Efficacy of oocyte vitrification combined with blastocyst stage transfer in an egg donation program

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BACKGROUND: A successful oocyte vitrification program is important for women with various indications for assisted reproduction technology. The objective of this study was to report the outcome of vitrification of oocytes, obtained through an oocyte donation program, by evaluating the embryo development, pregnancy and implantation rates (IRs) after blastocyst transfer.

METHODS: A total of 1098 oocytes were obtained from 78 donors. There were 312 oocytes used in the study group (vitrified oocytes) and 786 used in the control group (fresh oocytes). There were 34 recipients who received blastocysts obtained from vitrified oocytes and 58 recipients who received blastocysts from fresh oocytes. The fertilization rate, cleavage rate, embryo quality, pregnancy rate (PR) and IR were compared between groups.

RESULTS: Vitrified oocytes showed a survival rate of 89.4%. There was no difference in the fertilization rate (76.1 and 87.5%), Day 2 cleavage rate (96.3 and 98.0%) or blastocyst formation rate (41.3 and 45.3%) for the study and control groups, respectively. PRs, IRs and miscarriages rates (MRs) were similar for the study group compared with the control group (PR: 61.8 versus 60.0%; IR: 43.9 versus 42.9%; MR: 9.5 versus 5.9%).

CONCLUSIONS: The developmental competence of embryos obtained from vitrified oocytes is not affected by the vitrification procedure, since they preserve the potential to be fertilized and to develop into high-quality blastocysts, similar to embryos from fresh oocytes. The successful clinical outcome indicates the use of this procedure for oocyte donation programs and for oocyte storage in general.

Key words: oocytes / vitrification / embryo development / ICSI / blastocyst

Introduction

At present, embryo cryopreservation methods are useful tools in assisted reproduction technology, increasing success rates after IVF cycles.

Human oocyte cryopreservation is an attractive addition to the range of fertility treatments presently offered. Since the very first pregnancy from a frozen oocyte was achieved in humans by Chen (1986), the slow cooling method has been applied with varying success (Van Uem et al., 1987; Porçu et al., 1997; Nawroth and Kissing, 1998; Young et al., 1998; Allan, 2004; Levi et al., 2006). There are many reports about the cryopreservation of human oocytes with good fertilization and cleavage rates (Chen, 1986; Gook et al., 1995) but with variable results on pregnancy rates (PRs) per cycle (Tucker et al., 1998; Porçu et al., 2000; Fabbri et al., 2001; Winslow et al., 2001; Borini et al., 2004). A recent meta-analysis on slow freezing revealed that the clinical PR per transfer with this method was 20.6 and 2.3% per thawed oocyte (Oktay et al., 2006).

Novel approaches to slow freezing have been introduced (Fabbri et al., 2001; Boldt et al., 2006; Borini et al., 2006a,b, 2007) with improved oocyte survival rates and subsequent embryo development, but with limitations in terms of the implantation rate (IR) per thawed oocyte. As an alternative to slow freezing, the vitrification procedure has been recently applied for human oocytes and embryos (Vajta and Nagy, 2006; Al-Hasani et al., 2007) allowing improvements in oocyte survival, fertilization, embryo development and clinical outcomes (Kuwayama et al., 2005; Kuwayama, 2007; Lucena et al., 2006; Selman et al., 2006; Antinori et al., 2007; Yoon et al., 2007; Chian et al., 2008; Cobol et al., 2008; Nagy et al., 2009). This procedure uses very high cooling rates and high concentrations of cryoprotectants in which a solution or specimen solidifies to form a glass-like, or vitreous, structure without any ice crystal formation during cooling. This state is maintained throughout the whole warming procedure (Kuwayama, 2007).

The difficulties associated with oocyte cryopreservation are mostly related to the special structure and sensitivity of this cell, the exposure time to the cryoprotectant solutions, the concentration of the...
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Cryopectectants, the equilibrium temperature and the extra- and intracellular ice formation that affect the viability (Fabbri et al., 2001) and physiology of oocytes (Gardner et al., 2007).

