Comparison of Two Different Fixed Doses of Follitropin-β in Controlled Ovarian Hyperstimulation: A Prospective Randomized, Double Blind Clinical Trial

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A prospective randomized, double blind, single centre study was conducted to compare the efficacy, efficiency and clinical side effects of daily fixed dose regimen of,-either 100 IU or 200 IU of recombinant follicle stimulating hormone(rFSH) Follitropin β in down-regulated women undergoing controlled ovarian hyperstimulation(COH) for either conventional in vitro fertilization(IVF) or intracytoplasmic sperm injection(ICSI). A total of sixty women were randomly allocated according to the criteria for the treatment by either 100 IU(n = 30) or 200 IU(n = 30) of FSH. Although more cycle cancellations due to low response were observed in the 100 IU group (n = 9 vs n = 2), two cases of mild and moderate ovarian hyperstimulation syndrome were noted in the higher dose group. Subjects in the group treated with 200 IU appeared to yield more follicles > 17 mm (4.4 vs 3.3, p = 0.05) and more oocytes compared to the group treated with 100 IU (9.2 versus 6.0 oocytes, NS). The total dosage required to develop at least three follicles according to the protocol was significantly lower in the group treated with 100 IU (1203.33 versus 2106.67, P < 0.0001). In conclusion, a fixed daily dose of 200 IU of rFSH Follitropin β compared to a fixed daily dose of 100 IU is more effective in terms of follicles > 17 mm development and the number of oocytes retrieved along with a lower cancellation rate, but less efficient as indicated by a higher total rFSH dose needed.

Keywords : *Follitropin, Ovarian hyperstimulation, Infertile, IVF, recombinant FSH, Oocyte, Clinical trial*

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Follicle-stimulating hormone (FSH) is an essential pituitaty gonadotrophic hormone regulation gametogenesis in both males and females[1]. Human pituitary gonadotrophins have been extracted, purified and introduced for clinical use as therapeutic agents since the early 1960s[2]. Their main clinical indications in females are stimulation of the follicular development to restore fertility of anovulators and the stimulation of multiple follicular development in ovulatory patients undergoing assisted reproductive techniques (ART). Human gonadotropins extracted from postmenopausal urine (hMG) containing amounts of urinary human FSH and LH became the standard preparations[3]. Ten years later, a preparation of urinary human FSH, practically devoid of LH activity, was developed to replace hMG for ART[4,5]. Most urinary gonadotrophin preparations share a number of disadvantages like the contamination of urinary proteins both FSH and LH, batch-to-batch inconsistency and the logistic problems associated with urine collection, leading to the development of recombinant gonadotrophins recently[6]. One recombinant FSH (rFSH) preparation, Follitropin β, (Puregon; NV Organon, Oss The Netherlands) has been extensively evaluated, in both pharmacokinetic dynamic study[7]. Clinical studies of safety, efficacy and dosage of the product have also been documented[8,9]. In a recent study of randomized controlled ovarian hyperstimulation (COH) with 225 IU urinary follicle stimulating hormone FSH (Metrodin-HP) vs Follitropin-β 150 IU/day in a fixed-dose regimen comparable efficacy in patients undergoing in vitro fertilization (IVF) and embryo transfer (ET) was documented[10]. The increased potency of rFSH and the financial costs associated with ART (Out 1995) render it of paramount

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importance that an optimal gonadotrophin dose in selected of COH. The aim of the present report was to evaluate the efficacy, efficiency and clinical side effects of treatment with a daily fixed-dose treatment of 100 IU or 200 IU of Puregon in down-regulated infertile women undergoing controlled ovarian hyperstimulation in IVF-ET programme.

Material and Method

Study population

Ninety five (n = 95) infertile women were enrolled for this study at the Division of Reproductive Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine, King Chulalongkorn Memorial Hospital. Sixty women were selected according to the following criteria. Inclusion criteria: Good mental and physical health, aged 25-38 years at the time of screening with a body mass index (IBM) between 18 and 29 kg/m^2. All women had to have clinically normal cycles with length of 24-35 days a cause of infertility potentially treatable by IVF and intracytoplasmic sperm injection (ICSI). Exclusion criteria: Infertility endocrine abnomalities such as hyperpolactinemia, polycystic ovarian syndrome, absence of ovarian function, as well as previous ART in which fewer than three oocytes were retrieved. A subject was considered to be eligible if all inclusion and none of the exclusion criteria were met.

