Cryopreservation of all prezygotes in patients at risk of severe hyperstimulation does not eliminate the syndrome, but the chances of pregnancy are excellent with subsequent frozen–thaw transfers

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In-vitro fertilization patients (n = 15) at risk of ovarian hyperstimulation syndrome (OHSS) (oestradiol ≥4500 pg/ml on the day of human chorionic gonadotrophin administration and 25 or more follicles of intermediate or large size) underwent aspiration of all follicles and cryopreservation of all fertilized oocytes at the pronuclear stage. Patients were monitored for up to 2 weeks post-retrieval. Subsequent transfer of cryopreserved–thawed embryos was performed in programmed cycles using exogenous oestrogen and progesterone for endometrial preparation. Two patients (13%) developed OHSS necessitating hospitalization and vaginal aspiration of ascitic fluid. Two other patients (13%) developed moderate OHSS requiring ascitic fluid vaginal aspiration in the office setting, with dramatic improvement of the condition. Subsequent transfer of cryopreserved–thawed embryos yielded a clinical pregnancy rate of 58% per transfer and ongoing or delivery rates of 42 and 67% per transfer and per patient respectively. By eliminating pregnancy potential with cryopreservation of all prezygotes and examining the pregnancy potential with subsequent cryopreserved–thawed transfers, it is concluded that OHSS is reduced, but not eliminated for patients at risk. Subsequent transfer of cryopreserved–thawed prezygotes in a programmed cycle with exogenous steroids yields an excellent pregnancy rate.

Key words: cryopreservation/embryos/IVF/ovarian hyperstimulation syndrome (OHSS)

Introduction

One of the primary goals of assisted reproductive technology (ART) is to achieve some degree of ovarian stimulation. However, too vigorous a response can lead to ovarian hyperstimulation syndrome (OHSS) which is a potentially life-threatening iatrogenic complication. The incidence of severe OHSS is only 1–2% (Golan et al., 1989; Smitz et al., 1990), yet this remains the major significant source of morbidity and mortality in ART. Many preventive strategies have been evaluated including early ovarian puncture, glucocorticoids, i.v. albumin, and the prolonged use of gonadotrophin-releasing hormone agonist (GnRHa). No method has consistently demonstrated superiority in prevention of this syndrome.

Several large studies have shown that the incidence and duration of severe OHSS is greatest in those patients who conceive (Golan et al., 1989; Forman et al., 1990; Asch et al., 1991). Patients receiving human chorionic gonadotrophin (HCG) for luteal support are also at an increased risk (Smitz et al., 1988; Forman et al., 1990). It has been concluded that development of this syndrome is limited to cycles with exposure to endogenous or exogenous HCG (Schenker and Weinstein, 1978; Navot et al., 1992). Consequently, the most effective prevention is to avoid pregnancy via cycle cancellation and the withholding of HCG (Smitz et al., 1988). Although the safest approach, cycle cancellation is frustrating and costly to the infertile patient.

One approach which minimizes HCG exposure without forfeiting oocyte retrieval was described by Amso et al. (1990). Oocyte retrieval was performed with elective cryopreservation of all resulting pre-embryos. Avoiding fresh embryo transfer minimized further HCG exposure during the cycle at risk and all participants subsequently conceived from thawed embryo transfer. Based on this approach, we adopted a strategy to identify prospectively patients at risk for severe OHSS, minimize their exposure to HCG, and not compromise their chances of pregnancy from oocytes developed within that cycle. Starting in 1992, we sought to reduce the incidence of severe OHSS using the following steps. Patients at risk were identified before HCG administration by specific serum oestradiol and ultrasound criteria, ovulation was triggered with a reduced HCG dose, retrieval was followed by cryopreservation of all pre-embryos and the patients observed pelvic rest for 2 weeks.

We report our experience with this protocol, including the risk of severe OHSS in patients who have bypassed fresh embryo transfer. The outcome of subsequent frozen–thawed transfers is presented and a high pregnancy rate was observed.