Proper organization of the oocyte cytoskeleton, particularly of its microtubular fraction, is essential for normal spindle formation and chromosome segregation (Nottola et al., 2007). The meiotic spindles are crucial for the events following fertilization in the completion of meiosis, second polar body formation, migration of pronuclei and formation of the first mitotic spindle (Schatten et al., 1985). The meiotic spindle has been shown to be extremely sensitive to temperature variation (Chen et al., 2000, 2001; Van der Elst et al., 1988). Several studies evaluating the effect of vitrification and slow freezing on the meiotic spindle integrity and chromosome alignment (Kuwayama, 2001; Katayama et al., 2003; Silber et al., 2003; Munné et al., 2006; Cao et al., 2009) showed less damage in vitrified oocytes (as observed in fresh oocytes without cryopreservation) compared with cryopreserved oocytes by slow freezing. Additionally, studies of Cobo et al. (2008), Chen and Yang (2009) and Ciotti et al. (2009) reported that spindle recovery was faster in vitrification than in slow freezing (1–2 h after thaw procedure). However, studies of Cotcchio et al. (2009) and Martinez-Burgos et al. (2010) showed that the vitrified–warmed oocytes maintain a metaphase II (MII) spindle with a bipolar organization, but the chromosomal alignment appears to be partly compromised.

Other biological characteristics of human oocytes that might confer susceptibility to damage during the cryopreservation procedure are: the low permeability coefficient of the plasmatic membrane of oocytes, which makes the penetration of cryoprotectant substances more difficult, their intra-cytoplasmic lipids which make them more sensitive to freezing than embryos (Ruffing et al., 1993), precocious oocyte activation induced by exposure to cryoprotectants which may disturb future development (Larman et al., 2006; Gardner et al., 2007), loss of high mitochondrial polarity associated with a significantly reduced capacity to up-regulate the levels of intracellular free calcium after thawing (Jones et al., 2004), microvacuolization in the ooplasm and ultrastructural alterations in specific oocyte microdomains linked to a reduced developmental potential of mature cryopreserved oocytes (Nottola et al., 2007).

There is a significant decrease in the women’s fertility with advancing age (Van Noord-Zaadstra et al., 1991; Perheentupa and Huhtaniemi, 2009; Steiner, 2009) due to a reduction in the ovarian follicular reserve and a greater prevalence of chromosomal alterations in the oocyte which lead to a significant reduction in the IRs (Bar-Hava et al., 1999; Munné et al., 2002) and an increase in miscarriage rates (Munné et al., 1995; Nybo Andersen et al., 2000). In addition, there are other conditions affecting fertility potential including premature ovarian failure, unexplained recurrent implantation failure and inherited conditions (Sauer and Kavic, 2006).

Oocyte donation is a successful and well-established treatment of age-related female infertility, where the oocyte and subsequent embryo qualities are optimized by donated oocytes from young women (Wong et al., 1996), resulting in high PRs and good obstetrical outcomes observed in recipients (Sauer and Kavic, 2006; Budak et al., 2007). Moreover, the clinical outcome of oocyte donation requires a receptive endometrium, usually prepared by exogenous hormone replacement (Pados et al., 1994; Borini et al., 1995), and a well-synchronized replacement of good-quality embryos. For the synchronization, several strategies have been employed with varying levels of success, but certainly the most crucial factor is the availability of oocyte banks. The drawbacks of oocyte donation, such as long waiting subject to the availability of a suitable donor, are responsible for the poor efficiency of oocyte donation programs, causing great stress and discomfort in patients. Furthermore, current regulations demand that donors are tested to avoid the transmission of infectious diseases (Guidelines for gametes and embryo donation, 2008). All these limitations could be solved with the establishment of efficient banks of donated cryopreserved oocytes (Nagy et al., 2009).

The cryopreservation of human oocytes solves the legal restrictions and ethical problems (Parmegiani et al., 2008) associated with the cryopreservation of embryos in patients undergoing IVF procedures. Likewise, it may offer the possibility of extending the reproductive capability of young women with malignant diseases in cases where the treatment involving surgery, chemotherapy or radiotherapy may compromise the ovarian reserve (Yang et al., 2007; Gidoni et al., 2008; Porçu et al., 2008). Cryopreservation can also offer alternatives for infertile patients who are subject to ovarian hyper-stimulation syndrome, those who are poor responders to ovarian stimulation or women who are undergoing premature ovarian failure (Li et al., 2005).