Assessment

In all eligible women routine medical history assessments, physical and pelvic examination as well as endoscopic evaluation of pelvic organs were performed. Basic hematology testing, urinalysis, hepatic and renal functions were evaluated. Baseline hormonal profiles, including FSH, LH, PRL E_2 were also measured on day 3 of the pretreatment cycle. Prior to rFSH (Puregon®) injection, a serum E_2 concentration of less than 50 pg/ml had to be present. When adequate stimulation was achieved i.e., at least three leading follicles with a diameter of 17 mm, 10,000 IU of human chorionic ganodotropin (hCG, Pregnyl, Organon) was given. Approximately 36 hours later, oocyte retrieval was performed by transvaginal ultrasound-guided follicle aspiration. Retrieved oocytes were counted and graded according to the protocol. Either conventional IVF or ICSI were carried out according to sperm quality.

Study design

This is a prospective randomized, double blind clinical trial comparing the efficacy and efficiency of two different fixed doses of Follitropin β (Puregon®-32489 N.V. Organon, OSS, The Netherlands) COH and IVF. The study was approved by the Ethical Committee of Faculty of Medicine, Chulalongkorn University and all infertile couples had signed an informed consent and consent form before participating in this study. The present study was conducted in full compliance with the Declaration of Helsinki (and revisions), Guideline for Good Clinical Practice and local rules.

Pretreatment, buserelin acetate nasal spray 600 mg per day for pituitaty down-regulation was started in the mid-luteal phase. When estradiol serum levels were $\leq$ 50 pg/ml, treatment with rFSH was started until at least three follicles $\geq$ 17 mm in diameter had developed. The maximum treatment period was two weeks. HCG (Pregnyl, 100,000 IU) was trigger final oocyte maturation. After oocyte pickup and IVF or ICSI, a maximum of three embryo was replaced.

Statistical analysis

Data analysis included all subjects who received at least an injection of Puregon®. Dichotomous variables were analyzed by the chi-square test with Yates correction. For continuous variable, T-test was performed, whereas Levene statistics were used to test for the homogeneity of group variance. The relationship between hormonal levels and follicular sizes was determined by means of Pearson Correlation statistics. P-value of < 0.05 was considered statistically significant.

Sample size consideration

On the assumption of pooled standard deviation and difference in means, the total number of mature oocytes retrieved between 100 IU and 200 IU group was 4.6 and 6.4, respectively. A sample size of 30 subjects per group was obtained with a power of 80% and a significance level of 5% (two-side test).

Hormonal assay and Pregnancy test

Blood samples were centrifuged at 1000 G for 15 min and separated serum was stored at -20°C until assayed. LH, FSH, PRL and β-hCG concentration were measured by solid phase, two-sites fluoroimmunometric assay, sandwich technique of a commercial kit(Delfia Kit, Wallac Oy Turku, Findland). Intra-and inter-assay variation was less than 2.5% and 3.2% for LH, less than 2.8 and 2.0% for FSH, less than 2.0% and 3.4% for PRL and less than 4.1% and 4.6% for β-hCG, respectively. Serum estradiol and progesterone concentrations were measured by a solid phase fluoro-

immunoassay, competition method, Delfia commercial kit. Intra and inter-assay variations were less than 5.7% and 9.7% for E_2 and less than 7.3% and 10.1% for progesterone.

Results

All women were down regulated two weeks prior to administration of recombinant FSH treatment. Both groups had comparable demographic and infertility characteristics (Table 1). The main cause of infertility was the female factors(n = 14 vs n = 15 in the low and high dose group, respectively) with mean duration of infertility of 5.9 years for the 100 IU group and 5.4 years for the 200 IU group. Of the women (n = 30) who started in the 100 IU group, nine subjects (n = 9) were cancelled due to low response while in the 200 IU group, eight subjects (n = 8) were cancelled due to low response (n = 2), failure to fertilize (n = 2) and postponement of embryo transfer (n = 4). In the last four subjects the embryos were transferred in subsequent cycles of which three conceived and delivered healthy children.

