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Pilot study of the optimal protocol of low dose step-up follicle stimulating hormone therapy for infertile women

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Abstract

Purpose: To evaluate the optimized protocol of low dose follicle-stimulating hormone (FSH) therapy that has a starting dose of 50 IU/62.5 IU with a small increment dose (12.5 IU) for women with World Health Organization (WHO) II ovulatory disorder and unexplained infertility.

Methods: Anovulatory women with WHO group II ovulatory disorder (ovulation induction [OI] patients, n = 29), and with an unexplained infertility (ovarian stimulation [OS] patients, n = 21) were enrolled. The protocol of low dose step-up FSH therapy was optimized for the starting dose as 50 IU (body mass index [BMI] < 20 group) and 62.5 IU (BMI ≥ 20 group) with the increment dose of 12.5 IU. Study outcomes were ovulation, monofollicular development and other variables.

Results: In the OIpatients, the ovulation rate was 100% (BMI < 20 group) and 90.9% (BMI ≥ 20 group). Monofollicular development was 80.0% (BMI < 20) and 77.3% (BMI ≥ 20). The pregnancy rate was 60% (3/5 BMI < 20) and 18.2% (4/22 BMI ≥ 20). There was no multiple pregnancy. In the OSpatients, the ovulation rate was 100%. Monofollicular development was 85.7% (BMI < 20) and 76.6% (BMI ≥ 20). No pregnancy was achieved in the OSpatients.

Conclusion: Optimized protocol of low dose FSH therapy setting a starting dose 50 IU/62.5 IU by BMI with an increment dose of 12.5 IU was safe and highly effective in WHO group II anovulatory patients. However, this protocol seemed uneffective for patients with unexplained infertility.

KEYWORDS
low dose FSH, ovarian stimulation, ovulation induction, polycystic ovary syndrome, WHO group II

1 | INTRODUCTION

Gonadotropin is a hormone that controls ovarian function in women. Abnormal secretion of gonadotropin results in an ovulatory disorder that is a common cause of infertility in women. Since human menopausal gonadotropin (hMG), which contains follicle stimulating hormone (FSH) and luteinizing hormone (LH), was established as a medical drug, it has been used broadly and successfully for patients

with World Health Organization (WHO) group I and II ovulatory disorders.[1] Purified preparation of FSH was also generated as a drug for WHO group II patients who did not need additional LH activity because their serum levels of LH remained adequate for the follicular development. Although hMG and FSH were derived from the urine of menopausal women, recombinant FSH has been generated using biotechnology and has contributed to the improvement of the quality and the clinical availability of the product. These gonadotropin drugs are used not only for ovulation induction (OI) in ovulatory disorders but also for ovarian stimulation (OS) in unexplained infertility[2] and OS in assisted reproductive technology (ART).[3]

On the other hand, infertility treatment, especially gonadotropin therapy, has brought serious side effects such as multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). In the 1970s, multiple pregnancy and severe OHSS rate were reported to be 36.3% and 3.9%, respectively, in 41 clomiphene-resistant PCOS patients treated with hMG-hCG.[4] Multiple pregnancy has a negative impact on maternal health and the long-term outcome of the infants, as well as imposing a significant burden on neonatal practice. OI by gonadotropin easily leads to multiple follicular development and multiple ovulation which are responsible for the multiple pregnancy. Controlling the serum FSH level suitable for monofollicular development is not easy because the negative feedback system does not work adequately in anovulatory patients, and therefore the dose of hMG or FSH often becomes excessive and multiple follicles develop. Based on the FSH threshold concept,[5] minimizing the dose of FSH seems to be effective in facilitating monofollicular growth and reducing the risk of multiple pregnancy associated with OI. Since a chronic low dose FSH regimen was reported in 5 PCOS women in 1984,[6] this type of regimen for OI has been reported to reduce multiple pregnancy.[7-10] In the low dose step-up regimen, the starting daily dose of FSH was 37.5 IU, 50 IU or 75 IU. The daily dose was increased by almost half of the starting dose; 25 IU or 37.5 IU, if no follicle developed after 7 days of administration, or after 14 days in the "chronic" protocol. Dose increment was considered weekly usually up to 4 times. The incidence of multiple pregnancy following OI is reported to be 5.7% in low dose FSH therapy.[10] However, this percentage might not be sufficient in comparison with recent ART, where elective single-embryo transfer (eSET) has become common and the multiple pregnancy rate has markedly reduced to 3.1% in fresh cycles and 3.2% in frozen cycles in 2015 in Japan.[11,12] Additionally, FSH or hMG, with or without intrauterine insemination (IUI), has been used for OS as an empiric therapy in patients with unexplained infertility who have normal FSH secretion.[2] OS, injecting FSH to women with unexplained fertility, results in higher serum levels of FSH allowing multiple follicular development, which might expand the chance of conceiving as well as increase a risk of multiple pregnancy.

There is evidence that women with a lower body mass index (BMI) are more likely to show an excessive ovarian response to OI, whereas women with a higher BMI generally require higher total gonadotropin doses for inducing follicular development and ovulation.[13] The devise used for subcutaneous self-injection (Gonalef® Pen; Merk Serono Co., Ltd., Tokyo, Japan) of recombinant FSH was recently renewed, and this has allowed us to adjust the FSH dose in smaller steps of 12.5 IU. In this study, we planned and tested an optimized protocol of FSH therapy that has an individualized starting dose based on BMI and a fine incremental doses as low as 12.5 IU for infertile women with an ovulatory disorder and unexplained infertility in order to facilitate a monofollicular ovarian response.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was a prospective interventional trial.

2.2 | Patients

Fifty infertile patients were enrolled in this study, including anovulatory women with WHO group II ovarian insufficiency for whom OI was indicated (OI patients, n = 29), and eumenorrheic women with a diagnosis of unexplained infertility for whom OS was indicated (OS patients, n = 21). These subjects were divided into groups by BMI, (BMI < 20 groups and BMI ≥ 20 groups) for selecting the initial FSH dose in the treatment described below (Section 2.3.). Each patient received only one cycle of the low dose step-up FSH therapy in this study, because we believe that multiple cycles treatment to the same patient is not fair due to the dose individualization in the following cycles and/or dropout of the patients who experienced unfavorable outcome in the first cycle preventing precise estimation of the protocol.