Materials and methods

Subjects

A total of 15 infertile patients who underwent ovarian stimulation for in-vitro fertilization (IVF)/embryo transfer between November 1992 and February 1995 were identified as high risk for developing severe OHSS. Of these 15 patients, nine had tubal infertility, three had abnormal semen parameters and three suffered from unexplained
infertility. All patients received luteal phase GnRH (Lupron; TAP Pharmaceuticals, Abbott Park, IL, USA). Ovarian stimulation was performed using human menopausal gonadotrophin (HMG) or follicle stimulating hormone (FSH), alone or in combination (Pergonal and Metrodin; Serono Laboratories, Randolph, MA, USA) by a protocol previously described (Muasher et al., 1991). Oestradiol concentrations were measured by microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL, USA).

Each patient was identified as being at high risk of OHSS based on serum oestradiol $\geq$4500 pg/ml on the day of HCG and $\geq$25 follicles of intermediate (12–15 mm), and large (\geq 16 mm) size. In order to minimize patient exposure to exogenous HCG, each received 5000 IU of HCG (Profasi; Serono Laboratories). Transvaginal follicular aspiration was performed 34–36 h later and every effort was made to aspirate all ovarian follicles. No fresh transfers were performed in this group of patients. All pre-embryos which subsequently arose from mature oocytes were cryopreserved at the pronuclear stage. All patients were given warnings for severe OHSS and were followed carefully for up to 2 weeks post-transfer. Patients were asked to perform surveillance by daily weight measurements using the same scale, daily abdominal girth measurements, and in some cases, urine volumes were recorded daily. Each patient was asked to observe strict pelvic rest for 2 weeks. A summary of patient characteristics and stimulation cycle outcome is presented in Table I.

**Thawed embryo transfer protocol**

Cryopreservation was performed using a slow freeze, slow thaw protocol (Veeck et al., 1993). Patients receiving thawed embryo transfer underwent a protocol of endometrial preparation using exogenous steroids according to Muasher et al. (1991). Briefly, all patients had a minimum interval of 2 months separating their stimulation and thawed embryo transfer. GnRH suppression was achieved using leuprolide acetate (0.5 mg/day s.c.) 2–3 weeks after menstruation. Transdermal oestradiol patches (Estraderm®; Ciba Pharmaceuticals, Summit, NJ, USA) were administered with the onset of menses. The leuprolide acetate was discontinued on cycle day 15 and daily administration of progesterone in oil (50 mg i.m.) was initiated. Pre-embryos were thawed on day 16 and transferred on day 17 after evidence of syngamy and cleavage confirmed viability.

Thawing of pre-embryos was performed in the biological freezer and then thawed specimens were taken through a series of decreasing US. The rate of clinical pregnancy per transfer was 58% (14/concentrations of 1,2-propanediol for 5 min at each dilution (1.0, 2.4). The rate of ongoing gestation after evidence of syngamy and cleavage confirmed viability. Thawing of pre-embryos was performed in the biological freezer transferred is currently only subject to voluntary limits in the USA. The rate of clinical pregnancy per transfer was 58% (14/24). The rate of ongoing gestation $\geq$20 weeks or live delivery per transfer was 42% (10/24). There were no multiple gestations.

Two patients developed severe OHSS with onset occurring 4 and 5 days post-retrieval. Both presented with massive ovarian enlargement (>10 cm by ultrasound), severe haemoconcentration (haematocrit >50), hypovolaemia, electrolyte imbalances, pleural effusion and ascites. Both patients were hospitalized for i.v. hydration and correction of electrolytes. Transvaginal aspiration of ascitic fluid was performed in these two patients, yielding 3500 and 1250 ml, respectively. Hospitalization lasted 5 and 3 days respectively, with dramatic relief occurring after fluid aspiration in both patients. Two patients developed moderate OHSS with moderate ovarian enlargement (7–10 cm by ultrasound), moderate haemoconcentration (haematocrit 45–50), hypovolaemia, and ascites. Both were followed daily with monitoring of abdominal girth, weight, and urine output. These two patients had transvaginal aspiration of 1200 and 650 ml of ascitic fluid respectively. This procedure was performed in the office setting and hospitalization was not required. All remaining patients who experienced moderate discomfort were treated symptomatically with bed rest and analgesics. Symptoms resolved without further intervention.

