A Modified hMG-GnRH Method for the Induction of Ovulation in Infertile Women with Severe Hypogonadotrophic Amenorrhea

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Abstract. The objective of this study was to compare, in infertile women suffering from severe hypogonadotrophic amenorrhea, the therapeutic utility and the incidence of complications arising from fertility treatment by the conventional human menopausal gonadotropin/human chorionic gonadotropin (hMG-hCG) method, the hMG step-down method, the sequential hMG/gonadotropin-releasing hormone (GnRH) method and a new, modified hMG-GnRH method that has been developed by us. In the step-down method, the daily dose of hMG was decreased from 150 IU to 75 IU when the follicle diameter reached 11-13 mm. In the sequential hMG-GnRH, hMG injection was switched to pulsatile GnRH administration (20 μg/120 min SC), when the follicle diameter reached 11-13 mm. In our new modified hMG-GnRH, pulsatile GnRH was injected together with hMG. Daily hMG was stopped and the GnRH dosage was changed from 10 μg to 20 μg when the follicle diameter reached 11-13 mm. Initially, the three established methods were applied randomly to treat 34 cycles in 20 women; and subsequently, five patients who failed to conceive following treatment by sequential hMG-GnRH were then treated by the modified hMG-GnRH method. More than eight growing follicles and multiple pregnancies were observed during treatment by the conventional method. The incidence of ovarian hyperstimulation syndrome (OHSS) was 25.7% with the conventional method, 20.0% with the step-down method and 0% with the sequential hMG-GnRH method; however, the rate of ovulation was only 50% with the sequential hMG-GnRH method. By contrast, with the modified hMG-GnRH method, less than three growing follicles occurred in 81.8% of patients, there was a 100% rate of ovulation, and neither OHSS nor multiple pregnancies were observed. Moreover, the modified hMG-GnRH method induced pregnancy in 3 out of 5 patients. These data indicate that this new method is favorable for the treatment of severe hypogonadotrophic amenorrhea.

Key words: Severe hypogonadotrophic amenorrhea, Pulsatile GnRH administration, Human menopausal gonadotropin, Induction of ovulation, Ovarian hyperstimulation syndrome (OHSS)

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PROGRESS in inducing ovulation with gonadotropins has made pregnancy possible for infertile women suffering from hypogonadotrophic amenorrhea, in particular, those with severe hypogonadotrophic amenorrhea. However, gonadotropin therapy frequently causes complications, including ovarian hyperstimulation syndrome (OHSS) and multiple gestation. In order to reduce the risks of such complications, several new methods of gonadotropin therapy have been developed, such as follicle-stimulating hormone (FSH) step-down treatment [1, 2]. The step-down protocol of ovulation induction mimics more closely the physiology of normal cycles, and van Santbrink et al. [2] have shown that a step-down regimen for gonadotropin induction of ovulation may serve as a safe and successful treatment alternative in patients with clomiphene-resistant anovulation.

In spite of these efforts, satisfactory results have not been achieved to date. Recently, induction of
single ovulation by sequential FSH and pulsatile
gonadotropin-releasing hormone (GnRH) treatment
has been reported [3]. Here we have re-examined
this FSH-GnRH method, and have also developed a
modified hMG-GnRH method in order to obtain a
higher incidence of ovulation and to reduce the risk
of complications. In this study, we have compared
the therapeutic utility and the incidence of compli-
cations arising from treatment by the conventional
hMG-hCG method, the hMG step-down method, the
sequential hMG-GnRH method and our own newly
developed modified hMG-GnRH method.

Materials and Methods

1) Subjects and methods of gonadotropin treatment

For this study, we recruited 20 women with severe
hypogonadotropic amenorrhea, that is, hypothala-
mic progestin non-responsive hypogonadotropic ame-
norrhea, out of the infertile women visiting our out-
patient clinic. Patients with polycystic ovary syn-
drome were not included in this study. Informed
consent was obtained from all subjects before entry.

Four different kinds of gonadotropin treatment
were given to these patients: the conventional hMG
method, the hMG step-down method, the sequential
hMG GnRH method, and the modified hMG-GnRH
method.