World Health Organization group II ovarian insufficiency consisted of amenorrhea (amenorrhea more than 6 months), oligomenorrhea (menstrual cycle more than 38 days), and anovulatory cycles (normal cycle length of 25-38 days without ovulation) which were diagnosed using the Japan Society of Obstetrics and Gynecology (JSOG) criteria.[14] The OI group included 20 patients with polycystic ovary syndrome (PCOS). Diagnosis of PCOS was made using the criteria of JSOG[14], which means that the PCOS patients in this study also met the Rotterdam criteria.[15]

Unexplained infertility was defined as not having apparent causes of infertility such as ovarian, male, and tubal factors. The OS patients had a regular menstruation of 25-38 days cycle with a biphasic pattern of basal body temperature, and had normal luteal function (mid-luteal progesterone [P4] concentration >5 ng/mL besides luteal phase length >10 days before registration).[16]

Two of the OI patients enrolled were ineligible because they appeared to have WHO class I ovarian insufficiency. Therefore, these 2 patients were excluded, leaving 48 patients evaluable for the study. One of the 2 excluded patients was a 33-year-old woman with a BMI of 21.5 kg/m^2 who had a baseline LH of 1.4 IU/L, FSH of 3.3 IU/L, and estradiol (E2) <10 pg/mL. She was treated with FSH at a starting dose of 62.5 IU and achieved successful ovulation and pregnancy without dose increase. The other patient excluded was a 29-year-old woman with a BMI of 21 kg/m^2 who had a baseline LH of <0.1 IU/L,

FSH of 0.2 IU/L, and E2 of <10 pg/mL. FSH was initiated at a starting dose of 62.5 IU, but the patient failed to respond (no follicle ≥10 mm in diameter) despite dose escalation.

2.3 | Treatment schedule

Recombinant human FSH (Gonalef® Pen; Merk Serono Co., Ltd.) was administered subcutaneously by self-injection according to a newly designed low dose step-up regimen for OI or OS. The starting dose of FSH was determined by BMI as 50 IU for women with a BMI <20 kg/m^2 (BMI < 20 groups) and 62.5 IU for women with a BMI ≥ 20 kg/m^2 (BMI ≥ 20 groups). Each patient started the daily self-injection of FSH between Days 3 and 6 of the first menstrual cycle after enrollment. If sufficient follicular development did not occur (no follicles ≥10 mm in diameter), the dose of FSH was increased by 12.5 IU, which is the minimum dose in the device. If a follicle ≥10 mm in diameter was found, daily dose was not increased. If the follicle did not reach 18 mm in diameter within a week after it reached 10 mm in diameter or genital bleeding occurred during the treatment, the cycle was canceled. A decision about dose increment was made every 7 days and up to 4 increases were permitted. If a follicle ≥10 mm in diameter did not appear during the 35 days of injection, the cycle was canceled due to no response. Follicular development was monitored by transvaginal ultrasonography (US), which was performed on Day 7 of treatment and every 2-4 days thereafter. When the mean diameter of the dominant follicle reached 18 mm, a single dose of 5000 IU of human chorionic gonadotropin (hCG) was administered to induce ovulation. If 4 or more follicles ≥16 mm in diameter were detected by transvaginal US, FSH treatment was discontinued, and administration of hCG was cancelled to reduce the incidence of multiple pregnancy.[17] Additionally, IUI was scheduled on the day after the hCG injection based on their own choice considering the couple's convenience and the duration of infertility.

2.3.1 | Transvaginal ultrasonography

The ovaries and follicular development were evaluated using transvaginal US with a 7.5 MHz transducer (Sonovista FX; Siemens Healthcare K.K., Tokyo, Japan). Ovarian volume per ovary (OVPO) was evaluated by the same gynecologist (T. M.) at the starting day of the FSH treatment and determined by the following formula: 0.5 × length × width × thickness.[18] OVPO associates with serum anti-Müllerian Hormone (AMH) level in PCOS,[19] which reflects ovarian reserve and could influence on the ovarian response to FSH. OVPO ≥10 cm^3 was reported to be one of the definitive factors and had 100% specificity to distinguish PCOS.[18] All of the patients with OVPO ≥10 cm^3 in the present study had PCOS.

2.3.2 | Blood sampling and hormone assay

Blood samples were collected on the day of starting the treatment before the first FSH injection (initial; for measuring LH, FSH, E2, testosterone [T], and AMH), on the day of hCG injection (preovulatory phase; for measuring LH, FSH and E2), and after 7-8 days from hCG injection (mid-luteal phase; for measuring P4). Serum LH and FSH concentrations were measured using chemiluminescence immunoassay assay kits (ARCHITECT® LH II and FSH; Abbott Japan Co., LTD, Tokyo, Japan). Serum E2 and P4 concentrations were measured using the electrochemiluminescence immunoassay kit (ECLusys® Estradiol II and Progesteron III; Roche Diagnostics, K.K., Tokyo, Japan). The serum AMH concentration was measured using the electrochemiluminescence immunoassay kit (ACCESS AMH; Beckman Coulter, Inc., Brea, CA, USA). Serum LH, FSH, and T concentrations were used for diagnosing PCOS as well as monitoring the hormonal status during the treatment.

2.4 | Study variables

2.4.1 | Demographic and clinical characteristics

Demographic profile (age, height, weight, BMI), history of infertility (duration, previous treatment, the presence of male factor and tubal factor in order to diagnose unexplained infertility), serum levels of hormones (FSH, LH, E2, P4, T, and AMH), and transvaginal US finding including polycystic ovarian morphology and ovarian volume both of which are essential for the diagnosis of PCOS, were summarized.