We hypothesize that cryopreservation by the vitrification procedure does not affect the physiology and the developmental potential of vitrified oocytes, as evaluated through embryo development from the frozen oocyte to the blastocyst stage. Thus, the objective of this study was to report clinical outcomes obtained with vitrified oocytes compared with those obtained from fresh oocytes in a donation program in order to validate the effectiveness of the vitrification procedure at our center.

Materials and Methods

Patients

This is a prospective study that was done in the Laboratory of Assisted Reproduction at the Concebir Clinic (Lima, Peru) and it was approved by the Institutional Review Board (IRB) and the corresponding Ethics Committee. Written consent forms were received from all recipients and their partners included in this study.

The age of the oocyte donors was 18–29-year-old in the study group and 18–34-year-old in the control group. They underwent physical, gynecological and psychological examinations and there were no family histories of hereditary or chromosomal diseases. All participants had a normal karyotype and tested negative in a screening for sexually transmitted diseases.

From the 78 oocyte donors included, 20 were assigned to the study group (vitrified oocytes) and 58 to the control group (fresh oocytes). A total of 312 and 792 oocytes were obtained from study and control
groups, respectively. There were 34 recipients in the study group and 85 recipients in the control group.

Recruitment of oocyte donors was done based on recommendations given by other donors and the donation of their gametes was merely for altruistic reasons. The donors and their oocytes were randomly and consecutively assigned to each evaluated group. They were matched with their recipients according to phenotype and blood groups and the recipients were not aware of the origin of oocyte received (vitrified or fresh).

**Controlled ovarian stimulation and oocyte collection**

The menstrual cycles of oocyte donors were stimulated using recombinant FSH (rFSH) (Puregon®, Organon laboratories, Peru), HMG (Humegon®, Organon Laboratories, Peru) and GnRH antagonist (Orgalantrin® Organon laboratories, Peru) according to the stimulation protocols previously established and starting on Day 2 of the menstrual cycle until at least three follicles reached ~18 mm in diameter. The oocyte retrieval was performed by vaginal ultrasound 36 h after the i.m. application of hCG (Ovidrel® 250 μg, Serono Laboratories, Peru). For the procedure, the patient was under general anesthesia with 200 mg of Propofol iv (Diprivan® 1% P/V; AstraZeneca Laboratories, UK).

During follicular aspiration procedure, the oocytes were recovered in Global®-HEPES-buffered medium (IVFonline, Canada) supplemented with 10% vol/vol Serum Substitute Supplement (SSS; Irvine Scientific, USA). After retrieval, cumulus–oocyte complexes were trimmed of excess cumulus cells and maintained in ~200 μl drops of Global®. Fertilization medium (IVFonline, Canada) plus 10% SSS under oil at 37°C and an atmosphere containing 5.6% CO2, 5% O2 and 89.4% N2, for 2 h until the recovered oocytes were vitrified (study group) or for 5 h until the recovered oocytes were inseminated (control group). All 312 oocytes collected from donors of the study group were divided between the 34 recipients (9.18 ± 3.34 oocytes per recipient) and all 792 oocytes collected from of the control group were divided between the 85 recipients (9.25 ± 3.39 oocytes per recipient).

All collected oocytes were denuded enzymatically of cumulus cells with hyaluronidase (80 IU/ml; IVFonline, Canada) to assess nuclear maturity. Only the MII oocytes were vitrified, with Cryotop minimum volume vitrification methods (Kuleshova and Lopata, 2002; Katayama et al., 2003; Kuwayama et al., 2005) using commercial cryoprotectant solutions (Cecolfes, Colombia) and the Cryolock device (Biodiseño-Colombia), for a maximum period of 3 h after retrieval.

