >10    | <6 cells |
| Embryo grade (fragmentation) | G1–G2| G1–G2        | G3–G6 |
| Cleavage rate               | Appropriate | Appropriate | Arrested |
|                             | Too slow/fast | Too slow/fast | |

| Day 5/6 blastocyst quality ranking | Good | Poor | Arrested |
|-----------------------------------|------|------|---------|
| Day 5 (ET) stage                 | ≥ early blastocysts | Morula | Cleavage |
| Day 5/6 stage                     | ≥ 3 blastocysts    | ≤ 2 blastocysts | Morula |
| ICM grade                        | A/B | C    | —      |
| TE grade                         | A/B/C | A/B/C | —      |

ET = embryo transfer.

* An appropriate cleavage rate was defined as an increase of ≥3 cells and ≤7 cells from day 2 to 3. An increase of <3 cells from day 2 to 3 was considered too slow. Embryos that had the same cell number on day 2 and 3 were classified as “Arrested.”

† Only embryos that were transferred on day 5 were subjected to the day 5 (ET) stage quality categories. All other embryos were either frozen or discarded on day 5 or 6 and follow the day 5/6 stage grading scheme.

Haakman. Follitropin delta and embryo quality. Fertil Steril Rep 2020.
A total of 440 IVF/ICSI cycles were identified during the study period: 115 with follitropin delta and 325 with either follitropin alfa or beta used for stimulation. Once exclusion criteria were considered, 106 Delta-1 group cycles and 297 Control-1 group cycles were included (Fig. 1). Demographic characteristics of the 2 groups are listed in Table 2. The cohorts differed significantly in the proportion of cycles with a diagnosis of male factor infertility (29.2% in Delta-1 vs. 45.1% in Control-1, \( P = .004 \)) as well as in the incidence of advanced maternal age in each group (52.8% in Delta-1 vs. 41.8% in Control-1, \( P = .049 \)). More women with cycles in the control group were nulliparous (56.9% in Delta-1 vs. 44.3% in Delta-1, \( P = .026 \)), and more cycles in the control group utilized growth hormone (26.6% in Control-1 vs. 15.1% in Delta-1, \( P = .017 \)) and menotropin (99.3% in Control-1 vs. 67.0% in Delta-1, \( P < .001 \)). In the Control-1 group, 31.6% of the women had undergone a previous cycle of IVF compared with 28.3% of women in the Delta-1 group (\( P = .521 \)). The mean number of oocytes retrieved was calculated per injected oocytes for intracytoplasmic sperm injection cycles and per oocytes retrieved for IVF cycles.

**RESULTS**

A total of 440 IVF/ICSI cycles were included during the study period: 115 with follitropin delta and 325 with either follitropin alfa or beta used for stimulation. Once exclusion criteria were considered, 106 Delta-1 group cycles and 297 Control-1 group cycles were included (Fig. 1). Demographic characteristics of the 2 groups are listed in Table 2. The cohorts differed significantly in the proportion of cycles with a diagnosis of male factor infertility (29.2% in Delta-1 vs. 45.1% in Control-1, \( P = .004 \)) as well as in the incidence of advanced maternal age in each group (52.8% in Delta-1 vs. 41.8% in Control-1, \( P = .049 \)). More women with cycles in the control group were nulliparous (56.9% in Delta-1 vs. 44.3% in Delta-1, \( P = .026 \)), and more cycles in the control group utilized growth hormone (26.6% in Control-1 vs. 15.1% in Delta-1, \( P = .017 \)) and menotropin (99.3% in Control-1 vs. 67.0% in Delta-1, \( P < .001 \)). In the Control-1 group, 31.6% of the women had undergone a previous cycle of IVF compared with 28.3% of women in the Delta-1 group (\( P = .521 \)). The mean number of oocytes retrieved was higher in the Control-1 group than in the Delta-1 group (12.3 ± 7.7 vs. 10.4 ± 6.1 [±SD], respectively; \( P = .033 \)). There were no differences between the groups in the mean length of stimulation; numbers of follicles \( \geq 15 \text{ mm}, \geq 18 \text{ mm}, \text{ and} \geq 20 \text{ mm} \).
mm; mean estradiol level on the trigger day; or proportion of normal fertilization. An equal proportion of fresh transfers occurred on day 3 after fertilization in both the Control-1 and Delta-1 groups (Table 2).