2.4.2 | Outcomes of the treatment, efficacy, and safety

Treatment outcome was estimated by dose up times, total dose of FSH, and the days of FSH injection. Successful ovulation, clinical pregnancy (detectable gestational sac in the uterus by trasvaginal US), monofollicular development, number of developing follicles (≥16 mm in diameter), hCG cancellation due to an excessive (4 or more developing follicles ≥16 mm in diameter) or insufficient (no follicles ≥10 mm in diameter) response to FSH, multiple pregnancy, moderate or severe OHSS, and other side effects such as genital bleeding during the cycle and thromboembolic events associated with severe OHSS.

2.4.3 | Demographic and baseline parameters influencing/predicting treatment outcomes in OI

The relationship between the number of developing follicles (0-2) or daily dose of FSH that was effective to induce monfollicular development (FSH threshold) and multiple factors, such as demographic/baseline parameters and serum hormone concentrations during the study, was analyzed in OI patients. Thereafter, multiple regression analysis was tried using the factors that showed a significant relationship with the FSH threshold which is a final daily FSH dose in the cycle achieving monofollicular development.

2.5 | Statistical analysis

Difference in values between the groups were analyzed using Student's *t* test, Welch's *t* test, and Mann-Whitney's *U* test after

considering the variance and distribution. An association between 2 categorical variables of FSH dose increment was analyzed using the chi-squared test. Correlations between FSH threshold and variables were calculated using Spearman's rank order analysis or Pearson correlation test. The correlation between FSH threshold and variables was examined using multiple regression analysis. XLSTAT 2016.2 (ADDINSOFT, New York, NY, USA) with Microsoft® Excel for Mac 15.32 (Microsoft Japan Co., Ltd., Tokyo, Japan) was used for statistical analysis. All data are presented as mean + SD values. Statistical significance was defined as $P < .05$.

2.6 | Ethics

This study was approved by the Ethical Committee of Tokushima University Hospital (approval No. 2075) and written informed consent was obtained from all the participants.

3 | RESULTS

Background data of the subjects are shown in Table 1. There was no significant difference between the BMI < 20 group and BMI ≥ 20 group in the OI or OS patients. Although this study did not intend to compare the data of the OI patients with the OS patients, several elements in their background showed significant differences between them. The duration of infertility was longer in the OS patients than in the OI patients because anovulatory women in the OI would be aware that they had difficulty in conceiving, and therefore, would consult a clinic earlier than women with unexplained infertility in the OS. The BMI ≥ 20 group in OS had significantly higher mean age compared with the other 3 subgroups ($P < .01$). The OI patients showed relatively larger OVPO especially in BMI ≥ 20 patients ($P < .01$) and tended to have higher serum AMH concentration, because 20 of 27 in the OI patients were PCOS, having enlarged ovaries. All of the patients with OVPO ≥10 cm^3 in the present study were PCOS patients.

As for the administration of FSH in the OI patients, the BMI ≥ 20 group tended to be greater in the parameters than in the BMI < 20 group regarding the number of FSH doses increased, the total dose, or the number of days of FSH treatment, although the differences were not significant (Table 2). The dose up times in OI were summarized in Tables 3 and 4. Two or more dose increments were needed in 0% (0/5) of the BMI < 20 group and 22.7% (5/22) of the BMI ≥ 20 group (Table 3). When BMI ≥ 20 group was divided into 3 subgroups by BMI, 2 or more dose increments were needed in 10.0% (1/10), 28.6% (2/7), and 40.0% (2/5, both of these patients failed to respond to FSH) of 20 ≤ BMI < 25, 25 ≤ BMI < 30, and BMI ≥ 30 subgroups, respectively (Table 4). However, even with BMI ≥ 30, 2 patients did not require any dose increment. Difference between BMI subgroups was not significant by chi-squared test due to the low number of patients for the statistical analysis. On the other hand, in the OS patients, there was no significant difference between the BMI < 20 group and the BMI ≥ 20 group for the parameters of FSH administration (Table 2), and all the patients did not need 2 or more dose increments (Table 5).

As for the clinical outcome of the OI patients, ovulation rate was 100% in the BMI < 20 group and ≥90% in the BMI ≥ 20 group (Table 6). In the 2 patients who failed to achieve ovulation, no follicle grew over 10 mm in diameter even after 3 or 4 times of FSH dose escalations. The number of developing follicle (≥16 mm in diameter) was distributed from 0 to 2. Monofollicular development rate per cycle with developing follicle in the OI patients was 80.0% in the BMI < 20 group and 85.0% in the BMI ≥ 20 group. Monofollicular development rate per all cycles including cycles with no follicular development was 77.3% in the BMI group and 85.0% in the BMI ≥ 20 group. The mean number of developed follicles was as small as 1.20 and 1.15 in the BMI < 20 group and the BMI ≥ 20 group, respectively. Pregnancy rate was 60.0% (3/5) in

TABLE 1 Background of the subjects

	Ovulation induction (n = 27)		Ovarian stimulation (n = 21)	
	BMI < 20 (n = 5)	BMI ≥ 20 (n = 22)	BMI < 20 (n = 7)	BMI ≥ 20 (n = 14)
Initial dose	50 IU	62.5 IU	50 IU	62.5 IU
Age (years)	32.4 ± 3.3	31.9 ± 2.9	33.4 ± 3.0	37.0 ± 2.6[a]
Duration of infertility (years)	0.90 ± 0.87	1.47 ± 1.54	4.88 ± 3.40[b]	5.45 ± 4.21[c]
Height (cm)	158.2 ± 7.4	156.3 ± 6.0	159.1 ± 4.7	156.4 ± 4.4
Weight (kg)	47.0 ± 4.3	64.1 ± 14.2	47.9 ± 2.7	61.1 ± 8.8
Body mass index (kg/m^2)	18.7 ± 1.2	26.1 ± 5.0	18.8 ± 0.5	25.0 ± 3.8
OVPO (cm^3)	9.64 ± 3.21[d]	7.72 ± 3.13	5.30 ± 2.13	6.28 ± 1.92
OVPO ≥ 10 cm^3 (%)	20.0 (1/5)	22.7 (5/22)	0 (0/6)	0 (0/12)
AMH (ng/mL)	12.90 ± 8.64	8.53 ± 5.17	3.45 ± 1.55	3.03 ± 1.34

AMH, anti-Müllerian hormone; BMI, body mass index; OVPO, ovarian volume per ovary; SD, standard deviation.
[a]$P < .01$ vs other groups (mean ± SD).
[b]$P < .05$ vs OI BMI < 20 (mean ± SD).
[c]$P < .01$ vs OI BMI ≥ 20 (mean ± SD).
[d]$P < .05$ vs OS BMI < 20 (mean ± SD).

