

Comparison of two dosages of recombinant human follicle-stimulating hormone in Chinese women undergoing controlled ovarian stimulation: prospective randomised double-blind study

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Objective. To compare two dosages of recombinant human follicle-stimulating hormone for controlled ovarian stimulation.

Design. Prospective, randomised double-blind study.

Setting. Tertiary assisted reproduction unit, Hong Kong.

Participants. Forty subfertile Chinese women aged 24 to 38 years undergoing in vitro fertilisation. Entry criteria included good physical and mental health, and a body mass index between 18 and 29 kg/m². Exclusion criteria were subfertility caused by an endocrine abnormality, polycystic ovarian syndrome, or absent ovarian function; previous assisted reproduction treatment in which fewer than three oocytes were retrieved; prior hospitalisation due to severe ovarian hyperstimulation syndrome; chronic cardiovascular, hepatic, renal, or pulmonary disease; alcohol or drug abuse; and the administration of investigational drugs within the previous 3 months.

Intervention. Injection of recombinant follicle-stimulating hormone, 100 IU/d or 200 IU/d.

Main outcome measures. The number of oocytes, total dose of drug used, and pregnancy rates.

Results. Compared with the 20 women receiving 200 IU/d, the 20 who received 100 IU/d had a significantly lower median number of oocytes retrieved and median total dose of drug used (7.5 versus 15.0 [P<0.001] and 1200 IU versus 2000 IU [P<0.001], respectively). The pregnancy rates in the fresh cycles were similar (20%) in both groups, but the cumulative pregnancy rates in the 100 IU/d and 200 IU/d groups were 20.0% and 45.0% per stimulated cycle, respectively. The incidence of ovarian hyperstimulation syndrome in the 100 IU/d and 200 IU/d groups was 5.0% and 20.0%, respectively.

Conclusions. Use of 100 IU/d of recombinant follicle-stimulating hormone requires a lower total dose but results in the harvest of half the number of oocytes compared with when a dosage of 200 IU/d is used.

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Key words: Fertilization in vitro; FSH/administration & dosage; Ovulation induction; Pregnancy rate; Recombinant proteins/administration & dosage

Introduction

In vitro fertilisation (IVF) and embryo transfer involve the development of multiple follicles, egg collection, and the transfer of one or more embryos after fertilisation has occurred. Ovarian stimulation is used in the majority of assisted reproduction units to improve

success rates by increasing the number of oocytes and thus the number of embryos to be replaced. Gonadotrophin preparations that are available include urinary human menopausal gonadotrophin (HMG), which comprises follicle-stimulating hormone (FSH) 75 IU and luteinizing hormone (LH) 75 IU; urinary human FSH (75 IU); and recombinant human FSH.

There is still no consensus on the best starting doses of gonadotrophins. Most assisted reproduction units start with FSH in dosages of either 150 IU/d or 225 IU/d for the first 4 days of treatment, after which the dose is individualised according to the ovarian response. Recombinant human FSH has been shown

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to be more effective than urinary FSH in IVF/embryo transfer treatment.¹⁻³ Compared with urinary FSH, a significantly lower total dose of recombinant FSH is required and for a shorter period. Furthermore, a significantly higher number of large follicles, oocytes, and embryos are obtained with recombinant human FSH, which results in significantly more ongoing pregnancies.¹⁻³

Devroey et al⁴ first reported the use of a 100 IU starting dose of recombinant human FSH (Puregon; NV Organon, Oss, The Netherlands) in a group of 43 unselected patients. A multicentre European trial⁵ that compared 100 IU and 200 IU daily fixed-dose regimens of Puregon showed similar pregnancy rates per cycle started, despite more cycles being cancelled because of low responsiveness and a reduced number of oocytes in the 100 IU group. These findings suggest that serum FSH levels are sufficient to stimulate follicular growth, even in women receiving a lower starting dose of 100 IU FSH. Data are not available, however, for Asian or Chinese women, who tend to be slimmer than European women.