Endocrinological parameters measured on the screening cycle and day 1 of rFSH treatment in both groups were not statistically different (6.1 IU/L vs 6.8 IU/L for the screening cycle in the 100 IU and 200 IU group, and for day one 3.2 IU/L vs 3.5 IU/L, respectively, data not shown). Serum LH concentration on day one did not differ (2.1 IU/L vs 2.9 IU/L, data not shown). The follicular development and serum FSH, LH, progesterone and estradiol on the day of hCG administration of both groups are shown in Table 2. The number of follicles > 17 mm tended to be higher in the 200 IU group (p = 0.05). The number of oocytes retrieved, the rates of fertilization, implantation rates and viable pregnancies are also shown in Table 2 and 3.

The mean total dose for rFSH was 1203 IU in the 100 IU group and 2100 IU in the 200 IU group,

Table 1. Demographics and infertility characteristics

Demographic characteristic	100 IU (n=30)	200 IU (n=30)[a]
Mean age (years, SD)	34.7(3.14)	33.7(6.87)
Mean weight (kg, SD)	50.6(6.09)	51.8(4.49)
Mean height (cm, SD)	158.0(5.085)	158.4(5.79)
Mean body mass index (BMI)(kg/m^2,SD)	20.2(1.97)	20.7(2.22)
Cause of infertility		
Male factors (n)	10	9
Female factors (n)	14	15
Endometriosis (n)	8	4
Tubes (n)	5	9
Pelvic adhesions	1	2
Male and female factors (n)	6	6
Mean duration of infertility (years, SD)	6.0(3.2)	5.4(2.3)

a = no significance difference between the two groups

Table 2. Hormonal profiles, number of follicles on the day of hCG injection, number of mature oocyte and pregnancy rate

Variables	100 IU(n=21)	200 IU (n=22)	95% CI of Treatment difference	p-value
Follicles ≥ 15 mm(n)	5.1	6.7	-3.6 to 0.4	0.12
Follicles ≥ 17 mm(n)	3.3	4.4	-2.3 to 0.006	0.05
FSH(mean, IU/L)	6.1	10.0	-56.0 to -2.1	< 0.00
LH(mean, IU/L)	3.6	3.9	-2.5 to 1.9	0.79
Progesterone(mean, ng/ml)	1.4	3.2	-4.3 to 0.7	0.15
Estradiol(mean, pg/ml)	1858.2	2363.1	-1613.8 to 603.8	0.36
Treatment length(mean, days)	12.00 11.7	10.5	0.4 to 2.5	0.00
Total oocytes retrieval(n)	6.0	9.2	-6.4 to 0.05	0.54
Mature oocytes(n)	5.1	7.6	-5.2 to 0.4	0.08
Total embryos(n)	4.0	5.9	-4.0 to 0.3	0.09
Total dose(mean, IU)	1203.3	2106.7	-1061.4 to -745.3	< 0.00
Embryos transferred(n)	2.4	3.0	-1.05 to 0.08	0.02
Imp. per started cycle	36.7	20.0	-5.7 to 39.1	0.25
Imp. per embryo transfer(%)	21.6	9.1	-1.4 to 26.4	0.08
PR per started cycle(%)	23.3	13.3	-942.0 to 29.4	0.50
PR per treated cycle(%)	33.3	18.2	-10.66 to 40.0	0.43

PR = pregnancy rates, S = significant($p<0.05$)Imp = Implantation

Table 3 Outcome of pregnancies

Categories	100 IU (n = 21)	200 IU (n = 22)
Singleton	5	3
Twins	1	1
Triplet	-	1
Ectopic	-	1
Blighted ovum	-	1
Biochemical (>25 IU/L)	3	5
Total	9	12

respectively, ($p < 0.001$). The treatment length was slightly different but significantly longer in the 100 IU treatment group (11.7 days) compared to the 200 IU group (10.5 days, $p < 0.008$). The mean number of oocytes retrieved was 6.0 in the 100 IU group, not significantly lower compared to 9.1 in the 200 IU.

Safety

Two patients developed mild to moderate ovarian hyperstimulation syndrome(OHSS) in the 200 IU group. The clinical symptoms subsided after postponing the embryo transfer. No OHSS was observed in the 100 IU group.