TABLE 2 Administration of follicle-stimulating hormone (FSH)

	Ovulation induction (n = 27)		Ovarian stimulation (n = 21)	
	BMI < 20 (n = 5)	BMI ≥ 20 (n = 22)	BMI < 20 (n = 7)	BMI ≥ 20 (n = 14)
Initial dose	50 IU	62.5 IU	50 IU	62.5 IU
Dose up times (times) (Mean ± SD)	0.20 ± 0.40	0.91 ± 1.35	0.29 ± 0.45	0.07 ± 0.26
Total dose of FSH (IU) (Mean ± SD)	625.0 ± 216.8	1111.4 ± 867.3	553.6 ± 244.6	573.2 ± 133.4
Days of FSH injection (days) (Mean ± SD)	12.0 ± 3.6	15.5 ± 10.2	10.4 ± 3.9	9.1 ± 1.9

BMI, body mass index; SD, standard deviation.

TABLE 3 Dose up times in ovulation induction patients

Dose up times	BMI < 20 (n = 5)	BMI > 20 (n = 22)
≤1		
0	4	13
1	1	4
≥2		
2	0	1
3	0	2[a]
4	0	2[b]

BMI, body mass index.
[a]One of the patients ended in no follicular development.
[b]One of the patients ended in genital bleeding.

TABLE 4 Dose up times in the subgroups of body mass index (BMI) ≥ 20 group in ovulation induction patients

Dose up times	20 ≤ BMI<25 (n = 10)	25 ≤ BMI<30 (n = 7)	BMI ≥ 30 (n = 5)
≤1			
0	7	4	2
1	2	1	1
≥2			
2	0	1	0
3	1	0	1[a]
4	0	1	1[b]

[a]This patient ended in no follicular development.
[b]This patient ended in genital bleeding.

TABLE 5 Dose up times in ovarian stimulation patients

Dose up times	BMI < 20 (n = 7)	20 ≤ BMI (n = 14)
≤1		
0	5	13
1	2	1
≥2		
2	0	0
3	0	0
4	0	0

BMI, body mass index.

the BMI < 20 group and 18.2% (4/22) in the BMI ≥ 20 group. All 7 pregnancies achieved in the OI patients were singleton. There was no cycle of hCG cancellation due to excessive response, and no incidence of moderate or severe OHSS and also no mild OHSS. The other adverse reaction to FSH was genital bleeding with no response of the ovary, which was reported in one patient receiving the OI treatment.

In the OS patients, ovulation rate was 100% in both the BMI < 20 group and the BMI ≥ 20 group. The number of developing follicle (≥16 mm in diameter) was distributed from 1 to 3. Monofollicular development rate in OS was 85.7% in the BMI < 20 group and 78.6% in the BMI ≥ 20 group. The mean number of developing follicles was as small as 1.14 and 1.36 in the BMI < 20 group and the BMI ≥ 20 group, respectively. Ten of 21 patients received IUI. No patient in the OS patients became pregnant. There was no cycle of hCG cancellation due to excessive response, and no incidence of moderate or severe OHSS. Mild OHSS occurred in 1 patient, who was a 35-year-old woman with a BMI of 21.87 kg/m^2. She had 3 developing follicles with an ovarian size of 7.67 cm × 4.72 cm by initial FSH dose of 62.5 IU and thereafter hCG was injected. There was no other side effect in the OS patients.

Serum hormone levels during FSH treatment was depicted in Figure 1. The E2 level increased significantly from the start of FSH treatment (initial) to the day of hCG injection (preovulatory) in all of the BMI groups of both OI and OS ($P < .05$ or $P < .01$). No group showed an increase in FSH level during FSH treatment, and OI BMI < 20 group tended to decrease it ($P < .1$). The LH level increased during FSH treatment in the OS groups and the difference was significant in the OS BMI ≥ 20 ($P < .05$) group. Mid-luteal P4 levels of OI BMI < 20, OI BMI ≥ 20, OS BMI < 20, and OS BMI ≥ 20 were 14.8 ± 5.67, 21.83 ± 13.29, 16.56 ± 6.16, and 14.27 ± 8.41, respectively, and were not significantly different among these groups. All patients showed normal luteal function during the study cycle.

In order to find a factor that influenced ovarian response to FSH treatment in the OS patients, the relationship between the number

TABLE 6 Clinical outcome of the treatment

	Ovulation induction (n = 27)		Ovarian stimulation (n = 21)	
	BMI < 20 (n = 5)	BMI ≥ 20 (n = 22)	BMI < 20 (n = 7)	BMI ≥ 20 (n = 14)
Initial dose	50 IU	62.5 IU	50 IU	62.5 IU
Ovulation (%)	100.0 (5/5)	90.9 (20/22)	100.0 (7/7)	100.0 (14/14)
Monofollicular development (%)	80.0 (4/5)	85.0 (17/20)	85.7 (6/7)	78.6 (11/14)
Number of developing follicles ≥16 mm	1.20 ± 0.40	1.15 ± 0.36	1.14 ± 0.35	1.36 ± 0.72
No response (%)	0 (0/5)	9.1 (2/22)	0 (0/7)	0 (0/14)
hCG cancel (%)	0 (0/5)	9.1 (2[a]/22)	0 (0/7)	0 (0/14)
Pregnancy (%)	60.0 (3/5)	18.2 (4/22)	0 (0/7)	0 (0/14)
Multiple pregnancy (%)	0 (0/3)	0 (0/4)	—	—
OHSS (%)[b]	0 (0/5)	0 (0/22)	0 (0/7)	0 (0/14)
Other side effects (%)	0 (0/5)	4.6 (1[c]/22)	0 (0/7)	0 (0/14)