In the conventional method, hMG (Pergonal®,
Teikoku Hormone Mfg. Co., Japan) was injected at
a dose of 150 IU every day beginning on the 5th day
of cycle. In the step-down method, hMG was inject-
ed as in the conventional method; and when the di-
ameter of the leading follicle reached 11–13 mm, the
daily dose of hMG was decreased to 75 IU. In the
sequential hMG-GnRH method, hMG was initially
injected as in the conventional method, and when the
diameter of the leading follicle reached 11–13 mm,
pulsatile GnRH (Hypocrine®; Tanabe Pharmaceutical
Co., Tokyo, Japan) was administered instead of
hMG. GnRH was injected subcutaneously (s.c.) at a
dose of 20 µg every 120 min using an automatic infu-
sion pump (Model SP31; Nipro Co., Osaka, Japan)
until the follicle matured sufficiently. In the modi-
fied hMG-GnRH method, pulsatile GnRH was ad-
ministered by s.c. injection of 10 µg every 120 min
beginning on 5th day of the cycle in addition to a dai-
ly dose of 150 IU hMG. When the leading follicle
reached 11–13 mm, daily administration of hMG was
stopped and the GnRH dosage was changed from 10
µg to 20 µg. In all four methods, human chorionic
gonadotropin was administered at a dose of 5000 IU
when the mean diameter of the dominant follicle
reached 18 mm.

The three established regimens were applied ran-
domly to 34 cycles of the 20 women as follows: the
conventional method was applied to 15 cycles; the
step-down methods to 7 cycles; and the sequential
hMG-GnRH methods to 12 cycles. Five women who
failed to become pregnant by the sequential hMG-
GnRH method were then treated by our modified
hMG-GnRH method.

2) Monitoring and Statistics

Serial pelvic US examination was performed using
a 7.5 MHz transvaginal transducer, and the mean
diameter was calculated from the sum of the longitudi-
nal and transverse diameters of the follicle divided by
2. The follicle that was more than 15 mm in the
mean diameter was defined as the growing follicle,
and the number of the growing follicles was counted
at the time of hCG administration. OHSS was rec-
ognized as patients with abdominal discomfort and
sonographic evidence for enlarged ovaries that ex-
ceeded 60 mm in diameter as reported by Mizunami
et al. [1].

Serum concentrations of LH, FSH, prolactin
(PRL) and estradiol (E2) were determined with
SPAC-S LH kit (Daichii Radioisotope Labs, Ltd.),
SPAC-S FSH kit (Daichii Radioisotope Labs, Ltd.),
SPAC-S prolactin kit (Daichii Radioisotope Labs,
Ltd.), and E2 kit Daiichi II (Daichii Radioisotope
Labs, Ltd.), respectively.

Significant differences between methods were test-
ed after analysis of variance (one-way ANOVA) with
a Mann-Whitney Rank Sum test. A P value of <0.05
was considered to be statistically significant. Data
are expressed as the mean ± SE.

Results

The conventional method, the step-down method
and the sequential hMG-GnRH method
All patients had normal body mass index, and no
history of excessive exercise, and all patients had normal levels of thyroid hormone, testosterone and PRL levels. Between the women being treated by the three established methods, there was no significant difference in mean parameters such as age, body mass index, basal levels of serum FSH and E2 (Table 1). Before the study, all patients were screened and those with other severe infertility factors, such as tubal occlusion and severe oligospermia, were excluded.

The number of days of treatment did not differ significantly among the three methods, nor did the rate of ovulation. The mean numbers of growing follicles in the sequential hMG-GnRH method were significantly less than in the conventional hMG method (P<0.01). The incidence of ovarian hyperstimulation syndrome (OHSS) was 33.3% in the conventional hMG method and 57.1% in the step-down method, but OHSS was not observed in the sequential hMG-GnRH method. In the conventional method, the incidence of multiple pregnancies was 50%, whereas in the hMG step-down and sequential hMG-GnRH methods, multiple pregnancies did not occur (Table 1).