Discussion

The present study represents a single-center, prospective, randomized double blind clinical trial of two different doses of Follitropin-β. Doubling the daily dosage from 100 IU and 200 IU, a borderline significantly larger number of follicles > 17 mm is achieved resulting in a non-significant increase of one third more retrieved oocytes per stared cycle. Furthermore, in the 100 IU regimen a mean of 1203 IU recombinant FSH were used, ending up with 2.4 transferable embryos, a vital pregnancy per started cycle of 23.3%, and no hospitalization due to OHSS. A lower dose of recombinant FSH not only decreases the costs but also reduces the adverse side effects. A similar outcome was found in a previous double-blind trial comparing 100 and 200 IU[9]. Where a dose response relationship was also seen.

It is obvious that the 200 IU group has a higher efficacy in terms of the number of mature oocytes retrieved, albeit not significant, and embryos transferred, statistically significant. Although the overall, number of fresh pregnancies was not higher in this group, it is likely that the pregnancy rate per single ovarian stimulation cycle including frozen-thawed embryo replacements will be greater in the high-dose group.

In the present study, the elevation of pregesterone concentration was lower in the 100 IU group compared to the 200 IU group (1.4 ng/ml vs 3.2 ng/ml). There is controversy whether elevated serum progesterone concentrations on the day of hCG administration could be detrimental of IVF outcome[9,11]. The implantation rate per embryo transfer was low in the 200 IU group (9.1% compared to 21.6% in the 100 IU group) which is similar to the findings in the study of Out et al[9].

The higher serum FSH levels and serum estradiol levels in the 200 IU group compared to the 100 IU group are merely the higher daily dose of administered rFSH and consequently the higher number of follicles developing in the group. The higher serum progesterone levels in the 200 IU group compared to the 100 IU group could be explained by the higher serum FSH levels leading to a greater FSH-induced LH receptivity in granulosa cells[12].

The cancellation rate was higher in the low-dose group(30% vs 6% in the high-dose group), but none of the subjects in the low-dose group developed OHSS compared to two subjects in the high-dose group.

In conclusion, a fixed daily dose of 200 IU of Follitropin β compared to a fixed daily dose of 100 IU is more effective in terms of follicles > 17 mm developing and the number of oocytes retrieved along with a lower cancellation rate, but less efficient as indicated by a higher total rFSH dose needed.

References

1. Hsueh AJ, Adashi EY, Jones PB, Welsh TH JR. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr Rev 1984; 5: 76-127.
2. Gemzell CA, Diczfalusy E, Tillinger KG. Human pituitary follicle-stimulating hormone I: clinical effect of a partly purified preparation. Ciba Foren Colloq Endocrinol 1960; 13: 191-200.
3. WHO Agents stimulation gonadal function in human. Report of a WHO scientific group WHO Tech Rep Ser 1973; 514-929.
4. Flamigni C, Venturole S, Dal Prato L, Porcu E. Purified FSH: Characteristics and application. In: Filecole M, Flamigni C, editors. Ovulation induction: Basic science and clinical advances. Elsevier Science 6V: Amsterdam 1994: 125-34.
5. Westergaard LG, Erb K, Laursen S, Rasmussen PE, Rex S. The effect of human menopausal gonadotropin and highly purified, urine derived follicle stimulating hormone on the outcome of in-vitro fertilization in down-regulated normogonadotropic women. Hum Reprod. 1996; 11: 1209-13.