BMI, body mass index; hCG, human chorionic gonadotropin; OHSS, ovarian hyperstimulation syndrome; OVPO, ovarian volume per ovary; SD, standard deviation.
[a]Cancel due to no follicular development.
[b]Moderate or severe OHSS.
[c]Genital bleeding (Mean ± SD).

of developing follicles (≥16 mm in diameter) and various factors, such as demographic and baseline parameters, were analyzed (Table 7). As a result, BMI was the only factor showing a significant correlation with the number of the developing follicles ($r = -.410$, $P < .05$). Among the OI patients who achieved monofollicular development, the relationship between the final daily dose of FSH that was effective to induce monofollicular development (FSH threshold) and demographic/baseline parameters was analyzed (Table 8). Body mass index was the only factor showing a significant correlation with the FSH threshold ($r = -.503$, $P < .05$), whereas age almost reached significance ($r = -.428$, $P = .053$). When a multiple regression analysis was performed using a model with the FSH threshold as a response variable and with BMI and age as explanatory variables, there was no significant association between the FSH threshold and BMI or age (Table 9). On the other hand, no parameter was significantly correlated with the number of developing follicles in the OS patients based on Spearman/Pearson correlation analysis (data not shown). As for AMH, although several factors (OVPO, PCOS, baseline serum LH level, duration of infertility and age) showed a significant correlation with the serum AMH level based on Spearman/Pearson correlation analysis in the OI patients ($P < .05$ or $P < .01$), none of the factors concerning FSH treatment (dose up times, initial and

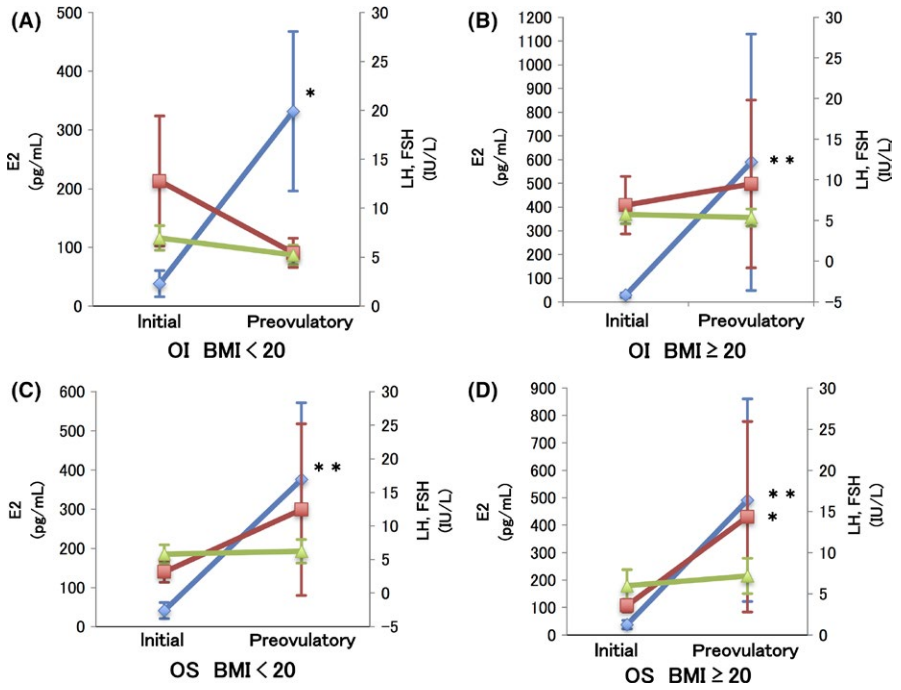

FIGURE 1 Hormonal transition during follicle-stimulating hormone (FSH) injection is depicted. A and B, showed data of ovulation induction (OI) for patients with World Health Organization (WHO) group II anovulation and C and D, showed data of ovarian stimulation (OS) for patients with unexplained infertility. FSH did not elevate during FSH injection in OI and OS. Data are presented as mean ± SE values; *$P < .05$ vs initial; **$P < .01$ vs initial; —◇—, E2; —□—, LH; —△—, FSH

TABLE 7 Relationship between the number of the developing follicles (0-2) and other factors in ovulation induction patients

	Correlation coefficient (*r*)	*P* value
Significant correlation		
Body mass index (kg/m^2)	−.410	.037
No significant correlation		
Initial estradiol (pg/mL)	.374	.062
Weight (kg)	−.319	.104
Preovulatory estradiol (pg/mL)	.294	.168
Mid-luteal progesterone (ng/mL)	.195	.371
Initial follicle-stimulating hormone (IU/L)	.154	.443
Duration of infertility (years)	−.128	.515
Age (years)	.127	.519
Preovulatory luteinizing hormone (IU/L)	−.112	.598
Type of irregular menstruation	.113	.564
Polycystic ovary syndrome	.090	.647
Ovarian volume per ovary (cm^3)	.051	.795
Initial luteinizing hormone (IU/L)	.032	.874
Height (cm)	.019	.924
Preovulatory follicle-stimulating hormone (IU/L)	−.017	.935

TABLE 8 Relationship between effective daily dose of follicle-stimulating hormone (FSH) and other factors in the cycles which single follicle developed in ovulation induction patients

	Correlation coefficient (*r*)	*P* value
Significant correlation		
Body mass index (kg/m^2)	.503	.024
No significant correlation		
Age (years)	−.428	.053
Ovarian volume per ovary (cm^3)	.312	.169
Initial FSH (IU/L)	−.263	.253
Weight (kg)	.220	.337
Mid-luteal progesterone (ng/mL)	−.163	.502
Preovulatory luteinizing hormone (IU/L)	−.159	.501
Polycystic ovary syndrome	.148	.509
Initial luteinizing hormone (IU/L)	−.126	.584
Preovulatory estradiol (pg/mL)	−.125	.596
Duration of infertility (years)	.117	.601
Initial estradiol (pg/mL)	−.111	.640
Type of irregular menstruation	−.048	.829
Preovulatory FSH (IU/L)	.008	.975
Height (cm)	.007	.976

total doses, and duration of the treatment) were correlated with the serum AMH level (data not shown).

