Continuation of GnRH agonist administration for 1 week, after hCG injection, prevents ovarian hyperstimulation syndrome following elective cryopreservation of all pronucleate embryos

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BACKGROUND: An approach consisting of elective cryopreservation of all embryos has been proposed for patients at risk of ovarian hyperstimulation syndrome (OHSS). Although elective cryopreservation can prevent pregnancy-induced late OHSS, it cannot prevent early OHSS. Early OHSS is reported to have been complicated with thromboembolism. The study was carried out to assess the efficacy with which the continued administration of GnRH agonist for 1 week after 5000 IU of hCG injection could prevent early OHSS. METHODS: This study employed an open controlled clinical trial at three centres for treatment of infertility in Sapporo. A total of 138 patients at risk of OHSS during IVF–embryo transfer from January 1, 1998 to December 31, 1999, were assigned in turn either to a group with elective cryopreservation of all pronucleate embryos (n = 68) or to one with continuation of GnRH agonist administration for 1 week after hCG injection following elective cryopreservation (n = 70). Subsequently, they were transferred in hormone replacement cycles. The development of severe OHSS (ascites, haemoconcentration) was compared between the two groups. RESULTS: A total of 10% of patients developed severe OHSS necessitating hospitalization because of a marked increase in ascites in the upper abdomen and the haemoconcentration in the elective cryopreservation alone group. On the other hand, none developed severe OHSS in the GnRH agonist continuation group. CONCLUSIONS: In our study, continuation of GnRH agonist for 1 week after hCG injection prevented severe early OHSS following elective cryopreservation of all embryos. This treatment is safe and cost-beneficial, and should be performed promptly for patients at risk of OHSS.

Key words: early OHSS/embryo cryopreservation/GnRH agonist/prospective study

Introduction

Severe ovarian hyperstimulation syndrome (OHSS) is a potentially lethal iatrogenic complication of assisted reproductive technology that can only be prevented by withholding hCG. For those at risk of OHSS, an approach consisting of elective cryopreservation of all embryos and subsequent transfer of them in non-stimulated cycles has been proposed (Amos et al., 1990). Wada et al. reported that 27% of patients developed OHSS after retrieval and cryopreservation, 7% having the severe form of this disease (Wada et al., 1992a). They also examined the consequences of continuing GnRH agonist for 2 weeks after hCG administration following the elective cryopreservation of embryos. They then gave 10 000 IU hCG to the patients to induce oocyte maturation. However, this treatment could not prevent OHSS (Wada et al., 1992b).

Therefore, it is impossible for elective cryopreservation of all embryos to eliminate early OHSS, although it can prevent pregnancy-associated late OHSS. It has been reported that even early OHSS is complicated by thromboembolism (Levy et al., 1996; Aboulghar et al., 1998).

Although we often employ cycle cancellation in patients at the highest risk of OHSS or embryo cryopreservation when patients are at increased risk, Queenan et al. have reported that elective cryopreservation of all embryos does not eliminate OHSS (Queenan et al., 1997). In this study, we present the results of an open controlled clinical trial to evaluate the efficacy of continuation of GnRH agonist for 1 week after low dose hCG administration in women at risk of OHSS compared with patients who had elective cryopreservation of all embryos.
Materials and methods

Between January 1998 and December 1999, 1073 patients entering centres for treatment of infertility (Sapporo Medical University Hospital, Tonom Hospital and Kamiya Ladies’ Clinic) for IVF attempts were thoroughly checked during the ovarian stimulation period in order to select those patients at risk of developing OHSS.

All three hospitals are in the centre of the Sapporo metropolitan area and the social features of the patients were quite similar. This study was approved by Institutional Review Board of Sapporo Medical University and the other institutions. Informed consent was obtained from each patient after the purpose and nature of the study had been fully explained. All patients included in this study presented a high level of serum estradiol (E2) on the day of hCG administration (E2 >3000 pg/ml) and a high number (≥20) of follicles of intermediate or large size (diameter >12 mm). A total of 138 women fulfilling the above-mentioned criteria were alternately assigned (patients with an odd hospital number to group A and those with an even number to group B) into two groups: (i) 68 patients (group A: control group) had elective cryopreservation of all pronucleate embryos obtained (two patients withdrew); and (ii) 70 patients (group B: study group) had continuation of GnRH agonist administration for 1 week after hCG injection and cryopreservation of all pronucleate embryos.

