Elective cryopreservation of all pronuclear oocytes after GnRH agonist triggering of final oocyte maturation in patients at risk of developing OHSS: a prospective, observational proof-of-concept study


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BACKGROUND: A bolus dose of GnRH agonist can substitute for hCG as a trigger for the resumption of meiosis in ovarian stimulation with GnRH antagonists, which has been suggested to reduce the risk of ovarian hyperstimulation syndrome (OHSS). As the efficacy of this measure in fresh embryo transfer (ET) cycles is unclear, we evaluated a new clinical concept of GnRH-agonist triggering. METHODS: In this prospective, observational proof-of-concept study, 20 patients considered at increased risk of developing OHSS (>20 follicles ≥10 mm or estradiol ≥4000 pg/ml, or a history of cycle cancellation due to OHSS risk or the development of severe OHSS in a previous cycle) after ovarian stimulation and concomitant GnRH-antagonist administration had final oocyte maturation triggered with 0.2 mg triptorelin s.c. All two pronucleate (2 PN) oocytes were cryopreserved by vitrification, and frozen–thawed ETs (FT-ETs) were performed in an artificial cycle. Main outcome measures were the cumulative ongoing pregnancy rate per patient and the ongoing pregnancy rate per first ET. Secondary outcomes included the incidence of moderate-to-severe OHSS. RESULTS: Of the 20 patients triggered with GnRH agonist, 19 patients underwent 24 FT-ETs in the observational period. The cumulative ongoing pregnancy rate was 36.8% (95% confidence interval: 19.1–59.0%). The ongoing pregnancy rate per first FT-ET was 31.6% (15.4–54.0%). No cases of moderate or severe OHSS were observed. CONCLUSIONS: The present study is the proof of the concept that GnRH-agonist triggering of final oocyte maturation in combination with elective cryopreservation of 2 PN oocytes offers OHSS risk patients a good chance of pregnancy achievement, while reducing the risk of moderate and severe OHSS.

Key words: frozen–thawed embryo replacement/GnRH agonist/GnRH antagonist/ovarian hyperstimulation syndrome

Introduction

Severe ovarian hyperstimulation syndrome (OHSS) is a rare and potentially life-threatening complication of ovarian stimulation for assisted reproduction (Mozes et al., 1965). Luteinization by human chorionic gonadotrophin (hCG) following ovarian stimulation is essential for OHSS to occur. As a result, the syndrome occurs exclusively post-ovulatory and is closely related to the life span of the corpus luteum.

It has been proposed that the administration of a GnRH agonist to induce final oocyte maturation instead of hCG, which is feasible in a GnRH-antagonist protocol (Fauser et al., 2002), may result in a reduced risk of OHSS (Itskovitz-Eldor et al., 2000; Kol and Itskovitz-Eldor, 2000). However, recent evidence indicated that GnRH-agonist administration is associated with a significantly reduced likelihood of ongoing pregnancy achievement in normal responder patients, when compared with the standard hCG treatment (Griesinger et al., 2006a).

The cause of the reported lower pregnancy likelihood after GnRH-agonist triggering and fresh embryo transfer observed in previous RCTs (Humaidan et al., 2005; Kolibianakis et al., 2005) in general patient population is unknown, but is likely to be associated to a defective luteal phase (Fauser et al., 2002; Nevo et al., 2003; Humaidan et al., 2005; Yding Andersen and Humaidan, 2005; Griesinger et al., 2007), despite luteal phase support with vaginal progesterone and oral estradiol (E2). Although it has been suggested that luteal phase support following GnRH-agonist triggering should consist of intramuscular progesterone combined with transdermal E2 (Engmann et al., 2005); as yet, an optimal protocol has

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not been established (Griesinger et al., 2006b). Potentially, a disturbed luteal phase after agonist triggering and fresh ET will also impair the likelihood of pregnancy in patients at risk of OHSS, despite a good prognosis in this patient group (young age, good ovarian response). Furthermore, late-onset manifestations of the syndrome in a GnRH-agonist-triggered cycle as a result of hCG exposure from an implanting embryo cannot be excluded (Chun, 2005).