4 | DISCUSSION

Multiple pregnancy is a major side effect of gonadotropin therapy that is derived from multifollicular development due to injection of excessive dose of FSH. Low dose step-up regimen of FSH was constructed as a safe method for WHO group II anovulatory patients, and has contributed to reduce the incidence of multiple pregnancy. We designed the new fine protocol of low dose step-up FSH therapy to reduce multifollicular development and the rate of multiple pregnancy. As a result, this protocol was highly effective to achieve monofollicular development and completely avoided the cancellation of the cycle due to excessive response and the complications of multiple pregnancy and/or OHSS, while having high rates of ovulation and pregnancy in WHO group II anovulatory patients.

Principle of low dose step-up FSH therapy was supported by FSH threshold concept.[5] In order to achieve monofolliclar development, we have to control serum FSH level just above the threshold level suitable only for the dominant follicle to grow. Otherwise, higher FSH level far from the threshold level allows the growth of non-dominant follicles. Therefore, starting dose should be less than the threshold and preferably close to it if predicted. Careful seeking of the threshold level during the FSH treatment, smaller increment dose is apparently preferable to avoid over dose. Both of starting dose and increment dose of FSH would be crucial factors in the low dose step-up protocol for monofollicular development. The new devise of FSH self-injection has enabled us to use fine adjustment of FSH dose. We decided to use 50 IU or 62.5 IU as an starting dose and 12.5 IU as an increment dose. Doses of 62.5 IU and 12.5 IU have not ever been used in the protocols of low dose step-up FSH therapy. Serum FSH level did not rise from initial to preovulatory phase during FSH injection, indicating that FSH level was controlled suitably by our new protocol.

Phase II study of recombinant FSH (follitropin alfa, GONALEF®/GONAL-f®; Merk Serono S.A.) in Japan was conducted with starting doses of 37.5 IU, 75 IU and 150 IU, with increment dose of 37.5 IU for WHO group II anovulatory women. Both of 37.5 IU and 75 IU

TABLE 9 The multiple regression analysis of the factors related to follicle-stimulating hormone threshold in ovulation induction group (n = 21)

Explanatory variable	Regression coefficient	Partial correlation coefficient	*P* value
Age(years)	−2.234	−.385	.094
Body mass index (kg/m^2)	0.322	.114	.632

groups showed favorable results in this phase II study; the rates of ovulation and pregnancy were 86.0% and 15.8% in 37.5 IU group, and 95.1% and 18.0% in 75 IU group. Rate of monofollicular development seemed higher in 37.5 IU group (64.9%) than in 75 IU group (50.8%), and rates of hCG cancellation and ovarian enlargement symptoms were lower in 37.5 IU group. However, because ovulation rate was lower and the days of FSH injection was longer in 37.5 IU group, 75 IU was selected as a starting dose for Japanese women in the phase II study.[19] The phase III study of follitropin alpha was conducted adopting the starting dose of 75 IU with increment dose of 37.5 IU. Monofollicular development was achieved only in 33.3% of the patients, and the rates of hCG cancellation and OHSS were 7.0% and 7.8%.[20] Results of the phase II and III trials suggested that 75 IU was somewhat excess as a starting dose for monofollicular development. Based on these data, optimized starting dose seemed to be located between 37.5 IU and 75 IU, which was a reason why we selected 50 IU and 62.5 IU as starting doses using the recent device with every 12.5 IU steps adjustable. There is another preparation of recombinant FSH, follitropin beta (follistim®; MSD K.K., Tokyo, Japan; formerly known as Banyu Pharmaceutical Co., Ltd., Tokyo, Japan, and Schering-Plough Corporation, Osaka, Japan.), which has been unfortunately withdrawn from the market in Japan in March, 2018. Phase II study of follitropin beta for Japanese women with WHO group II comparing the starting doses of 25 IU and 50 IU showed that 25 IU starting was insufficient because of lower ovulation rate (41.2%), and 50 IU starting showed relatively better result (57.2%) (Phase II study in Japan, internal report of MSD). In the phase III study of follitropin beta in Japan with starting dose of 50 IU and increment dose of 50 IU, ovulation rate was satisfactory (83.0%) but monofollicular development was achieved only in 28.3% (Phase III study in Japan, internal report of MSD), presumably due to large increment dose as 50 IU. Therefore, increment doses of 25 IU and 50 IU were compared in a low dose step-up protocol adopting a starting dose of 50 IU in another phase II study of follitropin beta in Japan. As a result, a 25 IU increment showed relatively higher rates of monofollicular development (39.3% vs 24.1%) and pregnancy (85.7% vs 75.9%) thn 50 IU increment although these differences were not statistically significant (Phase II study in Japan, internal report of MSD). A similar study in Europe and Canada including 158 women showed a significant result that the 25 IU increment group showed a significantly higher rate of monofollicular development (41.3% vs 21.8%) with fewer cancellations (5.0% vs 20.5%) than the 50 IU increment group.[21] Thus, increment dose of 50 IU seems too large and detract from the benefit of the favorable starting dose of 50 IU. Recent report suggested that relatively small increment dose of 25 IU also detracts benefit of extremely low starting dose of 25 IU that 15.8% of cycles was cancelled due to excessive response in spite of 62.6% of monofollicular development cycle.[22] Therefore, we think an increment dose would be the most important factor to get the FSH threshold level. The increment dose of our new protocol was as small as 12.5 IU, being adjustable in the recent devise, and this small dose would bring better results than previously tested protocols. However, a small increment rationally has the potential to prolong the treatment days due to multiple increments required to reach the FSH threshold level. Therefore, we had to set a starting dose close to the FSH threshold which differs individually. Previous studies in Japan suggested that 75 IU seemed excessive as a starting dose and 50 IU was insufficient for some of the patients. In general, lean anovulatory patients respond well to FSH and obese patients did not do so. BMI, ovarian volume, and menstrual pattern were reported to be independent predictive factors of the FSH threshold.[23] Especially, BMI is objective and easy to calculate. On the other hand, an intermediate dose between 50 IU and 75 IU is 62.5 IU that we can now set using the recent self-injection devise. We planned to use a starting dose as 50 IU for women with a BMI <20 and as 62.5 IU for women with a BMI ≥20 in the new protocol coupling with a smallest increment dose of 12.5 IU because lean women might respond well to 50 IU, and 62.5 IU might be excessive for them.