In group A, GnRH agonist treatment was continued until hCG administration. The ovarian stimulation protocol was similar for all patients. We used a down-regulation protocol with GnRH agonist 900 µg/day (buserelin acetate; Hoechst Japan, Tokyo, Japan) as a long or short protocol and FSH (Fertinome; Serono Japan, Tokyo, Chiba Pharmaceuticals, Tokyo, Japan) or hMG (Humegon; NV Organon, Oss, The Netherlands and Pargonal; Teikokuzoki, Tokyo, Japan). All patients were administered 5000 IU of hCG (hCG-Mochida; Mochida, Tokyo, Japan) 34 or 35 h before oocyte retrieval. After hCG injection, we checked physical signs, blood analysis and ascites retention by ultrasonographic examination every 1–2 days for 2 weeks. Cryopreservation of pronucleate embryos was performed using a modification of a previously described method (Veek et al., 1993). Patients received thawed embryo exogenous steroid according to Muasher et al. (Muasher et al., 1991). Briefly, all patients had a minimum interval of 2 months separating their stimulation and thawed embryo transfer. GnRH agonist suppression was initiated using buserelin acetate (900 µg/day) from the previous mid-luteal phase. Transdermal E2 patches (Estraderm TTS®; Chiba Pharmaceuticals, Tokyo, Japan) were administered with the onset of menses. Buserelin acetate was discontinued on cycle day 15 and then daily administration of vaginal progesterone suppository (400 mg/day) was initiated. Pre-embryos were thawed on day 16 and transferred on day 17 after evidence of synangy and cleavage had confirmed their viability. The patients who had frozen–thawed embryos transferred with a positive test of hCG continued the hormone replacement therapy (HRT) for a further 8 weeks.

Statistical analysis

Mann–Whitney U-test, Fisher’s exact test and χ²-test for independent means were used as appropriate. P < 0.05 was considered statistically significant.

Results

There were no significant differences in the demographic data between the two groups (Table I). The stimulation parameters were also similar in terms of the E2 level at hCG administration and number of follicles. Furthermore, the numbers of collected oocytes and fertilization rates of both groups were also similar. The patients of groups A and B underwent HRT for the thawed cycle. In total, clinical pregnancies were established in 22 patients (32%) in group A, and 20 patients (29%) in group B. Five pregnancies aborted in group A and six in group B during the first trimester. The peak haematocrit and the peak number of white blood cells/ml were significantly greater in group A than those in group B. Other complications were not different between the two groups.

Seven patients (10%) in group A developed severe OHSS necessitating hospitalization until 7 days after hCG injection because of a marked increase in ascites in the upper abdomen and the haemoconcentration (haematocrit: mean ± SD, 46.9 ± 2.1% (Table II)). The criteria for hospitalization were the same among the three participating centres. These seven patients also had leukocytosis (17319 ± 2088/mm³). Two of the seven patients received paracentesis. These two patients also had pleural effusion. They were given low dose dopamine because of oliguria. All seven patients were infused with albumin because of albuminaemia. All seven patients recovered within 5–10 days after admission without severe complications.

No patients suffered from severe OHSS in group B. There was only limited, or no, ascites retention after retrieval.

### Table I. Characteristics of patients with cryopreservation of all prezygotes. All data are mean ± SD, unless otherwise indicated

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient number</td>
<td>68</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Agea</td>
<td>32.3 ± 4.0</td>
<td>33.0 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>Amount of hMG or FSH (IU)b</td>
<td>1869.5 ± 501.6</td>
<td>2082.9 ± 781.4</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol (pg/ml) at hCGc</td>
<td>5817.9 ± 2280.0</td>
<td>5601.3 ± 3311.0</td>
<td>NS</td>
</tr>
<tr>
<td>Number of folliclesd</td>
<td>26.8 ± 6.2</td>
<td>28.8 ± 8.1</td>
<td>NS</td>
</tr>
<tr>
<td>Number of PCOS embryos</td>
<td>15</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Long/short down-regulation</td>
<td>40/28</td>
<td>51/19</td>
<td>0.082</td>
</tr>
<tr>
<td>protocol with GnRH agonistb</td>
<td>(NS)</td>
<td>(NS)</td>
<td></td>
</tr>
</tbody>
</table>

aMann–Whitney U-test.
bχ² for independent test.
cPOCS = polycystic ovarian syndrome; NS = not significant.

dχ²-test for independent test.
eAgreement between 3 centres.