Therefore, we decided to prospectively study the efficacy and safety of the following new protocol in OHSS risk patients: (i) triggering of final oocyte maturation with GnRH agonist instead of hCG in a GnRH-antagonist protocol, (ii) elective cryopreservation by vitrification of all two pronucleate (2 PN) oocytes and (iii) transfer of embryos in subsequent frozen–thawed ET cycle(s) (FT-ETs).

Materials and methods

The present study was a single-centre, prospective, observational proof-of-concept study conducted at a university-affiliated tertiary care centre. The observational period was from December 2004 up to and including September 2006. All participants provided informed consent after counselling about alternative options of OHSS prevention (coasting, cycle cancellation) routinely employed at our centre.

Patient population

Inclusion criteria were: (i) indication for IVF/ICSI; (ii) stimulation in a GnRH-antagonist protocol; (iii) increased risk of developing OHSS, defined as ≥20 follicles ≥10 mm or E2 ≥4000 pg/ml at the time of induction of final oocyte maturation or a history of cycle cancellation due to OHSS risk (same criteria as above) or the development of severe OHSS in a previous cycle (Golan et al., 1989); (iv) willingness to participate in the study.

Study protocol

Patients were enrolled in the study on the last day of monitoring follicular growth by serial ultrasound and hormone measurement (the day of GnRH-agonist administration) and were registered prospectively by a third-party telephone line registration system. When the leading follicle reached a mean diameter of ≥18 mm, GnRH agonist (triptorelin 0.2 mg) was injected s.c. and oocyte retrieval was performed 36 h later, followed by IVF/ICSI. The luteal phase was supported (to avoid spotting and premature menses) with either 90 mg micronized progesterone (Crinone® 8%) vaginally per day or 10 mg medroxy-progesterone acetate orally per day for 10–14 days. No further luteal phase medication was given.

A single assessment for signs and symptoms of OHSS in the luteal phase was performed on days 4–7 after oocyte retrieval and included a physical examination by a doctor, and blood analysis to detect haematocrit >45% (Navot et al., 1992). In case of abdominal pain or distension, transvaginal sonography was performed. Patients were advised to present themselves at any time point within 14 days after GnRH-agonist administration, in case of symptoms such as abdominal distension/pain, nausea, vomiting, diarrhoea or headache.

Oocytes at the pronuclear stage were cryopreserved by vitrification as previously described (Kuwayama et al., 2005a). All FT-ET cycles were performed after establishment of spontaneous menses in an artificial cycle as previously described (Bals-Pratsch et al., 1999). Briefly, preparation of the endometrium was performed for 14 days with transdermal E2 patches (Estraderm TTS® 100), followed by the addition of vaginal micronized progesterone (Crinone 8%) from day 15 onwards. ET happened on the third day of progesterone administration. Supplementation continued with intramuscular progesterone in case of pregnancy until 8 weeks of gestation.

Outcome measures

Main outcome measure: ongoing pregnancy rate reported cumulatively on a per-patient basis, as well as on a per-ET basis; secondary outcome measure: incidence of moderate-to-severe OHSS (Golan et al., 1989); fertilization rate (number of 2 PN oocytes/number of metaphase II oocytes injected), survival rate after cryopreservation (number of vital embryos/number of thawed 2 PN oocytes); cumulative embryo score. The cumulative embryo quality score (Steer et al., 1992) of the FT-ETs was assessed in a modified version as previously described (Ludwig et al., 2000). All data are presented descriptively with 95% confidence intervals (CIs) or ranges.

Sample size consideration

A lower margin of the CI equivalent to 15% ongoing pregnancy rate was defined as still sufficiently effective in the context of a risk–benefit evaluation for an OHSS high-risk population. Estimating an ongoing pregnancy rate of 35%, a sample size of 20 patients produces a 97% CI equal to the sample pregnancy rate of ±20%.