Our present data from the newly developed protocol with starting dose of 50 IU or 62.5 IU with 12.5 IU increment showed superior clinical outcome to the data of follitropin alfa starting at 75 IU with a 37.5 IU increment and the data of follitropin beta starting at 50 IU with a 25 IU increment previously reported in Japan for WHO II anovulatory women, as for ovulation rate, monofollicular development, hCG cancellation, and incidence of side effects such as multiple pregnancy and OHSS. The pregnancy rate seemed satisfactory and the number of days of injection was acceptable in most of the patients. Our data also seems better than those of the excellent conventional low dose step-up regimens using urinary FSH: an ovulation rate of 72% and a monofollicular development rate of 73% reported by Hamilton-Fairley et al,[7] ovulation rates of 73% or 71% and monofollicular development rates of 72% or 84% at starting doses of 75 IU or 52.5 IU reported by Homburg et al.[10] Thus, an ovulation rate of 90% and/or monofollicular development rate of 70% has been reported in the literature as an achievement of the low dose step-up FSH therapy. Our new optimized protocol achieved a higher rate of monofollicular development (80.0%/85.0% [BMI < 20/BMI ≥ 20]) with adequate conception rate (100.0%; 90.9%) with no hCG cancellation due to excessive response, no multiple pregnancy, or OHSS in patients with WHO group II ovulatory disorder, adding a further improvement on the previous excellent low dose protocols reported in the literature.

Frequent dose up of FSH for no response of the ovary results in a prolonged treatment period and brings disappointment to the patients. There were several OI patients in the BMI ≥ 20 group who required 2 or more dose up times. It seems that such patients were prevalent in higher BMI subgroups. However, more than half the patients of each BMI subgroup responded in initial step or one-time dose up step, indicating that starting doses of 50 IU in the BMI < 20 group and especially 62.5 IU in the BMI ≥ 20 group should be kept to prevent injecting an excessive dose. We tried to find factors affecting a number of developing follicles or FSH threshold in monofollicular development cycle. However, only BMI was a significant factor. To date, no effective prediction for FSH threshold has been reported although several factors are associated with FSH threshold.[22,23] It is a future challenge to find an effective factor to predict FSH threshold other than BMI. Meanwhile, the low dose step-up method would be a realistic method for ovulation induction in WHO group II patients to reach the FSH threshold during the treatment. For patients who require multiple dose up due to no response in the initial one or two cycles, adjusting the starting dose in the following cycle would be needed with reference to their own FSH threshold revealed in the initial cycle. Our optimized protocol with minimum increment dose and starting dose close to FSH threshold set by BMI would be a safe and effective modality, showing an insight for tactics modulating a low dose protocol.

Unexplained infertility is found in 15%-40%[24,25] of infertile couples. Ovarian stimulation by FSH has also been used as an empiric therapy for patients with unexplained infertility.[2,3] Patients with unexplained infertility have normal menstrual cycle, and OS by FSH frequently associated with multifollicular development, incidence of multiple pregnancy, and OHSS. In the recent study, 3 cycles of IUI with OS in patients with unexplained infertility brought 25/101 (24.7%) singleton live births, 4/25 (13.8%) multiple pregnancy, and no cases of OHSS.[26] Multiple pregnancy still seems to be problematic in OS treatment for unexplained infertility. In the present study, we tried mild stimulation in patients with unexplained infertility to prevent multiple pregnancy. As a result, no patient required 2 dose up times or more, and all patients ovulated with predominant monofollicular cycle (85.7% in BMI < 20 group and 78.6% in BMI ≥ 20 group). However, none of the 21 patients with unexplained infertility conceived. After the study, all 21 patients continued OS with or without IUI, and only 1 patient conceived to date in a total of 75 cycles with 4 cancelled cycles due to no response. It is assumed that mild stimulation by our protocol is not suitable for patients with unexplained infertility. If multiple ovulation itself might enhance the chance of pregnancy, reducing the incidence of multiple pregnancy might be misguided, and elective single-embryo transfer in ART would be the only solution of the problem especially for patients with relatively long infertility period such as our subjects in this study.

In conclusion, low dose, step-up treatment with FSH starting with a BMI-based starting dose (50 IU for BMI < 20 and 62.5 IU for BMI ≥ 20) with a small increment dose (12.5 IU) was a safe and effective protocol for OI in patients with WHO group II ovulatory disorder. In overweight and obese patients, who require 2 or more dose escalations, the starting dose of FSH for the following cycle should be individualized. On the other hand, mild stimulation using this protocol might not be useful for patients with unexplained infertility having relatively long infertility period.

DISCLOSURES

Conflicts of interest: The authors declare no conflict of interest. Funding: This study was supported by Merck Serono International S.A., Geneva, Switzerland and Merk Serono Co., Ltd., Tokyo, Japan. *Human and Animal Rights*: All the procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. This study was approved by the Ethical Committee of Tokushima University Hospital (approval No. 2075) and written informed consent was obtained from all the participants to be included in the study. This article does not contain any study with animal participants that have been performed by any of the authors.