### Table II. Effects of continuation of GnRH agonist on clinical findings. All data are mean ± SD, unless otherwise indicated

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of collected oocytesa</td>
<td>23.1 ± 4.2</td>
<td>22.6 ± 5.2</td>
<td>0.086</td>
</tr>
<tr>
<td>Peak haematocritb</td>
<td>39.9 ± 3.7</td>
<td>38.0 ± 1.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peak number of white blood cells/mlc</td>
<td>11858 ± 3055</td>
<td>10079 ± 1749</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Peak number of platelets (x10³/mm³)d</td>
<td>26.4 ± 4.7</td>
<td>26.9 ± 5.6</td>
<td>NS</td>
</tr>
<tr>
<td>Number of patients with severe ascitesb</td>
<td>7</td>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td>Rate of oocytes fertilized (%)e</td>
<td>52.8 ± 19.6</td>
<td>51.7 ± 19.9</td>
<td>NS</td>
</tr>
<tr>
<td>Number of pregnancy patientsf</td>
<td>22</td>
<td>20</td>
<td>NS</td>
</tr>
<tr>
<td>Number of miscarriage (first trimesterg)</td>
<td>5</td>
<td>6</td>
<td>NS</td>
</tr>
</tbody>
</table>

aMann–Whitney U-test.
bFisher’s exact probability test.
cχ² for independent test.
dχ²-test for independent test.
eAgreement between 3 centres.

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Moderate OHSS (haematocrit 40–45%) was seen in 25 patients in group A (haematocrit: 40.0–44.8%) and in 10 patients in group B (haematocrit: 40.0–41.8%). However, none of these patients required hospitalization. The difference was significant ($\chi^2$ for independent test, $P < 0.001$). On the other hand, continuation of GnRH agonist did not reduce the rates of oocytes fertilized and pregnancy (Table II).

**Discussion**

Continuation of GnRH agonist administration for 1 week after low dose hCG injection prevented severe OHSS following elective cryopreservation of all pronucleate embryos. This treatment reduced the increase in haematocrit, white blood cell count and ascites retention in patients at risk of OHSS.

Cryopreservation of all pronucleate embryos alone in patients at risk of OHSS did not eliminate the syndrome (Queenan et al., 1997). In contrast, Ferraretti et al. reported that elective cryopreservation of all pronucleate embryos might reduce the risk of OHSS in patients undergoing IVF treatment (Ferraretti et al., 1999). That study chose patients who had a serum E$_2$ concentration $\geq$1500 pg/ml on the day of hCG administration and $\geq$15 oocytes were collected. Although the definition of the risk of OHSS varies depending on the report, Ferraretti’s definition was quite different from ours. In our study, we selected patients who had blood E$_2$ $\geq$3000 pg/ml and $\geq$20 follicles. Actually serum E$_2$ was 3000–22550 pg/ml and 20–49 oocytes were retrieved in both groups.

On the other hand, Queenan et al. selected 15 patients who had blood E$_2$ $\geq$4500 pg/ml and $\geq$25 follicles (Queenan et al., 1997). In our study, group B included 39 of 70 patients who had E$_2$ $\geq$4500 pg/ml and 46 with $\geq$25 follicles. Therefore, we can say that the effects of continuation of GnRH agonist did not depend on the serum E$_2$ level or follicle number.

Although Wada et al. tried the continuation of GnRH agonist during the luteal phase for patients at risk of developing OHSS, they found that this treatment could not prevent OHSS (Wada et al., 1992). The major difference between their protocol and ours was the dose of hCG (10 000 and 5000 IU respectively). The status of OHSS is related to the effect of hCG. The amount of 5000 IU hCG is quite important to prevent severe OHSS, as they themselves reported in a later study (Brinsden et al., 1995).

Therefore, reduction of the hCG dose is one of the key points for the effectiveness of the continuation of GnRH agonist. In this study, cryopreservation of all embryos with continuation of GnRH agonist for 1 week after low dose hCG administration prevented severe ascites retention in group B.