No significant differences were identified between the Delta-1 and Control-1 groups in terms of the proportion of good-, intermediate-, and poor-quality embryos on day 3 after fertilization (Table 3). There were also no significant differences between cohorts in the proportions of poor and arrested blastocysts (day 5 and 6 after fertilization). A significant difference was noted in the proportion of good quality blastocysts, with the Delta-1 group having a lower proportion compared with the Control-1 group (median 0.11 vs. 0.22, respectively; \( P = .026 \)). This difference persisted even when controlling for potential confounding variables (Table 3). Clinical pregnancy and clinical implantation indices for both day 3 and day 5 fresh embryo transfers did not differ between the Control-1 and Delta-1 groups (Table 3).

For the secondary analysis, once day 3 after fertilization embryo transfer and vitrification cycles were excluded, the Delta-2 group included 43 cycles and the Control-2 group included 109 cycles (Fig. 1). There were no significant differences between the groups in the quality of embryos on day 3 after fertilization as well as at the blastocyst level (Table 3).

DISCUSSION
Our results suggest there was no difference in the embryo quality associated with IVF/ICSI cycles in which follitropin delta was used for stimulation compared with cycles in which follitropins alfa or beta were used. The clinical pregnancy indices with fresh transfer were comparable. The ESTHER-1 trial showed an improved safety profile with follitropin delta, with fewer excessive stimulation responses and fewer measures taken to prevent ovarian hyperstimulation syndrome (1, 10, 11). Our findings of equivalent cycle

### TABLE 3

Median proportions of good, intermediate, and poor-quality embryos on day 3 after fertilization and proportions of good, poor, and arrested blastocysts on days 5 and 6 after fertilization.

| Outcome variable | Analysis of Covariance$^a$ |
|------------------|-----------------------------|
|                  | Control-1 | Delta-1 | \( P \) value | B(SE) (95% CI) | \( P \) value |
| Primary analysis day 3 embryo stage |            |         |              |              |              |
| Cycles included  | Control-1 | Delta-1 | .746         | —             | —             |
| Good embryos (IQR) | 0.54 (0.33–0.75) | 0.50 (0.31–0.75) | .746 | — | — |
| Intermediate embryos (IQR) | 0.20 (0.00–0.36) | 0.25 (0.00–0.40) | .338 | — | — |
| Poor embryos (IQR) | 0.14 (0.00–0.33) | 0.05 (0.00–0.25) | .119 | — | — |
| Secondary analysis day 3 embryo stage |            |         |              |              |              |
| Cycles included  | Control-1 | Delta-1 | .156         | —             | —             |
| Good embryos (IQR) | 0.67 (0.50–0.80) | 0.60 (0.44–0.75) | .156 | — | — |
| Intermediate embryos (IQR) | 0.20 (0.10–0.31) | 0.29 (0.00–0.38) | .146 | — | — |
| Poor embryos (IQR) | 0.10 (0.00–0.17) | 0.11 (0.00–0.20) | .852 | — | — |
| Primary analysis blastocyst stage |            |         |              |              |              |
| Cycles included  | Control-1 | Delta-1 | .026         | —             | —             |
| Good blastocysts (IQR) | 0.22 (0.00–0.50) | 0.11 (0.00–0.38) | .026 | — | — |
| Poor blastocysts (IQR) | 0.38 (0.13–0.57) | 0.40 (0.25–0.67) | .137 | — | — |
| Arrested blastocysts (IQR) | 0.25 (0.00–0.50) | 0.22 (0.00–0.60) | .858 | — | — |
| Secondary analysis blastocyst stage |            |         |              |              |              |
| Cycles included$^b$ | Control-1 | Delta-1 | .121         | —             | —             |
| Good blastocysts (IQR) | 0.33 (0.17–0.56) | 0.26 (0.13–0.48) | .121 | — | — |
| Poor blastocysts (IQR) | 0.40 (0.26–0.55) | 0.40 (0.38–0.60) | .127 | — | — |
| Arrested blastocysts (IQR) | 0.17 (0.07–0.35) | 0.19 (0.10–0.43) | .512 | — | — |