Results

All 20 patients had at least one 2 PN oocyte available for cryopreservation after GnRH-agonist triggering. Of the 20 patients who were recruited to the study, 19 patients had embryos thawed (and at least one embryo surviving the thawing procedure) and underwent at least one ET by August 2006. One patient, who had seven 2 PN oocytes cryopreserved, had cancelled further treatment for personal reasons after oocyte retrieval and cryopreservation. In total, 24 ETs in 19 patients were performed.

Table I summarizes the demographic parameters. Five patients had no regular cycle, of which three patients had polycystic ovarian syndrome (PCOS) according to the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group criteria (2004). Seven patients had a previous pregnancy, with only one patient having delivered a child (in a previous partnership). Three patients had one or more previous cycles cancelled due to risk of OHSS. The majority of patients had a male indication for treatment, including three couples undergoing testicular sperm extraction. Two patients had higher grade endometriosis (revised American Fertility Society endometriosis stage III–IV). All patients underwent ICSI. Table II summarizes the stimulation characteristics of the 19 patients.

### Table I. Main demographic parameters of the study population (n = 20)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.1 ± 4.4</td>
<td>25.0–39.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.5 ± 8.9</td>
<td>53.0–82.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 2.9</td>
<td>20.0–29.7</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>35.7 ± 17.1</td>
<td>25–92</td>
</tr>
<tr>
<td>Cycle rank (#)</td>
<td>1.95 ± 1.6</td>
<td>1–5</td>
</tr>
</tbody>
</table>
**Table II.** Stimulation characteristics and embryo data (n = 19)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation (days)</td>
<td>10.0 ± 3.2</td>
<td>7–22</td>
</tr>
<tr>
<td>Total FSH (IU)</td>
<td>1926.3 ± 1025.8</td>
<td>1050.0–5025.0</td>
</tr>
<tr>
<td>FSH/day (IU)</td>
<td>186.8 ± 52.7</td>
<td>131.2–343.7</td>
</tr>
<tr>
<td>E₂ (pg ml⁻¹)</td>
<td>4193.3 ± 1569.5</td>
<td>838.0–7531.0</td>
</tr>
<tr>
<td>P₄ (ng ml⁻¹)</td>
<td>1.3 ± 0.5</td>
<td>0–4.2</td>
</tr>
<tr>
<td>Follicles (&gt;10 mm)</td>
<td>19.8 ± 6.7</td>
<td>6–35</td>
</tr>
<tr>
<td>COCs (n)</td>
<td>16.4 ± 6.4</td>
<td>4–29</td>
</tr>
<tr>
<td>Fertilization (%)</td>
<td>60.4 ± 28.0</td>
<td>12.5–100.0</td>
</tr>
<tr>
<td>2 PN cryopreserved (n)</td>
<td>7.4 ± 4.3</td>
<td>1–16</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>77.8 ± 21.2</td>
<td>33.3–100.0</td>
</tr>
<tr>
<td>Embryos transferred (n)ᵇ</td>
<td>2.3 ± 0.6</td>
<td>1–5</td>
</tr>
<tr>
<td>Modified cumulative embryo scoreᵇ</td>
<td>22.4 ± 12.3</td>
<td>4–56</td>
</tr>
<tr>
<td>Luteal phase Hct (%)</td>
<td>37.4 ± 3.4</td>
<td>28–42</td>
</tr>
</tbody>
</table>

E₂ and P₄, serum estradiol, progesterone and follicles > 10 mm in mean diameter, respectively, on the day of final oocyte maturation; COCs cumulus–oocyte complexes; 2 PN, oocytes at the 2 pronuclear stage; Hct, haematocrit.

ᵇDenominator here is 24 attempts to thaw 2 PN oocytes.

**Pregnancy**

Table III summarizes the pregnancy outcomes. Cumulative ongoing pregnancy rate was 36.8% (95% CI: 19.1–59.0). At the time of writing, two healthy singletons have been delivered and five pregnancies (including one twin pregnancy) are still ongoing. The implantation rate was 19.4 ± 31.7% (range 0–100%).