These data show that the conventional method induced high rates of ovulation and pregnancy, but caused higher numbers of growing follicles and more multiple pregnancies, including two sets of triplets and one of quintuplets out of six pregnancies. By contrast, the sequential hMG-GnRH method led to significantly decreased numbers of growing follicles, but also to a decreased rate of ovulation.

These data indicated that some patients with severe hypogonadotropic amenorrhea did not sufficiently respond to GnRH pulsatile therapy. The patients who failed to ovulate by the sequential hMG-GnRH method were therefore treated by the modified hMG-GnRH method.

The sequential hMG-GnRH method and the modified hMG-GnRH method

There was no significant difference in basal levels of serum LH, FSH and E2 between women treated by the sequential hMG-GnRH and the modified hMG-GnRH method (Table 1).

The number of days of treatment did not differ between the two methods, but the rate of ovulation was significantly higher in the modified hMG-GnRH method than in the sequential hMG-GnRH method

| Table 1. Background and clinical outcomes in patients treated by three established methods and the modified hMG-GnRH methods. Data are mean±SE. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Conventional method | Step-down method | Sequential hMG-GnRH | Modified hMG-GnRH method |
| No. of cases    | 11               | 6               | 7               | 5               |
| Age (y)         | 30.3±2.5         | 28.6±1.5        | 30.3±2.6        | 29.2±1.1        |
| Body mass index | 18.2±1.3         | 20.0±2.5        | 18.5±1.9        | 19.1±2.2        |
| LH (mIU/mL)     | 2.1±1.6          | 2.1±1.8         | 1.9±1.9         | 0.7±0.5         |
| FSH (mIU/mL)    | 4.8±3.5          | 5.9±4.1         | 4.6±3.9         | 1.5±2.2         |
| E2 (pg/ml)      | 15.4±11.1        | 18.8±16.2       | 16.7±13.5       | 11.9±6.2        |
| No. of cycles   | 15               | 7               | 12              | 11              |
| Days of treatment | 16.1±2.6       | 15.1±3.6        | 18.6±3.4        | 17.2±2.7        |
| Ovulation rate  | 80.0%            | 71.4%           | 50.0%           | 100%***         |
| No. of growing follicles | 5.2±4.4    | 3.0±1.2       | 2.3±2.0**       | 2.6±1.2         |
| Conception rate per cycle | 40.0%     | 0%             | 8.3%            | 27.3%           |
| Rate of OHSS    | 33.3%            | 57.1%           | 0%              | 0%              |
| Rate of multiple pregnancies | 50.0%     | 0%             | 0%              | 0%              |

**P<0.01 vs the conventional method
***P<0.001 vs Sequential hMG-GnRH method
(P<0.001). The mean number of growing follicles in the modified hMG-GnRH method did not differ from that in the sequential hMG-GnRH method.

The incidence of conception was 27.3% in the modified hMG-GnRH method compared with 8.3% in the sequential hMG-GnRH method. Neither OHSS nor multiple pregnancies were observed in either of the two methods (Table 1).

The numbers of growing follicles and the thickness of the endometrium

We compared the number of growing follicles among all four methods. More than eight growing follicles were observed only in patients treated by the conventional hMG method (33.3%). By contrast, less than three growing follicles were observed in 81.8% of the patients treated by the modified hMG-GnRH method (Fig. 1), which is a significant improvement over the conventional hMG method in which only 40.0% of the patients had less than three growing follicles (P<0.05).

We also compared the thickness of the endometrium at the time of hCG administration between the four methods. In the sequential hMG-GnRH method, the endometrium was significantly thinner than in the conventional hMG method (P<0.01); however, in the modified hMG-GnRH method, the endometrium was not significantly thinner than in either the conventional hMG method or the step-down method (Fig. 2).

Discussion

In our clinic, high rates of OHSS and multiple pregnancies have been observed in patients with severe
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hypogonadotropin amenorrhea and patients with polycystic ovary syndrome. Therefore, we re-examined treatment by the sequential hMG-GnRH method in patients with progesterin non-responsive hypogonadotropin amenorrhea, and compared it with treatment by both the conventional method and the step-down method.