**Oocyte vitrification**

A total of 283 MII oocytes corresponding to that of the study group were vitrified by minimum volume methods, which minimize the volume of vitrification solution thus increasing the cooling and warming rates and decreasing the chance of ice crystal nucleation/formation in the small sample (Rall, 1987; Vajta and Nagy, 2006). The vitrification procedure was performed at room temperature (24°C). The oocytes were equilibrated in 7.5% vol/vol ethylene glycol (EG) plus 7.5% dimethylsulfoxide (DMSO) in TCM199 medium for 15 min (equilibrium solution) and then placed in 15% EG plus 15% DMSO plus 0.5 mmol/l sucrose (vitrification solution, VS) for a maximum 1 min. The oocytes were loaded quickly onto the Cryolock in a minimum drop size of <0.1 μl of VS and immediately immersed directly in nitrogen liquid, at a cooling rate of approximately ~23 000°C/min (Kuwayama et al., 2005). A maximum three oocytes were placed by each Cryolock. All cryoprotectant solutions were supplemented with 20% vol/vol SSS.

**Oocyte warming**

Oocytes were warmed at fast warming rates of ~12 000°C/min (Kuwayama et al., 2005). The Cryolock was taken out of liquid nitrogen and quickly placed in 1.0 mol/l sucrose in TCM199 medium (shaving solution) for 1 min at 37°C. The oocytes were then placed in 0.5 mol/l sucrose in TCM199 medium (diluent solution) for 3 min at room temperature (24°C), followed by two consecutive 5-min flushes in TCM199 medium (washing solution). Oocytes were then immediately placed in Global®-Fertilization medium plus 10% SSS under oil at 37°C and an atmosphere containing 5.6% CO2, 5% O2 and 89.4% N2 for 2 h before ICSI. All warming solutions were supplemented with 20% vol/vol SSS.

**Insemination, fertilization and embryo culture**

In the study group, 2 h after warming, the viability of oocytes was evaluated microscopically based on the morphology of the oocyte membrane integrity. All viable oocytes were inseminated by ICSI with spermatozoa from the recipient’s husband. After the ICSI procedure (Day 0), all injected oocytes were cultured at 37°C in an atmosphere of 5.6% CO2, 5% O2 and 89.4% N2.

In the control group, ~5 h after oocyte retrieval, all MII oocytes were inseminated or injected, depending on the seminal characteristics, with spermatozoa from the recipient’s husband.

In both groups, fertilization was evaluated 16–18 h after injection by the presence of two pronuclei (Day 1). The zygotes were individually cultured under mineral oil, in 10-μl droplets of Global® medium (IVFonline, Canada) supplemented with 10% vol/vol SSS from Day 1 to Day 3. On Day 3, the embryos were moved to fresh 10-μl droplets of Global® medium +10% SSS.

On Day 2 and 3 the embryos were evaluated for cell number and fragmentation, on Day 4 for development to morula and on Day 5 for development to blastocyst and expansion.

**Embryo quality**

On Day 2 and 3 the embryos were evaluated for cell number, fragmentation and multinucleation. Good-quality Day 2 embryos were defined as those with 2–4 cells and ≤10% of fragmentation and absence of multinucleation. Good-quality Day 3 embryos were defined as those with 6–8 cells and ≤10% of fragmentation. Good-quality blastocysts were defined as having an inner cell mass (ICM) and trophoectoderm type A or B (Gardner et al., 1999). The ICM score was evaluated as type A = compact area, many cells present or type B = cells are loosely grouped. The trophoectoderm was scored as type A = many cells forming a tight epithelial network of cells or type B = few cells forming a loose network of cells.

**Blastocyst transfer**

Blastocysts were transferred on Day 5 in all recipients. There were 34 and 85 blastocyst transfers in the study group (vitrified oocytes) and control group (fresh oocytes), respectively. In the study group 1.94 ± 0.15 blastocyst/recipient (Mean ± SD) were transferred and in the control group 1.98 ± 0.15 blastocyst/recipient (Mean ± SD) were transferred. The blastocysts that were not transferred were cryopreserved or discarded according to their morphology. The blastocyst transfer was performed with a Frydman Ultrasoft catheter (CCD Laboratoire, Paris, France) that had been previously washed with culture medium. The catheter was completely filled with culture medium and the blastocysts filled in the last 10 μl of the catheter. All transfers were performed according to the methods previously described by Mansour (2005).
Pregnancy assessment
Biochemical pregnancy was assessed 12 days after the blastocyst transfer by measuring the hCG-beta subunit in blood. Clinical pregnancy was determined by transvaginal ultrasonography to detect gestational sacs and fetal heartbeats at ~21 and 28 days after transfer, respectively.