After a minimum interval of 2 months following retrieval, all 15 patients returned for thawed embryo transfer and the results are presented in Table II. To date, 24 frozen–thaw transfers have been performed for these patients. The post-thaw survival rate was 74%, which is consistent with our prior experience (Queenan et al., 1995). The mean number of pre-embryos transferred per patient was 3.8, which was thought to be a conservative figure, considering that, in our experience, frozen–thawed embryo transfers usually have a lower implantation rate than fresh transfers, and that the number of embryos transferred is currently only subject to voluntary limits in the US. The rate of clinical pregnancy per transfer was 58% (14/24). The rate of ongoing gestation $\geq$20 weeks or live delivery per transfer was 42% (10/24). There were no multiple gestations.

The rate of ongoing pregnancy or delivery per patient was 67% (10/15). There is reason to anticipate that this rate will continue to increase over time. Ten patients still have cryopreserved pre-embryos remaining (mean = 12 per patient).

### Table I. Characteristics of patients with cryopreservation of all prezygotes at the pronuclear stage

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>33 ± 1.2</td>
</tr>
<tr>
<td>Oestradiol on day of HCG (pg/ml)</td>
<td>5468 ± 1318</td>
</tr>
<tr>
<td>Range (pg/ml)</td>
<td>4600–7122</td>
</tr>
<tr>
<td>Peak oestradiol (pg/ml) (mean ± SD)</td>
<td>7022 ± 1640</td>
</tr>
<tr>
<td>Mature oocytes (mean)</td>
<td>29.7</td>
</tr>
<tr>
<td>Range</td>
<td>19–55</td>
</tr>
<tr>
<td>Immature oocytes (mean)</td>
<td>5.7</td>
</tr>
<tr>
<td>Prezygotes cryopreserved (mean)</td>
<td>19.4</td>
</tr>
<tr>
<td>Range</td>
<td>16–49</td>
</tr>
</tbody>
</table>

HCG = human choric gonadotrophin.

### Table II. Outcome of frozen–thawed embryo transfers

<table>
<thead>
<tr>
<th>Category</th>
<th>Patients (n)</th>
<th>Frozen–thaw transfers (n)</th>
<th>Frozen–thaw survival rate of prezygotes (%)</th>
<th>Clinical pregnancy/transfer (%)</th>
<th>Ongoing pregnancy or delivery/transfer (%)</th>
<th>Ongoing pregnancy or delivery/patient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>24</td>
<td>74</td>
<td>58 (14/24)</td>
<td>42 (10/24)</td>
<td>67 (10/15)</td>
</tr>
</tbody>
</table>

If pregnancy was confirmed by serum quantitative β-HCG 13 days after transfer, Estraderm® patches (0.2 mg replaced every 2 days) and progesterone (50 mg i.m. daily) were maintained. Patients were weaned from exogenous steroids once placental autonomy was determined by weekly serum oestradiol and progesterone monitoring. Pregnant patients were asked to have a pelvic ultrasound 4–6 weeks after transfer to determine viability and check for multiple gestations.
Discussion

No method of treatment has been able to eradicate severe OHSS from the practice of ART. Prevention of OHSS remains the best strategy for the practitioner. One approach is through identification of those at risk before triggering ovulation. During ovarian stimulation, a large cohort of intermediate and large follicles is a widely accepted risk factor for OHSS (Tal et al., 1985; Blankstein et al., 1987). Once identified, the HCG dose can be withheld, delayed or reduced. It should be remembered that severe OHSS is a potentially lethal iatrogenic complication of ART which can only truly be prevented when HCG is withheld. Our approach was to minimize endogenous and exogenous HCG exposure. We reduced the HCG dose at ovulatory trigger by 50% and avoided HCG for luteal support. The patients were not exposed to endogenous HCG since no fresh transfer was performed. OHSS occurs less often in patients given 5000 IU of HCG compared with those receiving a higher dose (Schenker, 1995). We treated all patients at risk with this reduced dose of HCG. When severe OHSS was encountered, we aggressively performed early transvaginal aspiration of ascitic fluid. In our experience, this provides instant symptomatic relief and decreases the chance of hospitalization or length of stay. We feel this step should be considered in all stable patients without severe coagulation disorder.