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REFERENCES

1. Lunenfeld B. Historical perspectives in gonadotrophin therapy. *Hum Reprod Update*. 2004;10:453-467.
2. Nandi A, El-Toukhy T. Stimulated intrauterine insemination for unexplained subfertility. *Lancet*. 2018;391:404-405.
3. Bahadur G, Homburg R, Muneer A, et al. First line fertility treatment strategies regarding IUI and IVF require clinical evidence. *Hum Reprod*. 2016;31:1141-1146.
4. Wang CF, Gemzell C. The use of human gonadotropins for the induction of ovulation in women with polycystic ovarian disease. *Fertil Steril*. 1980;33:479-486.
5. Brown JB. Pituitary control of ovarian function–concepts derived from gonadotrophin therapy. *Aust N Z J Obstet Gynaecol*. 1978;18:46-54.
6. Seibel MM, Kamrava MM, McArdle C, Taymor ML. Treatment of polycystic ovary disease with chronic low-dose follicle stimulating hormone: biochemical changes and ultrasound correlation. *Int J Fertil*. 1984;29:39-43.
7. Hamilton-Fairley D, Kiddy D, Watson H, Sagle M, Franks S. Low-dose gonadotrophin therapy for induction of ovulation in 100 women with polycystic ovary syndrome. *Hum Reprod*. 1991;6:1095-1099.
8. Homburg R, Levy T, Ben-Rafael Z. A comparative prospective study of conventional regimen with chronic low-dose administration of follicle-stimulating hormone for anovulation associated with polycystic ovary syndrome. *Fertil Steril*. 1995;63:729-733.
9. White DM, Polson DW, Kiddy D, et al. Induction of ovulation with low-dose gonadotropins in polycystic ovary syndrome: an analysis of 109 pregnancies in 225 women. *J Clin Endocrinol Metab*. 1996;81:3821-3824.
10. Homburg R, Howles CM. Low-dose FSH therapy for anovulatory infertility associated with polycystic ovary syndrome: rationale, results, reflections and refinements. *Hum Reprod Update*. 1999;5:493-499.
11. Irahara M, Kuwahara A, Iwasa T, et al. Assisted reproductive technology in Japan: a summary report of 1992-2014 by the Ethics

Committee, Japan Society of Obstetrics and Gynecology. *Reprod Med Biol*. 2017;16:126-132.
12. Saito H, Jwa SC, Kuwahara A, et al. Assisted reproductive technology in Japan: a summary report for 2015 by The Ethics Committee of The Japan Society of Obstetrics and Gynecology. *Reprod Med Biol*. 2017;17:20-28.
13. Bruna-Catalan I, Menabrito M. Ovulation induction with minimal dose of follitropin alfa: a case series study. *Reprod Biol Endocrinol*. 2011;9:142.
14. Japan Society of Obstetrics and Gynecology. *Glossary of Obstetrics and Gynecology*, 3rd ed. Tokyo, Japan: Japan Society of Obstetrics and Gynecology; 2013.
15. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81:19-25.
16. Schliep KC, Mumford SL, Hammoud AO, et al. Luteal phase deficiency in regularly menstruating women: prevalence and overlap in identification based on clinical and biochemical diagnostic criteria. *J Clin Endocrinol Metab*. 2014;99:E1007-E1014.
17. Loumaye E. *A phase III, open, randomized multicenter study to compare the safety and efficacy of recombinant human follicle-stimulating hormone (Gonal F) administered subcutaneously with that of urinary human follicle stimulating hormone (Metrodin) given intramuscularly, to induce ovulation in WHO group II anovulatory infertile women*. Internal Report. Geneva, Switzerland: Ares-Serono; 1995.
18. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. *Hum Reprod Update*. 2003;9:505-514.
19. Matsuzaki T, Munkhzaya M, Iwasa T, et al. Relationship between serum anti-Mullerian hormone and clinical parameters in polycystic ovary syndrome. *Endocr J*. 2017;64:531-541.
20. Taketani Y, Kelly E, Yoshimura Y, et al. Recombinant follicle-stimulating hormone (follitropin alfa) versus purified urinary follicle-stimulating hormone in a low-dose step-up regimen to induce ovulation in Japanese women with anti-estrogen-ineffective oligo- or anovulatory infertility: results of a single-blind Phase III study. *Reprod Med Biol*. 2010;9:99-106.
21. Leader A. Monofollicular ovulation induction study group. Improved monofollicular ovulation in anovulatory or oligo-ovulatory women after a low-dose step-up protocol with weekly increments of 25 international units of follicle-stimulating hormone. *Fertil Steril*. 2006;85:1766-1773.
22. Lan VT, Norman RJ, Nhu GH, Tuan PH, Tuong HM. Ovulation induction using low-dose step-up rFSH in Vietnamese women with polycystic ovary syndrome. *Reprod Biomed Online*. 2009;18:516-521.
23. Nyboe Andersen A, Balen A, Platteau P, Devroey P, Helmgaard L, Arce JC. Predicting the FSH threshold dose in women with WHO Group II anovulatory infertility failing to ovulate or conceive on clomiphene citrate. *Hum Reprod*. 2008;23:1424-1430.
24. Smith S, Pfeifer SM, Collins JA. Diagnosis and management of female infertility. *JAMA*. 2003;290:1767-1770.
25. Gelbaya TA, Potdar N, Jeve YB, Nardo LG. Definition and epidemiology of unexplained infertility. *Obstet Gynecol Surv*. 2014;69:109-115.
26. Nandi A, Bhide P, Hooper R, et al. Intrauterine insemination with gonadotropin stimulation or in vitro fertilization for the treatment of unexplained subfertility: a randomized controlled trial. *Fertil Steril*. 2017;107:1329-1335.

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