This prospective, randomised double-blind study aimed to compare the number of oocytes obtained, the total dose of FSH used, the pregnancy rate, and the cumulative pregnancy rate between two groups of subfertile Chinese women using 100 IU/d or 200 IU/d of recombinant human FSH (Puregon).

Methods

Participants

This study was part of a larger multicentre trial. Women were recruited from among those attending the Assisted Reproduction Unit at the Department of Obstetrics and Gynaecology, The University of Hong Kong, for IVF treatment from 26 May 1998 to 6 May 1999. Participants had to satisfy all inclusion and exclusion criteria. The inclusion criteria used were as follows: age between 18 and 39 years at the time of screening; cause of subfertility potentially treatable by IVF or intracytoplasmic sperm injection (ICSI); normal ovulatory cycles with a mean duration between 24 and 35 days; good physical and mental health; and a body mass index of between 18 and 29 kg/m². The exclusion criteria used were as follows: subfertility caused by endocrine abnormalities (eg hyperprolactinaemia), polycystic ovarian syndrome, or the absence of ovarian function; previous assisted reproduction treatment in which fewer than three oocytes were retrieved; prior hospitalisation due to severe ovarian hyperstimulation syndrome (OHSS); chronic cardiovascular, hepatic,

renal, or pulmonary disease; current alcohol or drug abuse, or a history thereof; and the administration of investigational drugs within 3 months prior to screening.

During the screening procedure, patients underwent a standard protocol of history-taking; a general and gynaecological examination; and investigations, which included haematological, biochemical, and hormonal tests. All eligible women received extensive counselling and were required to give written consent before participating in the study, which was approved by the Ethics Committee, Faculty of Medicine, The University of Hong Kong. The indications for conventional IVF treatment included tubal infertility, male-factor infertility, endometriosis, unexplained infertility, and mixed factors. Intracytoplasmic sperm injection was performed for couples in which the man had either severe semen abnormalities (<100 000 motile spermatozoa recovered after sperm preparation) or obstructive azoospermia, in which case surgically retrieved spermatozoa from the epididymis or testis were used.

Ovarian stimulation

The 'long' protocol of ovarian stimulation⁶ was used. Participants were pretreated with the gonadotrophin-releasing hormone (gonadorelin) analogue, buserelin (Suprecur; Hoechst AG, Frankfurt, Germany) 150 µg—one spray in one nostril—four times daily, from the mid-luteal phase of the menstrual cycle preceding the treatment cycle. Pituitary downregulation was confirmed by both transvaginal ultrasonography and the measurement of the serum 17-β-oestradiol (E₂) level on the second day of the treatment cycle. Concentrations of serum levels of FSH, LH, and progesterone were also measured before the administration of FSH. The serum hormone concentrations were measured using commercially available radioimmunoassay kits (E₂ levels: Diagnostic Products Corporation, Los Angeles, United States; FSH, LH, and progesterone levels: Chiron Diagnostics Corporation, Massachusetts, United States).

Participants were randomised to receive recombinant human FSH (follitropin beta [Puregon]) 100 IU/d or 200 IU/d. They were allocated a number from a randomisation list that corresponded with patients' boxes in which the medication was kept. The randomisation was done in blocks of four and was computer-generated using random numbers. The recombinant FSH was supplied as lyophilised spheres in two ampoules each containing FSH of either 50 IU or 100 IU in vivo bioactivity. The 50-IU and 100-IU ampoules were indistinguishable in appearance. The drug in the

two ampoules was reconstituted with 1 mL of solvent (0.45% normal saline) and given by subcutaneous injection.

The ovarian response was monitored by serial transvaginal ultrasound scanning and by measuring the serum E₂ concentration. Human chorionic gonadotrophin (HCG [Pregnyl; NV Organon, Oss, The Netherlands]) 10 000 IU was given by intramuscular injection when three leading follicles of >16 mm in diameter were detected. Transvaginal ultrasound-guided oocyte collection was scheduled 36 to 38 hours after the HCG injection. Oocytes were not collected if women who had been recruited subsequently did not meet the study criteria and their treatment cycle had to be cancelled. Blood was taken on the day of HCG administration to measure serum E₂, FSH, LH, and progesterone concentrations.