Note: Data are presented as medians with interquartile ranges (IQR). CI = confidence interval; SD = standard deviation; SE = standard error.

$^a$ Analysis of covariance was performed taking into account the following control variables: etiology of infertility (presence of endometriosis, ovulatory disorder, polycystic ovarian syndrome, advanced maternal age), previous in vitro fertilization attempt, use of growth hormone, use of menotropin, body mass index, and the ovarian reserve category.

$^b$ Secondary analysis was performed after the exclusion of cycles in which day 3 vitrification or transfer occurred.

Haakman. Follitropin delta and embryo quality. Fertil Steril Rep 2020.
outcomes contribute to the evidence on follitropin delta and support its position as an important alternative stimulation medication.

The findings of equivalent clinical implantation and clinical pregnancy indices between the 2 groups are consistent with the findings of ESTHER-1, where similar efficacy was reported between follitropin delta and follitropin alfa in ongoing implantation rates, ongoing pregnancy rates, and live birth rates. In that study, however, oocyte yield was equivalent between the groups under comparison [1]. A randomized, controlled, multicenter trial in 2014 found that a positive relationship existed between the dose of follitropin delta administered and the number of oocytes retrieved, this did not translate into an increase in the number of blastocysts [3]. Oocyte yield is, therefore, not necessarily a useful parameter for evaluating the performance of follitropin delta. The difference between the 2 groups in the number of oocytes retrieved was not reflective of the sample as cycles in which no oocytes were retrieved were excluded. The wide variation between groups in the use of menotropin was explained largely by possible differences in physician preference. Menotropin was not routinely used in cycles stimulated with follitropin delta.

Follitropin delta has a lower clearance compared with that of follitropin alfa, which is likely related to differences in glycosylation patterns, but a similar absolute bioavailability [4, 14]. In addition, its in vitro potency at the human FSH receptor was the same as that of follitropin alfa [14]. These pharmacodynamic traits may explain why the outcome parameters associated with the use of this medication have been equivalent to those of follitropin alfa, whereas the lower clearance and individualized dosing may contribute to the improved safety profile.

Our study was retrospective in design and consisted of a relatively small sample size. The study cohorts differed significantly in their demographic characteristics, and efforts were made to control for these differences when a difference in outcomes was observed. The ovarian reserve categories were based on antral follicle counts at the start of each stimulation cycle as AMH serum levels were unfortunately not available for all patients in the study. This could be considered a limitation because AMH is a better predictor of ovarian response [15, 16], and it is used in the formal dosing of follitropin delta [1]. The significant proportion of embryos that were either transferred or vitrified on day 3 after fertilization affected the evaluation of resulting blastocyst number and quality; secondary analysis was performed to account for this, albeit with a smaller sample size. A significant strength of our study was that it contributed important information on the quality of embryos and blastocysts associated with the use of follitropin delta for stimulation, a parameter that has not previously been reported.

In conclusion, stimulation with follitropin delta in IVF/ICSI cycles was associated with comparable embryo quality, clinical implantation, and clinical pregnancy incidence as compared to stimulation with follitropin alfa or beta. Further study of this association, with a greater number of cycles, in the future would be interesting to determine whether these results can be reproduced.