Blastocyst recipients
A total of 119 recipients were prepared for the blastocyst transfer, 34 for transfer of blastocysts obtained from vitrified-warmed oocytes (study group) and 85 recipients for transfer of blastocysts obtained from fresh oocytes (control group).

The endometrium was prepared with increasing doses of estradiol valerate (Progynova™, Shering Laboratories, Germany) as follows: 4 mg/day for the first 6 days of treatment, followed by 6 mg/day until before day the oocyte retrieval when the doses was incremented to 8 mg/day and maintained until the time of the pregnancy test. Micronized Progesterone (600 mg/day, vaginally; Geslutin™, Tecnofama, Peru) was started at Day 0 when the oocytes were inseminated. If the pregnancy test was positive, progesterone supplementation was continued until the 12 weeks of gestation.

Sperm collection
The semen samples were collected by masturbation from the recipients’ partners. Motile spermatozoa were separated from the seminal plasma by centrifugation through 1.0 ml 95 and 45% Isolate gradients (Irvine Scientific, USA). For oligospermic samples, the sperm were washed and resuspended in varying amounts of sperm washing medium depending on the initial concentration and motility and then placed into 10 μl drops of HEPES-buffered Global medium +10% SSS for ICSI.

Statistical analysis
Data were statistically analyzed using the χ² test and Student’s t-test as appropriate and differences were considered to be significant at P < 0.05. All statistical analysis was carried out using the statistic package Stata 10 (StataCorp, College Station, TX, USA).

Normal fertilization was calculated from the number of zygotes with two pronuclei divided by the number of mature oocytes inseminated by 100. Clinical PR per transfer was calculated from the number of patients with blastocyst transfer with at least one gestational sac divided by the total number of blastocyst transfer by 100. IR was calculated by dividing the number of gestational sacs observed by ultrasound at the 21st day post-transfer by the total number of blastocysts transferred by 100. Miscarriages were defined as the number of pregnancies with total loss of the gestational sac before the 20 weeks of gestation divided by the numbers of pregnancies by 100.

Results
The ages of oocyte donors were similar in both evaluated groups. The ages of recipients were similar in both groups. The mean days of stimulation were significantly greater for the study group donors than for the control group donors (9.15 ± 0.93 versus 8.47 ± 1.11; P < 0.05). Similarly, the oocyte donors of the study group compared with the control group donors had significantly higher mean rFSH treatment (1753.1 ± 276.8 versus 1583.4 ± 270.9; IU/l) and lower mean days of antag-GnRH treatment (1.5 ± 0.7 versus 1.8 ± 0.8; P < 0.05). The ages of recipients were similar in both groups (41.21 ± 5.39 versus 40.22 ± 5.53 years; data not shown).

Results obtained from study group (vitrified oocytes) and control group (fresh oocytes) are shown in Table I. A total of 312 and 786 oocytes were collected from donors of the study and control groups, respectively. There were 283 MII oocytes vitrified in the study group, resulting in the survival of 253 oocytes (89.4%). Two hundred and fifty-one and 695 oocytes from the study and control groups, respectively, were inseminated. The mean fertilization (2PN) was similar in both evaluated groups (study group: 87.5%). In the control group, 96.3% of zygotes underwent cleavage on Day 2, whereas for zygotes from the control group: 94.7%. In the study group, 96.3% of zygotes (2PN) was similar in both evaluated groups (study group: 76.1% versus control group: 87.5%). In the study group, 96.3% of zygotes underwent cleavage on Day 2, whereas for zygotes from the control group, the cleavage rate was 98%. Mean cell numbers on Days 2 and 3 were similar in both groups. The quality of embryos from vitrified oocytes was similar to that of embryos derived from fresh oocytes and 3 were similar in both groups. The quality of embryos from vitrified oocytes was similar to that of embryos derived from fresh oocytes and 3 were similar in both groups.