Spermatozoa were prepared by using a gradient separation kit (Isolate; Irvine Scientific, Santa Ana [CA], United States). Insemination by ICSI was performed 4 to 6 hours after oocyte collection, using metaphase II oocytes that had been denuded of their surrounding cumulus and corona radiata cells 2 hours after their collection by hyaluronidase treatment (approximately 100 U/mL) and by aspirating oocytes through a fine capillary. A maximum of three normally cleaving embryos were replaced into the uterine cavity 48 hours after oocyte retrieval. Excess good-quality embryos were frozen for subsequent transfer, if the woman was not pregnant during the same cycle. All fresh embryos were cryopreserved if the serum E₂ concentration on the day of ovulatory HCG injection exceeded 30 000 pmol/L, to reduce the risk of OHSS, which was graded as mild, moderate, or severe.⁷

The luteal phase was supported by injections of HCG 1500 IU on the day of embryo transfer and 6 days later. If, however, the serum E₂ concentration on the day of ovulatory HCG injection exceeded 18 000 pmol/L, vaginal pessaries of progesterone (Cyclogest; Cox Pharmaceuticals, Barnstaple, United Kingdom) 400 mg twice daily were used from the day of the transfer for 10 days. Serum E₂ and progesterone levels were measured 6 days after embryo transfer. A urine pregnancy test was done 16 days after the transfer. If the result was positive, an ultrasound examination was performed 10 to 14 days later to confirm intrauterine pregnancy and to determine the number of gestation sacs present.

Frozen-thawed embryo transfer cycles

Embryos were cryopreserved using a programmable freezer, with 1,2-propanediol as a cryoprotectant.

Frozen embryos were thawed at room temperature for 40 seconds and then at 30°C in a water-bath for 40 seconds. The cryoprotectant was then removed by washing the embryos successively through phosphate buffers containing decreasing concentrations of propanediol. The embryos were cultured in a CO₂ incubator for a short period before transfer. Any embryo with half or more of the blastomeres surviving the freeze-thaw process was transferred. After thawing, frozen embryos were transferred in natural, clomifene-induced, or hormonal replacement cycles.⁶

Statistical analysis

Oocytes were classified as mature or immature, and embryos were graded as type 1, 2, 3, or 4, according to published criteria.⁸ Types 1, 2, and 3 were considered to be transferable embryos. The fertilisation rate was defined as the proportion of oocytes that formed two pronuclei. Only metaphase II oocytes were counted in ICSI cycles, and only clinical pregnancies were considered. A clinical pregnancy was defined as the presence of one or more gestation sacs, and ongoing pregnancies were those pregnancies beyond 10 to 12 weeks of gestation. The mean implantation rate was the proportion of embryos transferred that resulted in the development of an intrauterine gestational sac.

The primary outcome measures were the number of oocytes obtained and the total dose of FSH used. Data on the age of the women, type of treatment (IVF /ICSI), duration of FSH use, number of follicles aspirated, number of oocytes fertilised, fertilisation rate, frequency of OHSS development, pregnancy rate, implantation rate, and the pregnancy outcomes were compared between the groups. The pregnancy rates in both fresh and frozen-thawed embryo transfer (FET) cycles were determined. As the data were not normally distributed, results were given as median (range). Statistical tests used were the Mann-Whitney *U* test, Fisher's exact test, and the Chi squared test. A *P* value (two-tailed) of <0.05 was considered to be statistically significant.