It has been reported that haematocrit can act as a biological marker of the severity of OHSS (Bergh and Navot, 1992; Navot et al., 1992; Fabregues et al., 1998) and the white blood cell count is also reported to be a prime parameter indicative of the severity of OHSS (Navot et al., 1996). Moreover, the occurrence of thromboembolic phenomena has been reported to be related to the rapid body fluid shift leading to haemococoncentration and increased blood viscosity (Shenker and Weinstein, 1978; Rizk and Aboughar, 1991; Elchalal and Schenker, 1997). Thus, we should be careful about an increase in haematocrit in patients at risk of OHSS. Cryopreservation of all embryos with continuation of GnRH agonist succeeded in reducing the haematocrit in group B. Actually, our treatment reduced the incidence of severe OHSS as well as that of moderate OHSS. This study also showed that the continuation of GnRH agonist prevented early OHSS without negative effects on pregnancy because the count of retrieved oocytes and fertilization, pregnancy and miscarriage rates were similar to those of the control group. GnRH antagonist is able to prevent OHSS. At present, the GnRH antagonist regimens have been reported to be associated with a lower pregnancy and implantation rate than the established GnRH agonist protocols (Godon, 2001).

The efficacy of GnRH agonist was not due to the duration of GnRH agonist administration itself, because it has been reported that OHSS still occurs when patients are treated by ‘coasting’ (Lee et al., 1998). GnRH agonist was not used in our study as an alternative to hCG for triggering ovulation, as has been reported previously (Imoedemhe et al., 1991; Lewit et al., 1996). GnRH agonist inhibits LH secretion from the pituitary during short and long protocol cycles. Of course, this effect is not sufficient to prevent OHSS because LH secretion is not a major factor in the induction of OHSS. In addition to the luteolytic effect produced by inhibition of LH secretion, the presence of GnRH receptor mRNA in human granulosa-luteal cells (Peng et al., 1994) and luteal cells (Popkin et al., 1983; Bramley et al., 1986) sustains the concept that GnRH agonist may have a local effect on the ovary in humans.

A recent cell culture study reported that $10^{-10}$ mol/l GnRH agonist directly reduces progesterone secretion and LH receptor and FSH receptor mRNA expression in human granulosa-luteal cells collected during oocyte retrieval from women undergoing IVF (Kang et al., 2001). These patients were given an hCG injection following GnRH agonist treatment before oocyte retrieval. These granulosa-luteal cells were still found to be sensitive to GnRH agonist treatment. We speculate that granulosa-luteal cells may also be sensitive to GnRH agonist after hCG injection in our study. Thus, GnRH agonist treatment from hCG injection to formation of the corpus luteum seems to be a key point. GnRH agonist is also reported to have a direct effect, causing apoptosis in granulosa cells from IVF patients (Zhao et al., 2000).

During luteal formation, vascular endothelial growth factor (VEGF), which induces angiogenesis and vascular permeability, is essential for angiogenesis (Ferrara et al., 1998). VEGF was also reported to be related to OHSS (McClure et al., 1994). One possible mechanism of GnRH agonist action may be a direct effect reducing VEGF expression in the ovary, as we previously found that GnRH agonist directly suppressed luteal VEGF mRNA expression during luteal formation in rats treated with excessive amounts of pregnant mares’ serum gonadotrophin (Kitajima et al., 1998). Fujii et al. tried the continuous administration of GnRH agonist for 2 weeks after oocyte retrieval to facilitate implantation, not to prevent OHSS (Fujii et al., 2001). They speculated that the effect of GnRH agonist might lie in the direct action of GnRH agonist through its receptor for the regulation of embryonic interactions. We did not prove the existence of a direct effect...
on the ovary in this study. The effect of the continuation of GnRH agonist to reduce the risk of OHSS may not be perfect, as rare cases of OHSS have been reported to follow sole administration of GnRH agonist before the start of FSH therapy (Weissman et al., 1998).

The direct effects of GnRH agonist on the human ovary are still controversial. Although we have to clarify the mechanism of the new method of using GnRH agonist for OHSS, the efficacy of GnRH agonist should be acceptable as a large number of patients (a total of 138 patients) at risk of OHSS were surveyed compared with previous reports. This treatment is safe and cost-beneficial, and should be performed promptly for patients at risk of OHSS.

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References

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