The Blastocyst Utilization Rate Is Reduced For Women At Risk Of Ovarian Hyperstimulation Syndrome During In Vitro Fertilization Treatment

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Abstract

Women at risk of ovarian hyperstimulation syndrome (OHSS) in an IVF programme, i.e. serum oestradiol >20,000 pmol/l or >20 oocytes collected, had all embryos cryopreserved to avoid exacerbation of the condition by pregnancy. This group was compared with oocyte donors (whose resulting embryos were all cryopreserved for quarantine reasons) and fertility patients not at risk of OHSS. As expected, the OHSS group had significantly more oocytes collected than the donors or non-OHSS patients (both p

Introduction

Ovarian hyperstimulation syndrome (OHSS) remains a potentially life threatening condition that results from an excessive ovarian response to stimulation by exogenous gonadotrophins [15], usually associated with cases of polycystic ovarian syndrome [23] or women with a spontaneously high rate of twinning [24]. The condition is exacerbated by increasing concentrations of human chorionic gonadotrophin (hCG) during pregnancy, and so a strategy employed by many in vitro fertilization (IVF) programmes is to cancel the embryo transfer and cryopreserve all embryos [4]. This reduces the severity of symptoms associated with OHSS to a more manageable level [26]. The increased number of oocytes collected from patients at risk of OHSS often yields a high number of zygotes and cleavage stage embryos for cryopreservation, and the transfer of these embryos in subsequent cycles is thought to give satisfactory pregnancy rates [8, 18, 19, 25]. However, a possible reduction of oocyte quality has been suggested [1, 2], although this is not always seen [7, 17]. These studies were limited by the technology of the day and often relied upon crude indicators of oocyte quality such as fertilization rates and the grading of early cleavage embryos on day 2 or day 3 at the time of transfer. Furthermore, pregnancy rates did not reflect the developmental potential of individual embryos as multiple embryos were usually transferred. The introduction of sequential media has now made the culture of embryos to day 5 or day 6 and cryopreservation of supernumerary blastocysts a standard and successful procedure in many laboratories, enabling the transfer of single embryos to reduce the risk of multiple pregnancy [10]. The identification of those embryos that form a usable blastocyst (i.e. suitable for transfer or cryopreservation) enables a utilization rate to be determined which is a better index of embryo quality and potentially a more informative endpoint [6]. The present study has therefore re-visited the issue of cryopreserving all embryos for women at risk of OHSS with the additional outcome measure of blastocyst utilization rate. A group of oocyte donors of a similar age that had zygotes frozen for the purposes of quarantine provided a suitable group for comparison (for the freezing variable) in addition to those IVF patients not deemed to be at risk of OHSS (control for infertile population of patients). The aim of this study was therefore to assess whether blastocyst utilization rates and subsequent pregnancy rates were compromised in patients at risk of OHSS in stimulation cycles.

Methods

Patients

Women treated between 2000 and 2007 were included if either their fresh or frozen/thawed embryos were cultured to the blastocyst stage.

Fertility patients. Women (n=137), aged between 22 and 44 years, had all embryos frozen because the serum estradiol was >20,000 pmol/l or >20 oocytes were collected, putting them at risk of OHSS. A parallel group of women (n= 1404) treated over the same period, aged 20-47 years and not at risk of developing OHSS, were also included.

Oocyte donors. Women (n=25) aged 22 to 44 years who donated oocytes were included for comparison, as all the resulting embryos were cryopreserved and stored for at least six months before use due to quarantine restrictions [9]. This acted as a control group for the freeze-all strategy on the basis that donor cycles give implantation rates in frozen embryo
transfer cycles similar to autologous cycles [21].

Ovarian Stimulation, Oocyte Collection and Embryo Culture

Ovarian stimulation used either a flare or pituitary desensitization protocol [12]. Recombinant follicle stimulating hormone (FSH) injections were given as either Puregen (Organon Australia Pty Ltd, Lane Cove, Australia) or Gonal-f (Merck Serono Australia Pty Ltd, Frenchs Forest, Australia) and were used in combination with a GnRH agonist, namely either Synarel (Pfizer Australia Pty Ltd, West Ryde, Australia) or Lucrin (Abbott Australasia, Botany, Australia). Follicular development was monitored by ultrasound and measurement of serum estradiol. A 5000IU dose of hCG (Pregnyl; Organon Australia Pty Ltd, Lane Cove, Australia) was administered when the leading follicle was at least 17mm in diameter, and trans-vaginal oocyte aspiration took place approximately 35 hours later. Sperm were added to oocytes 3-6 hours after retrieval either by conventional IVF or ICSI. All embryos were frozen at the zygote or blastocyst stage with a proportion of cycles freezing zygotes were exposed to 1.5M propanediol with 0.1M sucrose for up to 10 mins before loading. Zygotes were loaded into CBS™ High Security Straws (Cryo Bio Systems, Paris, France) which were heat sealed, placed into a rate controlled freezer (Kryo 10 Series III; Planer PLC, Sunbury-on-Thames, UK) and cooled at -2°C/min to -6.5°C where manual seeding and culturing the remainder to blastocyst. Embryos were cultured from 2000 to 2006 in Quinn’s Advantage (Sage Biopharma, Bedminster, USA) media and subsequently in SIVF media (Cook Australia Pty Ltd, Brisbane, Australia).