Results

A total of 40 women were recruited: 20 in the 100 IU/d FSH group and 20 in the 200 IU/d FSH group. No statistically significant differences were found between the two groups with regard to age, body mass index, type and duration of infertility, type of treatment received, and basal FSH level (Table 1). The number of oocytes obtained in the 100 IU/ group was significantly less than that in the 200 IU/d group (7.5 [range, 0-27.0] versus 15.0 [5.0-42.0];

Table 1. Demographic characteristics of participants receiving 100 IU/d or 200 IU/d Puregon regimens

Characteristic	Dosage		P value
	100 IU/d (n=20)	200 IU/d (n=20)	
Age (years)*	34.0 (26-38)	33.0 (24-36)	0.12 [†]
Body mass index (kg/m ²)*	21.5 (18.2-25.0)	22.1 (17.8-28.8)	0.17 [†]
Primary/secondary infertility	8/12	12/8	0.21 [‡]
Duration of infertility (years)*	4.0 (2-10)	4.8 (2-14)	0.28 [†]
Type of treatment			0.10 [†]
in vitro fertilisation	15	10	
intracytoplasmic sperm injection	5	10	
Basal FSH level (IU/L)*	6.2 (1.0-11.8)	6.7 (1.4-10.5)	0.48 [†]

* Values are expressed as median (range); other characteristics are given as No. of participants

[†] Mann-Whitney *U* test

[‡] χ^2 test

Table 2. Ovarian responses of participants receiving 100 IU/d and 200 IU/d Puregon regimens

Characteristic*	Dosage		P value [†]
	100 IU/d (n=20)	200 IU/d (n=20)	
Duration of stimulation (days)	13 (9-18)	11 (10-15)	0.08
No. of follicles			
>12 mm	7.5 (1.0-14.0)	13.5 (6-25.0)	0.002
>14 mm	7.5 (1.0-12.0)	11.0 (6.0-18.0)	0.013
>16 mm	4.5 (1.0-9.0)	6.5 (1.0-17.0)	0.049
17- β -Oestradiol (pmol/L) [‡]	8615 (1553-23600)	13053 (5760-25404)	0.028
Follicle-stimulating hormone (IU/L) [‡]	6.20 (4.10-8.40)	12.70 (1.20-17.20)	<0.001
Luteinizing hormone (IU/L) [‡]	1.55 (1.00-3.80)	1.30 (1.00-9.40)	0.36
Progesterone (nmol/L) [‡]	2.25 (0.40-9.70)	3.15 (1.00-9.60)	0.009
No. of mature oocytes	7.0 (0-24.0)	13.5 (0-38.0)	0.022
No. of oocytes fertilized	2.5 (0-19.0)	9.0 (4.0-27.0)	<0.001
Fertilisation rate (%)	80.0 (0-100)	76.4 (38.9-100)	0.82
No. of transferable embryos	3 (0-16)	8 (3-27)	0.001
No. of embryos replaced	1.5 (0-3.0)	2.0 (0-3.0)	0.035
No. of embryos frozen	0 (0-11)	3 (0-23)	0.014

* All values are expressed as median (range)

[†] Mann-Whitney *U* test

[‡] Serum concentrations on the day of injection of human chorionic gonadotrophin

$P < 0.001$, Mann-Whitney *U* test). The total dose of FSH used in the 100 IU/d group was also significantly lower than that in the 200 IU/d group (1200 IU [800-2100] versus 2000 IU [1800-2800]; $P < 0.001$, Mann-Whitney *U* test). The secondary indicators of ovarian response in the two groups are shown in Table 2. The duration of stimulation in the 100 IU/d group was 2 days longer than that of the 200 IU/d group, but this difference was not statistically significant. The numbers of follicles with a diameter of >12 mm, >14 mm, and >16 mm were significantly higher in the 200 IU/d group. The fertilisation rate was similar in both groups, but the number of transferable embryos and number of embryos frozen in the 100 IU/d group were significantly less than that in the 200 IU/d group.

There were no differences between the groups in serum E_2 , FSH, LH, and progesterone concentrations on the second day of the treatment cycle (data not shown). Serum E_2 , FSH, and progesterone concentrations in the 100 IU/d group were significantly lower

than those in the 200 IU/d group on the day of HCG administration (Table 2).