In this study, more than eight growing follicles and multiple pregnancies were observed only with the conventional method. The incidence of OHSS was 25.7% with the conventional method, 20.0% with the step-down method and 0% with the sequential hMG-GnRH method. Considering only these complications, the sequential hMG-GnRH method seems to be the best method; however, its rate of ovulation was only 50%.

Kuwahara et al. [3] reported an ovulation rate of 89.7% following treatment by the sequential method in hypogonadotropin anovulatory patients, who are characterized by a more than six-month absence of menstruation and by low or normal levels of gonadotropin levels. Their study group included women with severe as well as mild hypogonadotropin amenorrhea.

Intravenous pulsatile GnRH administration can successfully induce ovulation in patients with hypothalamic amenorrhea by restoring the ovarian physiology [4, 5]; however, the disadvantages of intravenous therapy include local discomfort, the inconvenience of a forearm IV catheter, and the potential for thrombophlebitis. Therefore, s.c. administration of pulsatile GnRH is preferable, although drug absorption following s.c. administration is slower and lacks a definite serum peak, which may lead to prolongation of follicular phase and/or luteal phase defects in some cycles [6].

The sequential hMG-GnRH method was introduced to stimulate the physiological change in serum FSH levels. Daily injection of FSH initiates follicle development, and then pulsatile GnRH stimulates adequate pulsatile gonadotropin secretion from the pituitary to induce further maturation of the dominant follicle. The sequential method needed fewer days of GnRH pump treatment than did the conventional treatment of GnRH alone; and the sequential method achieved single follicular development without complications [3].

In this study, serum concentrations of FSH, LH and E2 were examined before treatment, at the time when the leading follicle was 11–13 mm in diameter and at the time of hCG administration in four patients who were treated by both the sequential hMG-GnRH and the modified hMG-GnRH method (results not shown). Here we found that serum levels of FSH decreased in the late follicular phase during the sequential hMG-GnRH method, as also reported by Kuwahara et al. [3], who also reported that levels of serum FSH did not decrease in the conventional FSH method during follicle stimulation. These factors result in a significant reduction in the number of growing follicles, because an augmented sensitivity for FSH allows several follicles to gain dominance when a decrease in FSH is prevented in the late follicular phase [7].

The modified hMG-GnRH method stimulates follicles through both hMG and s.c. pulsatile GnRH administration, and then induces further follicular maturation by pulsatile GnRH administration alone. This method seems to be useful for patients with severe hypothalamic amenorrhea, because these patients lack intrinsic pulsatile secretion of GnRH and their pituitary gland does not respond to GnRH sufficiently; therefore GnRH priming is required before GnRH therapy alone [8]. In fact, levels of serum estradiol decreased markedly following the time of dominant follicle (size 11–13 mm) to the day of hCG administration in the sequential hMG-GnRH method, and serum estradiol increased by the time of hCG administration in the modified hMG-GnRH method in patients who did not ovulate following treatment by the sequential hMG-GnRH and who ovulated by the modified hMG-GnRH method (results not shown). These data support the idea that GnRH priming is required before GnRH pulsatile treatment in some patients with severe hypogonadotropin amenorrhea. In this study, the pulsatile GnRH was administered from 5th day of the cycle, but only a few days of the GnRH priming may be sufficient. Therefore, the duration of the GnRH priming requires further investigation.

An ovulation rate of 100% was obtained with the modified hMG-GnRH method. In addition, 81.8% of the patients developed less than three growing follicles, and no patients showed OHSS or multiple pregnancies. Moreover, this modified hMG-GnRH method induced pregnancy in 3 out of 5 patients who failed to conceive by the sequential hMG-GnRH method.
In summary, the modified hMG-GnRH method gave rise to the highest ovulation rate and did not increase the incidence of complications. We conclude that this new method is favorable for treatment for severe hypogonadotropic amenorrhea, and should be investigated further as a treatment for infertility.

References