**Abstract:** This study sought to evaluate whether a short or long protocol would be preferable for simplification of a fixed-schedule for *in vitro* fertilization and embryo transfer (IVF-ET) program without negatively affecting the pregnancy rate per cycle. All women were allocated, according to the ovarian stimulation protocol, into two groups. In Group 1 for the short protocol (41 occasions), the patients were treated with human menopausal gonadotropin (hMG) before pituitary desensitization using buserelin from cycle day 1. In the Group 2 for the long protocol (106 occasions), the patients received hMG after pituitary desensitization achieved with buserelin. The day and time of ovum pick-up (OPU) was fixed as a Wednesday. Ovarian stimulation was started on Monday, 10 days before the day of OPU, with a daily dose of 225 IU/hMG for the following 7 days. Ovulation was induced with human chorionic gonadotropin (hCG) on Tuesday, and OPU was performed on Wednesday; ET was carried out on Friday. No significant differences were found between the two groups in the incidence of canceled cycles [group 1: 7.3 % (3/41), group 2: 2.8 % (3/106)], mean number of oocytes recovered (6.4 ± 4.0, 5.8 ± 5.1), the fertilization rate [40.6 % (106/261), 44.3 % (272/614)], or the pregnancy rate per cycle [19.5 % (8/41), 18.9 % (20/106)]. These findings indicate that treatment with buserelin/hMG/hCG with either a short or long protocol is useful for a fixed-schedule IVF-ET program.

**Key words:** Fixed schedule, In vitro fertilization and embryo transfer (IVF-ET), Gonadotropin releasing hormone agonist (GnRHa), Human menopausal gonadotropin (hMG), Human chorionic gonadotropin (hCG)

**INTRODUCTION**

Recently much effort has been put into simplifying the clinical management of *in vitro* fertilization and embryo transfer (IVF-ET) cycles. In order to simplify the IVF-ET program, we used a fixed schedule of ovarian stimulation and ovum pick-up (OPU) with buserelin, human menopausal gonadotropin (hMG), and human chorionic gonadotropin (hCG). We applied two treatment protocols: a short protocol and a long protocol. The selection of protocols depended on schedule convenience. This study establishes and evaluates both a short and long protocol for fixed-schedule ovarian stimulation and OPU in order to determine the efficacy and application in terms of pregnancy rates per cycle.

**PATIENTS AND METHODS**

**Patients**

Data were analyzed from female patients undergoing fixed-schedule ovarian stimulation and OPU in the IVF-ET program at the...
Yamanashi Medical University between 1993 and 1994. A total of 85 patients completing 147 ovarian stimulation cycles were included. All study patients gave their written consent before entering the study. The patients ranged in age from 26 to 46 years and in infertility period from 7 to 203 months. Couples included in the study met one of the following criteria: tubal damage (36.4%), endometriosis (20.0%), male factor (21.2%) or unexplained infertility (27.1%). Couples whose infertility was due to a severe male factor (concentration of motile spermatozoa < 1,000,000/ml) or abnormal menstrual cycles were excluded from the study.

**Ovarian Stimulation Protocol**

Patients were allocated to two groups according to the ovarian stimulation protocol. Group 1 received a short protocol, and group 2 received an early follicular long protocol, as described below. Allocation was based on the time interval between the first day of the menstrual period and the scheduled first day of the ovarian stimulation. Although precise randomization of the short and the long protocols would have been preferable, it was not possible in our study. Consequently, by the end of the study, group 2 completed more cycles than group 1.

**Group 1: (Short protocol)**

Forty-one occasions were included. In this group hMG was begun before pituitary desensitization was achieved. The start day of hMG administration varied between day 0 and day 12 of the menstrual cycles.

**Group 2: (Long protocol)**

One hundred six occasions were recorded. In this group hMG was started after pituitary desensitization was achieved. Pituitary desensitization was recognized as the absence of follicles with diameter > 5 mm and the absence of thick endometrium (< 4 mm) confirmed by vaginal ultrasonography. If pituitary desensitization was not achieved, administration of GnRHa was continued until desensitization was achieved, and then the day of OPU was re-determined.