Embryo cryopreservation

Zygotes were frozen using a previously published slow rate freezing protocol [14, 22]. Following 10 mins of incubation at room temperature in 1.5M propanediol, zygotes were exposed to 1.5M propanediol with 0.1M sucrose for up to 10 mins before loading. Zygotes were loaded into CBS™ High Security Straws (Cryo Bio Systems, Paris, France) which were heat sealed, placed into a rate controlled freezer (Kryo 10 Series III; Planer PLC, Sunbury-on-Thames, UK) and cooled at -2°C/min to -6.5°C where manual seeding took place, then -0.3°C/min to -30°C and -50°C/min to -190°C before cryostorage. Blastocysts were frozen using a previously published protocol [3, 22], being held at room temperature for 10 mins in 5% glycerol, followed by 10 mins in 9% glycerol with 0.2M sucrose and sealed in CBS™ High Security Straws. The blastocysts were cooled in the rate controlled freezer at -1°C/min to -6.5°C for manual seeding, then -0.3°C/min to -36°C and plunged directly into liquid nitrogen for cryostorage. All embryos were stored with standard operating procedures and according to West Australian law [5, 16].

Embryo Thawing

Embryos were thawed as described in the previously published protocols mentioned above [3, 14, 22]. Briefly, embryos were removed from the liquid nitrogen, held in air for 30 seconds and then submerged in 30°C water for 30 seconds. The zygotes were rehydrated using SIVF Cleavage Thaw Kit (Cook Medical, Brisbane, Australia) and then transferred into cleavage medium (Cook Medical, Australia) where they were cultured until day 3. Embryos were either transferred on day 3 or moved into blastocyst medium (Cook Medical, Brisbane, Australia) until day 5 or 6. The blastocysts were rehydrated using SIVF Blastocyst Thaw Kit (Cook Medical, Brisbane, Australia) and then transferred into blastocyst medium (Cook Medical, Brisbane, Australia) until transfer.

Embryo Transfer and Pregnancy Detection

Embryos were transferred in either a hormone replacement therapy (HRT) cycle or a Natural/Low Dose Stimulation cycle [11]. HRT cycles consisted of a combination of estradiol valerate (Progynova; Bayer Schering Pharma, Pymble, Australia) at 3mg t.d.s. throughout the cycle, and progesterone pessaries at 400mg t.d.s. after a minimum of 7 days and the endometrial thickness is >8 mm. Embryos were thawed in synchronization with the patient’s cycle, being either 2 or 6 days post hCG trigger in natural or stimulated cycles or 1 to 5 days after starting progesterone pessaries in HRT cycles. Thawed zygotes were cultured to blastocyst stage and assessed for transfer or re-freezing. Blastocysts were thawed on the day of transfer. Embryo transfers were performed under ultrasound-guidance when the patient had a half-full bladder. Serum hCG was measured 12 days after the embryo transfer, with a positive pregnancy test result being recorded if the hCG concentration >25iu/l. The pregnancies were monitored weekly by serum hCG, estradiol and progesterone, and an ultrasound performed at 7 weeks to confirm the presence of a fetal heart. The patient was then referred back to their obstetrician for continuing care.

Statistical analysis

Proportions were compared with the χ² test, and Yates’ correction for continuity was applied if >20% of expected frequencies were

Results

Ovarian stimulation and oocyte collection

The details of the cycles in which the oocytes were collected are shown in Table 1. The OHSS group were significantly younger than those without OHSS (F2, 2991=9.373, p<0.05). Embryo culture to blastocyst stage

The culture to the blastocyst stage from either fresh or frozen zygotes is shown in Table 2. The blastocyst
utilization rate of fresh zygotes was different between the groups ($\chi^2=72.6$, p

Outcomes of transfer cycles

The positive and clinical pregnancy rates of fresh or frozen/thawed blastocysts are shown in Table 3. No fresh blastocysts were transferred in the donor or OHSS groups because of the freeze-all strategy. Following the transfer of frozen/thawed blastocysts, there was no significant differences between the three groups of women in terms of the incidence of positive pregnancy test results ($\chi^2=0.4$, p=0.80) or clinical pregnancy rates ($\chi^2=1.7$, p=0.43).

Discussion

Elective cryopreservation of embryos for patients at risk of OHSS is common practice [4], eliminating further exposure to exogenous and endogenous hCG particularly if pregnancy occurred. Once the symptoms have resolved, the transfer of the cryopreserved embryos still provides a good chance of achieving a pregnancy without the potentially life threatening complications of OHSS [25].