Severe OHSS is rare in the absence of HCG. Cryopreservation of all embryos can minimize further exposure to exogenous and endogenous HCG. Moderate to severe OHSS still occurs in a significant number of patients (Salat-Baroux et al., 1990); however, Wada et al. (1992) have suggested that the severity of symptoms is reduced. In our programme, the reduced amount of β-HCG given to trigger ovulation (5000 IU) was sufficient to allow development of severe OHSS in two out of 15 (13%) patients at high risk. In a similar protocol, Wada et al. (1992) reported 27% developed OHSS after retrieval and cryopreservation, 7% having the severe form of this disease. Pattinson et al. (1994) achieved a 1.4% rate of severe OHSS through cycle cancellation in patients at highest risk or cryopreservation when patients were at moderate risk. The low incidence of severe OHSS is admirable. However, 14% (11/80) of patients at risk were cancelled.

Cycle cancellation has been a logical approach, in that several authors observed that hyperstimulated cycles yielded a poor chance for conception (Gidley-Baird et al., 1986; Forman et al., 1988). Through the experience with cryopreservation and oocyte donation, it is now known that egg quality is not harmed in a hyperstimulated cycle. Cryopreservation gives the opportunity to defer pregnancy and conserve pregnancy potential in the form of stored embryos.

We found that the pregnancy potential of frozen embryos from hyperstimulated cycles is equal to or possibly even superior to that of standard thawed embryo transfer. Pattinson et al. (1994) found a 25.2% pregnancy rate per transfer which was significantly better than their normal frozen transfer rate. Frederick et al. (1995) achieved a 31.8% pregnancy rate per cycle and 58.3% pregnancy rate per patient in thawed transfers from hyperstimulated cycles. We compared our results with a large series of patients treated under the identical steroid cycle thawed embryo transfer protocol which we have previously reported (Queenan et al., 1994). Our findings are similar to those of Frederick et al. (1995) in that our steroid prepared thaw transfer protocol has achieved a 30% clinical pregnancy rate (70/230) compared with this subpopulation that have a 58% pregnancy rate under the same transfer protocol. The possibility that these embryos are of a higher quality is intriguing, but further studies and greater numbers are needed to confirm this observation.

We believe the protocol presented identifies patients early and is somewhat aggressive in its approach. All patients received HCG and no cycles were cancelled. Further modifications could possibly lessen the incidence of severe OHSS, such as i.v. albumin (Asch et al., 1993) or avoiding HCG by the use of GnRH agonists. Moderate to severe OHSS is rare in the absence of HCG. Cryopreservation can minimize further exposure to exogenous and endogenous HCG. Moderate to severe OHSS still occurs in a significant number of patients (Salat-Baroux et al., 1990); however, Wada et al. (1992) have suggested that the severity of symptoms is reduced. In our programme, the reduced amount of β-HCG given to trigger ovulation (5000 IU) was sufficient to allow development of severe OHSS in two out of 15 (13%) patients at high risk. In a similar protocol, Wada et al. (1992) reported 27% developed OHSS after retrieval and cryopreservation, 7% having the severe form of this disease. Pattinson et al. (1994) achieved a 1.4% rate of severe OHSS through cycle cancellation in patients at highest risk or cryopreservation when patients were at moderate risk. The low incidence of severe OHSS is admirable. However, 14% (11/80) of patients at risk were cancelled.

References


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Received on January 29, 1997; accepted on April 28, 1997