At the time of writing, seven patients still have surplus 2 PN oocytes cryopreserved: three non-pregnant patients and four women with ongoing pregnancy or delivery.

**Ovarian hyperstimulation syndrome**

No patient developed signs or symptoms of clinically relevant OHSS II–III (0%, 95% CI: 0.0–16.1). No patient showed luteal phase haemoconcentration (Table II). One patient presented at the clinic in an unscheduled visit on the day following GnRH-agonist triggering with lower abdominal pain due to enlargement of the ovaries; however, symptoms vanished within 2 days, and no ascites and haematoconcentration were observed while the patient was serially followed throughout the luteal phase.

**Discussion**

The results from the present proof-of-concept study suggest that GnRH-agonist triggering of final oocyte maturation offers patients a good chance of pregnancy, when the ET is performed in an FT-ET cycle.

The rationale of elective cryopreservation of oocytes after GnRH-agonist triggering is based on previous publications (Humaidan et al., 2005; Kolibianakis et al., 2005), which have indicated that the likelihood of pregnancy achievement is significantly impaired in fresh ET cycles, when the luteal phase is supported with vaginal progesterone and oral E₂. Although these trials have not been performed in OHSS risk patients, it is still likely that the mechanisms accounting for the lower pregnancy likelihood in normal responder patients after agonist triggering will also impair pregnancy likelihood in patients at risk of OHSS.

Table III. Pregnancy outcome

<table>
<thead>
<tr>
<th>Outcome</th>
<th>% (n)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical PR/patient</td>
<td>5.3 (1/19)</td>
<td>0.9–24.6</td>
</tr>
<tr>
<td>Ongoing PR/patientᵇ</td>
<td>36.8 (7/19)</td>
<td>19.1–59.0</td>
</tr>
<tr>
<td>Ongoing PR/first ET</td>
<td>31.6 (6/19)</td>
<td>15.4–54.0</td>
</tr>
<tr>
<td>Ongoing PR/ET</td>
<td>29.2 (7/24)</td>
<td>14.9–49.2</td>
</tr>
</tbody>
</table>

PR, pregnancy rate.

ᵇPresented here is the cumulative pregnancy rate resulting from 24 ETs in 19 patients.

Indicative of this notion are previous reports on GnRH-agonist triggering, followed by fresh ET in OHSS risk patients: in a publication by Itskovitz-Eldor et al. (2000), eight patients were triggered with 0.2 mg triptorelin s.c. and received luteal phase support with daily injections of 50 mg progesterone in oil and 2 mg E₂ orally; no patient conceived. In a paper by Kol and Muchtar (2005), six patients considered at OHSS risk were triggered with 0.2 mg triptorelin s.c. and received luteal phase support with daily 600 mg micronized vaginal progesterone and 4 mg vaginal E₂; one patient conceived. In a paper by Babayof et al. (2006), 15 PCOS patients were agonist triggered and received luteal phase support with daily 50 mg i.m. progesterone and 4 mg oral E₂; one patient conceived. Similarly, Orvieto et al. (2006) recently reported impaired pregnancy rates in OHSS risk patients triggered with agonists. The reason for the disappointing outcome after GnRH-agonist triggering and fresh ET has not been elucidated, but is likely to be associated with a defective luteal phase, possibly in combination with insufficient luteal phase support (Engmann et al., 2005). As in all the trials so far, ET has happened on day 3, possibly blastocyst transfer on day 5 or 6 could overcome an impaired luteal phase plus distorted endometrial maturation.