Embryo transfer was performed in 16 cycles of the 100 IU/d group and 18 cycles of the 200 IU/d group. In the 100 IU/d group, three cycles were cancelled owing to poor ovarian response, and another cycle was cancelled because of failure of fertilisation. In the 200 IU/d group, no cycle was cancelled because of a poor ovarian response, but embryo transfer was postponed in two cycles because the women had a moderate degree of OHSS. Despite the lower mean number of embryos replaced in the 100 IU/d group compared with the 200 IU/d group (1.5 versus 2.0; $P = 0.035$), no significant differences were observed between the two groups directly after fresh transfer, in the clinical pregnancy rate per cycle or per transfer, implantation rate, and multiple pregnancy rate (Table 3). Two miscarriages occurred in the 200 IU/d group, whereas none occurred in the 100 IU/d group; the ongoing pregnancy rates per cycle were 10% and 20%, respectively. Mild and/or moderate OHSS developed in four women

Table 3. Outcomes of fresh cycles in participants receiving 100 IU/d or 200 IU/d Puregon regimens

Outcome measures	Dosage		P value*
	100 IU/d (n=20)	200 IU/d (n=20)	
No. of embryo transfers (cycles)	16	18	
Clinical pregnancy rate per cycle (%)	20.0 (4/20)	20.0 (4/20)	1.0
Clinical pregnancy rate per transfer (%)	25.0 (4/16)	22.2 (4/18)	1.0
Implantation rate (%)	16.7 (5/30)	11.4 (5/44)	0.735
Multiple pregnancy rate (%)	25.0 (1/4)	25.0 (1/4)	1.0
Pregnancy outcome			0.214
clinical abortion	0	2	
ongoing	4	2	
No. of cases of ovarian hyperstimulation syndrome			
mild	1	2	
moderate	0	2	
Total	1	4	0.352

* Fisher's exact test

Table 4. Outcome of frozen-thawed embryo transfer cycles in participants who were not pregnant in the fresh cycle

Outcome measure	100 IU/d	200 IU/d	P value*
No. of cycles with frozen embryos	7	15	0.265
No. of patients attempting FET†	4	11	
Total No. of FETs attempted (cycles)	6	18	
No. of embryos thawed	13	54	
No. of embryos lysed (% of No. thawed)	3 (23)	3 (5)	0.115
No. of pregnancies	0	5	
No. of embryos still frozen	9	39	

* χ^2 test

† FET frozen-thawed embryo transfer

in the 200 IU/d group, whereas only one woman in the 100 IU/d group had mild OHSS (Table 3).

Table 4 shows the outcomes of frozen-thawed embryo transfers that were performed up to the end of December 1999 in women who were not pregnant in the fresh cycle. There was no statistical difference in the number of FET cycles and the proportion of embryos that lysed on thawing. The pregnancy rate in FET cycle, was 27.8% per transfer, and the cumulative pregnancy rates per stimulated cycle in the 100 IU/d and 200 IU/d FSH groups were 20.0% and 45.0%, respectively (P=0.338, Fisher's exact test).

Discussion

Follicle-stimulating hormone is the key gonadotrophic hormone during the follicular phase, and only very low amounts of LH are needed in the different stages of follicular development and function. Excessive levels of LH in the early or late follicular phase have adverse effects on fertilisation, implantation, pregnancy rates, and early embryonic development.^{9,10} Gonadotrophin preparations extracted from postmenopausal urine are of low purity and may be contaminated up to 95% with urinary proteins. The impurity may explain the high incidence of local allergic reactions¹¹ and batch-to-batch variation¹² associated with the use of human

menopausal gonadotrophins. Urinary proteins may also have negative effects on follicular recruitment and development.¹³ Two recent meta-analyses^{14, 15} have also suggested that the use of urinary or recombinant FSH is associated with significantly higher clinical pregnancy rates than HMG and that recombinant FSH is superior to urinary FSH in terms of the pregnancy rate.