6. Shoham Z, Insler V. Recombinant technique and gonadotropins production: new era in reproductive medicine. Fertil Steril. 1996; 66: 187-201.
7. Porchet HC, le Cotonnec JY, Loumaye E. Clinical pharmacology of recombinant human follicle-stimulating hormone III. Pharmacokinetic-pharmacodynamic modeling after repeated subcutaneous administration. Fertil Steril. 1994; 61: 687-95.
8. Out HJ, Driessen SG, Mannaerts BM, Coelingh Bennink HJ. Recombinant follicle-stimulating hormone (Follitropin beta, Puregon) yields higher pregnancy rates in in vitro fertilization than urinary gonadotropins. Fertil Steril 1997; 68: 138-42.
9. Out HJ, Lindenberg S, Mikkelsen AL, Eldar-Geva T, Healy DL, Leader A. et al. A prospective, randomized, double-blind clinical trial to study the efficacy and efficiency of a fixed dose of recombinant follicle stimulating hormone (Puregon®) in women undergoing ovarian stimulation. Hum Reprod. 1999; 14: 622.
10. Hoomans EH, Andersen AN, Loft A, Leerentveld RA, van Kamp AA, Zech H. A prospective, randomized clinical trial comparing 150 IU recombinant follicle-stimulating hormone(Puregon) and 225 IU highly purified urinary follicle stimulating hormone (Metrodin-HP) in a fixed-dose regimen in women undergoing ovarian stimulatioin. Hum. Reprod. 1999; 14: 2442.
11. Harada T, Yoshida S, Katagiri C, Takao N, Ikenari T, Toda T. et al. Reduced implantation rate associated with a subtle rise in serum progesterone concentration during the follicular phase of cycles stimulated with a combination of a gonadotropin-releasing hormone agonist and gonadotropin. Hum Reprod. 1995; 10: 1060-4.
12. Ubaldi F, Camus M, Smitz J, Bennink HC, Van Steirteghem A, Devrocy P. Premature luteinization in in vitro fertilization cycles using gonatropin-releasing hormone agonist(GnRH-a) and recombinant follicle stimulating hormone(FSH) and GnRH-a and urinary FSH. Fertil Steril. 1996; 66: 275-80.

การศึกษาเปรียบเทียบการใช้ยาฟอริโทรปิน-เบต้า สองขนาด ในการกระตุ้นการตกไข่

กำธร พฤกษานานนท์, สมชาย สุวจณกรณ์, วิสันต์ เสรีภาพงศ์, ประมวล วีรุตมเสน

การศึกษาเปรียบเทียบประสิทธิภาพ ประสิทธิผล และผลข้างเคียงทางคลินิก ของการใช้ยากระตุ้นการตกไข่ ฟอริโทรปิน- เบต้า ขนาด 100 หรือ 200 หน่วย ในการกระตุ้นการเจริญเติบโตของรังไข่ เพื่อการปฏิสนธินอกร่างกาย สตรีอาสาสมัคร 60 คน ที่มารับการรักษาภาวะมีบุตรยาก ถูกสุ่มให้ได้รับยาขนาด 100 หน่วย (30ราย) หรือ 200 หน่วย (30ราย) ของยา ฟอริโทรปิน-เบต้า พบว่าในกลุ่มที่ใช้ยาขนาด 100 หน่วย มีการกระตุ้นไม่สำเร็จมากกว่า (9 ราย เทียบกับ 2 ราย) การเกิดภาวะแทรกซ้อนจากการกระตุ้นมากเกินไป ไม่พบในกลุ่มที่ใช้ยา 100 หน่วย แต่พบในกลุ่มที่ใช้ยา 200 หน่วย 2 ราย อย่างไรก็ตาม ในกลุ่มที่ใช้ยาในขนาดสูง จะได้ฟอลิเคิล ขนาดมากกว่า 17 มม.จำนวนมากกว่า(4.4 เทียบกับ 3.3,p=0.05) และได้จำนวน ไข่ มากกว่ากลุ่มที่ใช้ขนาด 100 หน่วย (9.2 เทียบกับ 6.0, NS) จำนวนรวมของยาที่ใช้ในการกระตุ้นให้ได้ไข่อย่างน้อย 3 ใบ ในกลุ่มที่ฉีดครั้งละ 100 หน่วยจะน้อยกว่าอย่างมีนัยสำคัญทางสถิติ (1203.33 เทียบกับ 2106.67) กล่าวโดยสรุป การใช้ยาฟอริโทรปิน-เบต้าฉีดในขนาดวันละ 200 หน่วย จะมีประสิทธิภาพมากกว่าในเชิงของจำนวนฟอลิเคิลที่มากกว่า 17 มม. จำนวนไข่ที่เก็บได้ก็มากกว่า และ อัตราการกระตุ้นไม่สำเร็จมีน้อยกว่าในขณะเดียวกัน ประสิทธิผลจะน้อยกว่าการฉีดครั้งละ 100 หน่วย เนื่องจาก จำนวนรวมของยาทั้งหมดที่ใช้มากกว่า