Clinical outcomes are shown in Table II. The mean number of blastocysts available for recipients of the study group was significantly lower than that for the recipients of the control group (2.45 ± 1.23 versus 3.33 ± 1.93; P < 0.05). In the study group, a total of 66 blastocysts were transferred to 34 recipients with a mean of 1.94 blastocysts. In the control group, a total of 168 blastocysts were transferred...
The present study has shown the feasibility and efficiency of oocyte vitrification. The data obtained demonstrated that oocyte vitrification procedures result in high recovery and post-warming survival. Similar fertilization rates, preimplantational embryo development and clinical outcomes were observed with vitrified and fresh donated oocytes. Similar results were shown by Lucena et al. (2006) and Cobo et al. (2008).

Different devices have been employed for rapid cooling and vitrification of oocytes and embryos. These include the whole plastic straws of 0.25 ml (Rall and Fahy, 1985; Kasai et al., 1990; Vanderzwalmen et al., 2002), nylon loops (Lane et al., 1999; Yeoman et al., 2001; Begin et al., 2003; Murakaida et al., 2003), copper or gold electron microscope grids (Martino et al., 1996; Chen et al., 2001), nylon mesh (Matsumoto et al., 2001), open pulled straws (OPS) (Vajta et al., 1998; Lopez-Bejar and Lopez-Gatius, 2002; Chen et al., 2003) and versions derived from OPS such as the super thin OPS (Isachenko et al., 2000), glass micropipettes (Kong et al., 2000; Hochi et al., 2001), flexible denudation pipettes (Lieberman et al., 2002) and small diameter plastic micropipettes (Cremades et al., 2004). Each type has specific characteristics which influence the total size and volume of cryoprotectants, cooling and warming rates, likelihood of losing the specimen, fracture or explosion, contamination risks, ease of use, labeling, storage and sterilization, loading costs and availability.

The vitrification system designed by Kuwayama et al. (2005) allows the samples to be loaded in very small volumes of vitrification solution, allowing a fast transfer of temperature assumed to be 15 to −15 ºC (Martino et al., 1996). Kuwayama’s model avoids damage to the oocyte’s spindles and may also be helpful in avoiding zona pellucida and embryo fracture damage. Once oocytes are loaded onto a device (Cryotop), almost the entire loading solution is removed by aspiration before direct immersion into liquid nitrogen; thus, the final volume is <0.1 µl. Using this device with the minimum volume methods, higher cooling rates can be achieved (23 000 ºC/min), which can facilitate vitrification with less concentrated cryoprotectants. A higher warming rate can also be achieved (12 000 ºC/min), which prevents devitrification and the chilling injury (Kuwayama et al., 2005; Chen and Yang, 2007).

In the present study, the vitrification procedures were all carried out using the Cryolock device, which has a similar design and handle to those of Cryotop. The Cryolock has been designed and developed specifically for use in vitrification procedures with slots in the ends and top for better manipulation, and to avoid the risk that these loosen or fall during their use or storage (Biodesho-Colombia). On the other hand, the Cryotop presents a thinner film compared with Cryolock, but this difference does not significantly affect survival and PRs when vitrified embryos are warmed and transferred to patients (Lazcano et al., 2010). Several studies using Cryotop and minimum volume methods showed excellent results in survival rates (89–96%), fertilization rates (87–91%), cleavage rates (50–94%) and PRs (41–57%) after warming of vitrified human oocytes (Katayama et al., 2003; Kuwayama et al., 2005; Lucena et al., 2006; Cobo et al., 2008). In our study, similar results were obtained using Cryolock, thus showing that the efficacy of the vitrification procedure is not completely dependent of the device used. Furthermore, the results obtained during vitrification procedures using both devices clearly illustrate a major improvement when compared with those obtained with slow freezing protocols.