The present study is the first report in the literature on the concept of combining agonist triggering with elective cryopreservation of 2 PN oocytes. A prerequisite for this concept to work clinically is a cryopreservation method, which is effective enough in terms of oocyte survival not to impair the chance of subsequent ET(s) by higher order oocyte loss due to the freezing–thawing procedure. The newly developed vitrification method (Kuwayama et al., 2005b) used in the present study resulted in a mean 78% survival rate of 2 PN oocytes. The concept of cryopreservation at the 2 PN stage is furthermore supported by several reports in the literature which have indicated that the developmental potential of oocytes cryopreserved at the 2 PN stage is higher than that of the early cleaved embryos (Veeck et al., 1993; Horne et al., 1997; Senn et al., 2000). A further theoretical basis for splitting ovarian stimulation and ET temporally in OHSS risk patients is the possibility of the occurrence of a late-onset form of the disease in a pregnant woman due to revival of the multiple corpora lutea by early embryonic hCG. This is especially relevant, as late-onset forms tend to be more often severe and of longer duration (Papanikolaou et al., 2005b). Indeed, one of the rare cases...
of severe OHSS after agonist triggering reported in the literature occurred as a late-onset OHSS in a pregnant woman (Chun, 2005).

Some heterogeneity can be noted in the stimulation characteristics (Table II), which mostly results from the heterogeneity of management prior to GnRH-agonist triggering. Because of a more moderate stimulation in patients with previous imminent severe OHSS or development of OHSS, in three patients <10 oocytes were retrieved. Excluding these three patients widens the 95% CI for OHSS incidence from 0.0–16.1 to 0.0–18.4. Still, the present report provides further important evidence that GnRH-agonist triggering is associated with a decreased incidence of clinically relevant OHSS. For GnRH-agonist-based stimulation, a threshold value of \( \geq 18 \) follicles \( \geq 11 \) mm on the day of hCG administration has recently been reported to have 83% specificity in predicting severe OHSS requiring hospitalization (Papanikolaou et al., 2006); a criterion that was fulfilled by 14/20 (70%) of the patients in the present cohort (data not shown). The observed absence of OHSS II–III in the present cohort of 20 high-risk patients is, therefore, quite reassuring. For a better quantification of the reduction in OHSS risk through GnRH-agonist triggering, a trial would be necessary in which patients at OHSS risk were randomized to hCG or GnRH-agonist. As such a trial would be unethical (Kol, 2006), observational studies such as the present one are the only way to further substantiate the concept of OHSS prevention by GnRH-agonist triggering (Griesinger et al., 2006b).

The trial is confirmatory for OHSS risk patients as regards the fertilization capacity of the oocytes retrieved after GnRH-agonist triggering (Table II), which is similar to what has been reported previously for PCOS and normal responder patients (Fauser et al., 2002; Humaidan et al., 2005; Kolibianakis et al., 2005; Babayof et al., 2006).

Part of the good outcome (for German standards: DIR, 2004) in terms of ongoing pregnancy achievement in the present study can be attributed to the low early pregnancy loss rate. Although it is likely that, for the most part, patient selection accounts for this effect, it can also be speculated about two further effects: (i) it has been proposed that excessive steroid production as a side effect of ovarian stimulation might have a negative influence on the endometrial receptivity (Devroey et al., 2004; Papanikolaou et al., 2005a), and on the potential of ongoing embryo implantation in case endometrial advancement occurs (Kolibianakis et al., 2002). Therefore, an ET in an artificial cycle that is unaffected from ovarian stimulation might be of benefit to patients, who have a severely altered endocrine situation in the follicular phase, e.g. excessive steroid production (Table II); (ii) it has been indicated that early pregnancy loss in early-onset OHSS patients is higher in comparison to non-OHSS cycles (Papanikolaou et al., 2005b). The absence of OHSS in the present cohort might, therefore, have facilitated a good outcome.

In conclusion, the present study is the proof of the concept that GnRH-agonist triggering of final oocyte maturation in combination with elective cryopreservation of 2 PN oocytes offers patients at increased risk of OHSS a good chance of pregnancy while reducing the risk of moderate-to-severe OHSS. Although recent publications have indicated different ways to overcome the luteal phase deficiency encountered by GnRH-agonist triggering (Engmann et al., 2005; Humaidan et al., 2006; Krause and Ohlinger, 2006), we suggest elective cryopreservation of 2 PN oocytes as a measure of first choice until preliminary promising results on fresh ET after GnRH-agonist triggering in OHSS risk patients (Engmann et al., 2005) have been confirmed.

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