REFERENCES

1. Andersen AN, Nelson SM, Fauser BC, García-Velasco JA, Klein BM, Arce JC, et al. Individualized versus conventional ovarian stimulation for in vitro fertilization: a multicenter, randomized, controlled, assessor-blinded, phase 3 noninferiority trial. Fertil Steril 2017;107:387–96.e4.
2. Bergandi L, Canosa S, Caroso AR, Paschero C, Gennarelli G, Silvagno F, et al. Human recombinant FSH and its biosimilars: clinical efficacy, safety, and cost-effectiveness in controlled ovarian stimulation for in vitro fertilization. Pharmaceuticals 2020;13:1–20.
3. Arce JC, Andersen AN, Fernández-Sánchez M, Visnoha H, Bosch E, García-Velasco JA, et al. Ovarian response to recombinant human follicle-stimulating hormone: a randomized, antimullerian hormone-stratified, dose-response trial in women undergoing in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2014;102:1633–40.e5.
4. Olsson H, Sandström R, Grundemar L. Different pharmacokinetic and pharmacodynamic properties of recombinant follicle-stimulating hormone (rFSH) derived from a human cell line compared with rFSH from a non-human cell line. J Clin Pharmacol 2014;54:1299–307.
5. Lunenfeld B, Bilger W, Longobardi S, Alam V, D’Hooghe T, Sunkara SK. The development of gonadotropins for clinical use in the treatment of infertility. Front Endocrinol 2019;10:1–15.
6. Williams RS, Vensel T, Sistrom CL, Kipersztok S, Rhoton-Vlask A, Drury K. Pregnancy rates in varying age groups after in vitro fertilization: a comparison of follitropin alfa (Gonal F) and follitropin beta (Folllistim). Am J Obstet Gynecol 2003;189:342–6, discussion 346–7.
7. Tulppala M, Aho M, Tuuri T, Vilksa S, Foudila T, Hakala-Ala-Petilä T, et al. Comparison of two recombinant follicle-stimulating hormone preparations in in-vitro fertilization: a randomized clinical study. Hum Reprod 1999;14:2709–15.
8. Harlin J, Csemiczky G, Wramsby H, Fried G. Recombinant follicle stimulating hormone in in-vitro fertilization treatment-clinical experience with follitropin alpha and follitropin beta. Hum Reprod 2000;15:239–44.
9. Brinsden P, Akagbosu F, Gibbons LM, Lancaster S, Gourdon D, Engrand P, et al. A comparison of the efficacy and tolerability of two recombinant human follicle-stimulating hormone preparations in patients undergoing in vitro fertilization-embryo transfer. Fertil Steril 2000;73:114–6.
10. Bosch E, Havelock J, Martin FS, Rasmussen BB, Klein BM, Mannaerts B, et al. Follitropin delta in repeated ovarian stimulation for IVF: a controlled, assessor-blind Phase 3 safety trial. Reprod Biomed Online 2019;38:195–205.
11. Fernández-Sánchez M, Visnoha H, Yuzpe A, Klein BM, Mannaerts B, Arce JC, et al. Individualization of the starting dose of follitropin delta reduces the overall OHSS risk and/or the need for additional preventive interventions: cumulative data over three stimulation cycles. Reprod Biomed Online 2019;38:528–37.
12. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26:1270–83.
13. Gardiner DK, Balaban B. Assessment of human embryo development using morphological criteria in an era of time-lapse, algorithms and ‘OMICS’: is looking good still important? Mol Hum Reprod 2016;22:704–18.
14. Koechling W, Plaksin D, Croston GE, Jeppesen JV, Macklon KT, Andersen CY. Comparative pharmacology of a new recombinant FSH expressed by a human cell line. Endocr Connect 2017;6:297–305.
15. Nelson SM, Klein BM, Arce JC. Comparison of antimullerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. Fertil Steril 2015;103:923–30.
16. Illdoromti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update 2015;21:698–710.