### Table II Clinical outcomes in the study and the control group.

<table>
<thead>
<tr>
<th>Study group</th>
<th>Control group</th>
<th>P</th>
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<tbody>
<tr>
<td>Recipients</td>
<td>34</td>
<td>85</td>
</tr>
<tr>
<td>No. of warmed oocyte/recipient (Mean ± SD)</td>
<td>7.44 ± 2.08</td>
<td></td>
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<tr>
<td>No. of injected oocytes/recipient (Mean ± SD)</td>
<td>7.38 ± 2.07</td>
<td>8.18 ± 3.03</td>
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<tr>
<td>No. of fertilized oocytes/recipient (Mean ± SD)</td>
<td>5.62 ± 1.94</td>
<td>7.15 ± 2.87</td>
</tr>
<tr>
<td>No. of blastocyst/recipient (Mean ± SD)</td>
<td>2.45 ± 1.23</td>
<td>3.33 ± 1.93</td>
</tr>
<tr>
<td>No. total blastocyst transferred</td>
<td>66 (1.94 ± 0.24)</td>
<td>168 (1.98 ± 0.15)</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>61.8</td>
<td>60.0</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>43.9</td>
<td>42.9</td>
</tr>
<tr>
<td>Single pregnancy (%)</td>
<td>61.9</td>
<td>58.8</td>
</tr>
<tr>
<td>Twin pregnancy (%)</td>
<td>38.1</td>
<td>41.2</td>
</tr>
<tr>
<td>Miscarriages (%)</td>
<td>9.5</td>
<td>5.9</td>
</tr>
<tr>
<td>Biochemical pregnancy rate (%)</td>
<td>4.6</td>
<td>3.8</td>
</tr>
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</table>

Note: Values in parentheses are mean blastocyst transferred/recipient.

*P* < 0.05 compared to the control group.

Discussion

Since the first pregnancy achieved with a vitrified and warmed human oocyte (Hong et al., 1999) and the first birth of a healthy baby (Yoon et al., 2000), vitrification results have improved significantly during the last decade (Kuwayama et al., 2005, 2007; Lucena et al., 2006; Selman et al., 2006; Antinori et al., 2007; Yoon et al., 2007; Chian et al., 2008; Cobo et al., 2008; Sher et al., 2008; Nagy et al., 2009; Kim et al., 2010; Rienzi et al., 2010; Ubaldi et al., 2010).

Vitrification is the glass-like solidification of a solution at low temperature, without ice crystal formation. This is possible through the extreme elevation in viscosity during freezing and is achieved by increasing the freezing and warming rates and/or increasing the concentration of the cryoprotectants (Fahy et al., 1984). Unlike slow freezing, vitrification results in the total elimination of ice crystal formation, with the inside of the cells being vitrified and the outside of the cells in the surrounding solution (Rall, 1987).
Cleavage rate, cleavage stage embryo morphology, cytoplasmic fragmentation and multinucleation have been shown to be important markers of embryo quality and viability that may be observed over time during in vitro culture. After the advent of extended embryo culturing, higher IRRs have been reported because of better embryo selection compared with earlier developmental stages and because of better synchronization between the embryo developmental stage and uterine environment. A common practice in several laboratories has been the daily evaluation of embryo quality from Day 2 up to the blastocyst stage. However, this practice of daily evaluation plus Day 3 medium changes may have negative effects on developmental competence of embryos cultured in vitro. During the present study the embryo quality was evaluated daily from Day 2 up to the blastocyst stage as a way to evaluate and obtain data about the effects of the vitrification procedures on developmental competence of MII oocytes to reach the blastocyst stage.

There are some reports of vitrification with autogenous oocytes (Yoon et al., 2000, 2003; Chian et al., 2005; Kuwawama et al., 2005; Selman et al., 2006; Antinori et al., 2007; Almodin et al., 2010) and donated oocytes (Lucena et al., 2006; Cobo et al., 2008, 2010) but with embryo transfer at Days 2 and 3. In the present study, all embryo transfers were carried out at the blastocyst stage, allowing evaluation of the effect of the vitrification procedure on the oocyte viability from fertilization to blastocyst stage and, more importantly, the morphological characteristics of blastocysts at Day 5. All parameters evaluated were similar for patients whose embryos proceeded from vitrified oocytes and patients whose embryos proceeded from fresh oocytes. These results confirm that oocytes conserve their capacity for fertilization and potential to reach the blastocyst stage after being vitrified, similar to fresh oocytes.

Finally, our observations demonstrate that vitrification using the Cryoloop device and minimum volume methods is an efficient procedure for oocyte cryopreservation. The developmental competence of vitrified MII oocytes to reach the blastocyst stage is similar to that observed with fresh oocytes. In conclusion, the vitrification procedure represents the best current alternative for creation of reliable oocyte banks.

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