All patients were given a gonadotropin-releasing hormone agonist (GnRHa) buserelin acetate (Suprecur; Hoechist Japan, Tokyo, Japan), 300 µg in a nasal spray three times daily, beginning on the first day of the menstrual cycle and continuing until the day of hCG (Gonatropin; Teikoku Hormone MFG. Co., Ltd., Tokyo, Japan) administration. We planned that the date of OPU would be fixed on a Wednesday for all patients. Ovarian stimulation commenced on a Monday, 10 days before the day of OPU, and was continued until the next Sunday. The standard dose of hMG (Pergonal; Teikoku Hormone MFG. Co., Ltd., Tokyo, Japan) was 3 ampoules (225 IU) per day for 7 days, but 4 and more ampoules per day were given, if necessary, according to the patient's history. On Friday, day 5 of treatment, the dosage of hMG was adjusted according to the follicular development determined by vaginal ultrasonography. The course of ovarian stimulation never exceeded 7 days.

On Monday, after the 7-day course of ovarian stimulation, a vaginal ultrasound scan was performed. When there was at least one follicle with a mean diameter of ≤ 10 mm, the patient was hospitalized, and 10,000 IU of hCG was injected intramuscularly on Tuesday morning at about 6:00 a.m., approximately 40 hours after the last injection of hMG. In 119 cycles (81.0%), at least two follicles reached a mean diameter of ≥ 16 mm. When there were no follicles with a mean diameter of ≤ 10 mm on the day of the scan, these cycles were canceled. At
the same time, serum estradiol (E₂) and progesterone (P₄) levels were measured. These results were used for subsequent data analysis and had no influence on the timing of OPU.

Ovum pick-up was performed by transvaginal aspiration on Wednesday at about 3:00 p.m., 34 hours after hCG administration. The retrieved oocytes were inseminated with 100,000 motile sperm per oocyte 5 hr after OPU and cultured in human tubal fluid medium (HTF) (GIBCO, Grand Island, NY) supplemented with 10 % heat-inactivated maternal serum. Inseminated oocytes were checked for the presence of pronuclei on Thursday, 18 hr after insemination. Fertilized oocytes were transferred to a growth medium, HTF with 20 % serum. ET was carried out on Friday, 48 hr after OPU. The fixed schedule is illustrated in Fig. 1.

The luteal phase was supported with hCG and progesterone. 1000 IU of hCG was administered intramuscularly on days 3, 5, 8, and 14 after OPU, and dydrogesterone (Duphaston; Daiichi Pharmaceutical Ltd., Tokyo, Japan), 30 mg per day, was administered orally for 14 days after OPU.

Serum and urine concentrations of hCG were measured 14 days after OPU and any pregnancies were confirmed by ultrasonography 14 to 21 days later. Only clinical pregnancies were recorded (i.e., presence of gestational sac, or ectopic pregnancy). Biochemical pregnancies were excluded.

Statistical Analysis

Statistical analysis was performed using unpaired t-test and chi-squared analysis to examine relationships between the groups. A probability value < 0.05 was considered statistically significant.

RESULTS

Table 1 shows the clinical characteristics of the patients participating in the treatment pro-
There were no significant differences in the patients’ ages or duration of infertility between the two groups. The number of patients suffering from endometriosis in group 2 was significantly higher than in group 1. In the other causes of infertility, no significant differences were found between the groups. The dose of hMG and response to ovarian stimulation are shown in Table 2. There were no significant differences in the mean number of hMG ampoules per cycle or response to ovarian stimulation (i.e., the thickness of endometrium, serum E₂, serum E₂ per follicles, serum P₄ and number of follicles on the day immediately following the 7-day course of ovarian stimulation). As can be seen from Table 3, no significant differences were found between the two groups in the incidence of canceled cycles, mean number of oocytes recovered per cycle, the fertilization rate or the pregnancy rate per cycle. In the pregnancy cases with the short protocol, the starting day of hMG administration was distributed between day 0 and day 11 of the cycles.