The results of this study show a dose-response relationship between FSH and the number of oocytes obtained; this finding has also been shown by Out et al.⁵ The number of oocytes that were obtained in the 200 IU/d FSH group was twice that obtained in the 100 IU/d group (15.0 versus 7.5). Consequently, the number of mature oocytes, fertilised oocytes, and transferable embryos in the 200 IU/d group was higher. The increase in the number of oocytes in this group was mainly due to an increase in the number of medium-sized follicles (ie ranging from 12 to 16 mm in diameter), which was associated with higher FSH levels on the day of HCG (Table 2). The progesterone levels on the day of HCG injection were significantly higher in the 200 IU/d group and the increased levels are likely to be related to a greater FSH exposure, thus leading to an increased FSH-induced LH receptivity in granulosa cells.¹⁶

The pregnancy rates per cycle and per transfer were similar in both groups, despite a slightly higher number

of embryos being replaced during the fresh cycle in the 200 IU/d group. When FET cycles were also included for consideration, however, the cumulative pregnancy rate among the women in the 200 IU/d group was double that of the 100 IU/d group. The difference was not statistically significant, probably because of the small sample number. The increase in the cumulative pregnancy rate was due to there being more frozen embryos in the 200 IU/d group than in the 100 IU/d group (median number [range], 3 [0-23] versus 0 [0-11]). Furthermore, more fresh cycles in the 200 IU/d group had frozen embryos.

The cycle cancellation rates due to poor ovarian response were 15% (3/20) in the 100 IU/d group and 0% in the 200 IU/d group. A similar rate of cycle cancellation in the 100 IU/d group was noted by Out et al.⁵ Approximately 10% of the cycles in patients undergoing ovarian stimulation in this study were cancelled because of poor ovarian response. The cancellation rate can be highly variable for different centres, however, because it depends on the characteristics of patients selected, the type and protocol of gonadotrophin-releasing hormone agonist used,¹⁷ and the dosage of gonadotrophin given. A higher starting dose of FSH may reduce the possibility of cycle cancellation but can lead to a higher risk of the development of OHSS. A clear relationship between the total amount of FSH given and the incidence of OHSS has been shown by Out et al.⁵ In this study, the incidence of OHSS was just 5% in the 100 IU/d group, but it was 20% in the 200 IU/d group. The difference was not statistically significant, however, probably because of the small numbers of patients in this study.

The findings of this study of Chinese women support the observations in Europe that in a significant proportion of women, the FSH threshold can be surpassed even on a 100 IU fixed daily dose, and result in multiple follicular development.^{4,5} The number of oocytes obtained, the duration and dosage of FSH used, and the body mass index of the participants in this study were similar to those reported by Out et al.⁵ Minimal ovarian stimulation in assisted reproduction is advocated because of the risks associated with OHSS.¹⁸ We have also shown that the pregnancy rate may decrease in cycles in which serum E₂ levels are lower than 20 000 pmol/L.⁶ A logical approach to determine the dose of gonadotrophin during ovarian stimulation is to predict the ovarian response and tailor the dosage accordingly.

Women who are older or obese, or who smoke are at risk of a poor ovarian response.¹⁹ Serum FSH levels

in the early follicular phase and stimulated FSH levels after a challenge test have been extensively evaluated to predict the ovarian responses during ovarian stimulation.¹⁹ Ultrasound assessment of the ovarian volume^{20,21} and the number of antral follicles²²⁻²⁴ has been recently used to predict the ovarian response. A higher dose of gonadotrophin could be tried in women who are more likely to have a poor response to gonadotrophin stimulation.

In conclusion, a 100 IU fixed daily dose regimen of recombinant FSH requires a lower total dose of FSH but results in the harvest of fewer oocytes, compared with when a 200 IU fixed daily dose is given. The pregnancy rates in the fresh cycle are similar in both groups. The chance of cycle cancellation, the risk of the development of OHSS, and the results of FET should be taken into consideration when deciding the starting dose of recombinant FSH. A better prediction of ovarian response to stimulation is helpful in the decision-making process.

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