The extended culture of embryos to the blastocyst stage allows selection of viable embryos reaching that developmental milestone, making a single embryo transfer more attractive as a means of limiting multiple pregnancy [10]. The present study has confirmed a similar implantation potential of embryos frozen in cases of OHSS compared with donated oocytes or non-OHSS patients. The acceptable pregnancy rates are in agreement with the reports of others for OHSS patients [8, 18, 19] and donated oocyte recipients [21]. However, the present study has revealed a reduced blastocyst utilization rate in cases at risk of OHSS compared to the oocyte donors and non-OHSS patients. A low blastocyst formation rate has been described previously for OHSS patients where the zygotes were electively cryopreserved but unfortunately there was no control group to compare against [20]. Given that embryo quality can be the product of oocyte quality [13] these results imply that embryo quality is compromised as a result of poor oocyte quality in patients with OHSS. Interestingly, a decrease in oocyte quality has been suggested elsewhere [1, 2], albeit based upon reduced fertilization rates, although this has been refuted by others [7, 17]. The current demonstration of a reduced blastocyst utilization rate does not mean that the freeze-all strategy should be abandoned, but rather that it should be recognized that more eggs collected does not necessarily mean more embryos available for transfer.

In summary, the present study has shown that the blastocyst utilization rate for women at risk of OHSS is reduced compared to oocyte donors and fertility patients not at risk of OHSS. This implies that there is a reduced oocyte quality associated with the exaggerated response which is not revealed by other parameters such as fertilization rates or early cleavage morphology. The blastocysts that are transferred for those women at risk of OHSS have an implantation potential that is no different to that of the other women. The freeze all strategy is a practical solution to an iatrogenic problem, but the collection of large numbers of oocytes from these women does not necessarily mean that they obtain a proportionately high number of usable blastocysts.

Acknowledgement(s)

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19.Queenan Jr JT, Veeck LL, Toner JP, Oehninger S, Muasher SJ. 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. Hum Reprod 1997; 12: 1573-1576.
The collection of oocytes and their fertilization for oocyte donors, non-OHSS and OHSS fertility patients.

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Non-OHSS patients</th>
<th>OHSS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. collections</strong></td>
<td>27</td>
<td>2822</td>
<td>145</td>
</tr>
<tr>
<td><strong>Female age at collection</strong></td>
<td>33.4 ± 0.9</td>
<td>35.4 ± 0.1</td>
<td>33.9 ± 0.4</td>
</tr>
<tr>
<td><strong>Total FSH dose (iu)</strong></td>
<td>2179.6 ± 164.3</td>
<td>2573.2 ± 30.8</td>
<td>1963.0 ± 65.6</td>
</tr>
<tr>
<td><strong>No. oocytes collected</strong></td>
<td>11.0 ± 1.3</td>
<td>8.7 ± 0.1</td>
<td>18.2 ± 0.8</td>
</tr>
<tr>
<td><strong>No oocytes fertilized normally</strong></td>
<td>7.8 ± 0.9</td>
<td>5.7 ± 0.1</td>
<td>12.9 ± 0.6</td>
</tr>
<tr>
<td><strong>Fertilisation rate</strong></td>
<td>210/297 (70.7%)</td>
<td>16005/24585 (65.1%)</td>
<td>1872/2633 (71.1%)</td>
</tr>
</tbody>
</table>

+Total oocytes fertilized normally * 100% / Total oocytes collected
Illustration 2

Table 2

<table>
<thead>
<tr>
<th>Fresh zygotes</th>
<th>Frozen zygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>Non-OHS</td>
</tr>
<tr>
<td>No. zygotes thawed</td>
<td>-</td>
</tr>
<tr>
<td>No. zygotes survived/cultured</td>
<td>11</td>
</tr>
<tr>
<td>(82.3%)</td>
<td>(85.0%)</td>
</tr>
<tr>
<td>No. blastocysts</td>
<td>0</td>
</tr>
<tr>
<td>- transferred</td>
<td>6</td>
</tr>
<tr>
<td>- frozen</td>
<td>6</td>
</tr>
<tr>
<td>- total</td>
<td>6/11</td>
</tr>
<tr>
<td>Utilization rate+</td>
<td>(54.8%)</td>
</tr>
</tbody>
</table>

+Total No. blastocysts utilized * 100%/Total No. zygotes cultured
The pregnancy rates following transfer of blastocysts to oocyte recipients, women not at risk of hyperstimulation in the cycle of oocyte collection, and women originally at risk of OHSS.

<table>
<thead>
<tr>
<th></th>
<th>Fresh blastocysts</th>
<th>Frozen blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recipients</td>
<td>Non-OHS OHSS</td>
</tr>
<tr>
<td>No. transfers</td>
<td>0</td>
<td>1483 0</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td>0</td>
<td>1593 0</td>
</tr>
<tr>
<td>No. positive pregnancy tests</td>
<td>-</td>
<td>566 -</td>
</tr>
<tr>
<td>No. on-going pregnancies</td>
<td>-</td>
<td>439 (29.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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