**DISCUSSION**

During the past decade, one major development in IVF-ET has been the simplification of therapy. With regard to ovarian stimulation, the combination of GnRHa and hMG allows simplification of clinical management for IVF-ET. This combination prevents premature LH surges^{1,2}, and allows flexibility in the timing of hCG administration^{3}. In previously reported efforts for simplification of therapy, Zorn *et al.*^{4}
demonstrated that the use of norethisterone to control the timing of a preceding menstrual cycle with a flare-up protocol led to a weekend OPU of only 2.5 cycles in 119 cycles and a pregnancy rate of 26 per ET. Vauthier and Lefebvre described cycle programming with the use of two formulations of triptoreline. This was developed to avoid working on Sunday. They reported pregnancy rates of 31 and 27, respectively. Some authors have shown that the use of GnRHa allowed the administration of hCG to be delayed and made it possible to avoid OPU on weekends. Abdalla et al. reported that delaying the administration of hCG, using the short protocol of GnRHa administration, allowed the operative procedure to be performed on weekdays without adversely affecting the overall outcomes; in this way the overall cost was reduced. Dimitry et al. scheduled OPU on either 5 or 3 days a week with a delayed administration of hCG in the long protocol.

We had to perform OPU on a certain day of the week, as an elective operative procedure, due to the restricted availability of operating rooms. For this reason, our fixed schedule of IVF-ET, which was more rigid in comparison with previous reports, was designed. All OPU were performed on Wednesdays, so that ET could also be done on Fridays. Our fixed 7-day ovarian stimulation protocols were tailor to the target day of OPU without delayed administration of hCG.

The GnRHa are usually administered according to two different protocol treatments, a short and a long one. We applied both protocols to this fixed procedure. The selection of protocols depended on the schedule convenience, as described above. The comparative merits of the two protocols remain controversial. In numerous studies, the long protocol was found to be associated with a higher number of follicles, higher E2 levels on the day of hCG administration, and a higher number of retrieved oocytes when compared to the short protocol. On the other hand, similar results in both protocols were observed in some studies. In the short protocol, the initial flare-up effect seems to result in an increase of LH concentrations, possibly detrimental to folliculogenesis. It has been shown that 5% to 10% of cycles in the short protocol may still be complicated by a premature LH surge. In contrast, use of the long protocol seems to effectively suppress endogenous LH secretion. Therefore, Tan et al. reported that when the short protocol was used, accurate timing of hCG remained critical, and the

Table 3. Outcomes of Treatments

<table>
<thead>
<tr>
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<th>Group 1</th>
<th>Group 2</th>
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<tr>
<td>No( % ) of canceled occasions</td>
<td>3/ 4 (7.3)</td>
<td>3/ 10 (2.8)</td>
</tr>
<tr>
<td>No( % ) of cycles with failed oocytes recovery</td>
<td>0/ 38 (0)</td>
<td>5/ 10 (4.9)</td>
</tr>
<tr>
<td>Mean no. of oocyte recovered per occasion ( mean ± SD )</td>
<td>6.4 ± 4.0</td>
<td>5.8 ± 5.1</td>
</tr>
<tr>
<td>No. ( % ) of occasions with failed fertilization</td>
<td>1/ 38 (2.6)</td>
<td>11/ 98 (11.2)</td>
</tr>
<tr>
<td>Fertilization rate ( % )</td>
<td>106/ 26 (40.6)</td>
<td>272/ 61 (44.3)</td>
</tr>
<tr>
<td>No. ( % ) of ET*</td>
<td>40/ 41 (97.6)</td>
<td>87/ 10 (82.1)</td>
</tr>
<tr>
<td>No. of occasions with failed fertilization</td>
<td>8/ 41 (19.5)</td>
<td>20/ 10 (18.9)</td>
</tr>
<tr>
<td>No. of ET*</td>
<td>40/ 41 (14.6)</td>
<td>17/ 10 (16.0)</td>
</tr>
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*P < 0.05
long protocol was superior to the short protocol in the outcomes of IVF-ET\(^8\). Concerning pregnancy rates, numerous studies showed no significant difference between the two protocols, although there was a trend of better results with the long protocol\(^{10-12}\). Concerning the cost of an IVF-ET cycle, the short protocol seems to be superior to the long one since the short protocol requires fewer days for stimulation, doses of gonadotropins, and doses of GnRHa\(^{12}\).

In our study, both protocols led to similar ovarian responses and outcomes of IVF-ET. We speculate that our rigid schedule might be responsible for the similar outcomes, but we could not clarify the relationship, because we had not used the conventional protocol. We found that rigid programming was equally possible under both protocols. These findings indicate that the treatment using GnRHa plus hMG in either the short or the long protocol is useful for fixed-schedule IVF-ET. In conclusion, the short or long protocol for the fixed schedule simplified IVF-ET without negatively affecting the pregnancy rate per cycle.

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