Egg donation of vitrified oocytes bank produces similar pregnancy rates by blastocyst transfer when compared to fresh cycle

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Abstract

Purpose Advances in reproductive techniques, mainly the introduction of oocyte vitrification, have provided the opportunity to conceive from oocyte banks. The aim of this study was to compare the clinical outcomes of fresh and vitrified oocytes in an egg donation program following blastocyst transfer.

Methods This retrospective observational study included 504 oocyte donation cycles. All donor women were younger than 30 years of age. The recipient cycles were divided into two groups: fresh oocytes ($n = 78$) or vitrified oocytes ($n = 426$). All oocytes were fertilized by ICSI using ejaculated sperm, followed by blastocyst transfer. Endometrium preparation was performed with estradiol valerate plus micronized progesterone according to standard protocols.

Results Recipients were of similar age (fresh 42.0 ± 4.5 years vs vitrified 41.8 ± 4.8 years; $p = 0.790$). The fresh group received more mature oocytes for injection compared to the vitrified group (10.1 ± 2.8 vs 9.2 ± 2.2 ; $p = 0.005$). The two pronuclei (2PN) rate (74.5 vs 77.4%; $p = 0.195$) and blastocyst rate (48.8 vs 51.6%; 0.329) were similar between the fresh and

vitrified groups, respectively. The rates of clinical pregnancy were 60.9% in the fresh and 59.0% in the vitrified groups ($p = 0.771$).

Conclusions Our findings suggest that vitrified oocytes result in similar pregnancy rates when compared to fresh oocytes with blastocyst transfer in an egg donation program. Moreover, vitrified oocytes may allow for a better cycle schedule, starting with a lower number of oocytes to be fertilized. Therefore, we hypothesize that egg banks with vitrified oocytes could be safely utilized in an egg donation program.

Keywords Oocyte donation · Oocyte vitrification · In vitro fertilization · Blastocyst transfer · Pregnancy rates

Introduction

Although the first human birth from a frozen oocyte was reported in 1986 [1], the American Society of Reproductive Medicine (ASRM) removed the experimental label of oocyte vitrification only in 2013. Historically, the success of in vitro fertilization (IVF) treatment using cryopreserved oocytes has been low, probably due to spindle misalignment and errors in chromosomal arrangement, as a consequence of ice formation during the process. Only recently have improvements in cryopreservation methods, such as vitrification, significantly improved oocyte survival and pregnancy rates [2], allowing the use of this technique for different circumstances.

Oocyte cryopreservation may be used not only as storage for supernumerary oocytes but also to give patients at risk of losing ovarian function a chance as well as for egg donation programs. Successful oocyte cryopreservation has the potential to simplify oocyte donation programs. The coordination of donor fresh cycles and recipients can be inconvenient,

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whereas the use of cryopreserved oocytes may provide more choices in selecting a donor and flexible cycle [2]. Indeed, data in the literature support the equivalence of pregnancy outcomes in cycles using fresh or vitrified oocytes [3, 4], reassuring live birth rates [5, 6], and the use of oocyte vitrification in the setting of donor oocyte cycles [7–10].

However, the safety and efficacy of oocyte cryopreservation need to be ensured before universal donor oocyte banking could be recommended worldwide [2]. Our service began in 2013 as an oocyte bank using the vitrification method for an egg donation program. The aims of this study were to demonstrate the clinical outcomes of an egg donation banking program after 3 years and to compare our results from fresh oocytes to vitrified oocytes following blastocyst transfer.

Materials and methods

This study was a retrospective observational study that evaluated IVF cycles with an oocyte donation (OD) program at the Huntington Reproductive Medicine Center, Sao Paulo, Brazil, between 2013 and 2015. Institutional review board approval was not required for this study since all procedures are routinely performed. Written informed consent was obtained from all patients before treatment, in which they consented to the retrospective use of their data in scientific publications.

The women who elected to participate in our oocyte donation program did so due to previously failed cycles, advanced maternal age, or diminished ovarian reserves. Anonymous donors from our egg bank were matched with their recipients according to their phenotype and blood groups.

Oocyte donors and ovarian stimulation

All donors were healthy female volunteers under the age of 32 years, with body mass indices between 18 and 30 kg/m², antral follicle counts ≥ 10 , normal karyotypes, and the absence of endometriosis, who were screened and tested for infectious diseases and consented to anonymous OD according to local regulations for oocyte donation programs. Controlled ovarian stimulation was achieved using standard protocols. Briefly, ovarian stimulation was performed using rFSH (Gonal, Merck-Serono or Puregon, MSD) combined with or without hMG (Menopur, Ferring) starting on the 2nd or 3rd day of the menstrual cycle, and monitored by ultrasound for dose adjustments based on the ovarian response. GnRH antagonist (Orgalutran, MSD) was used for pituitary blockage when at least one follicle attained a diameter of 14 mm. The final follicular maturation trigger was obtained by the administration of the GnRH agonist triptorelin (Gonapeptyl, Ferring) when patients presented at least two follicles ≥ 20 mm, and half of the follicle cohort had a 14-mm or larger diameter. Oocyte

retrieval was performed 35 h after trigger under anesthesia, using transvaginal ultrasound guidance.

Laboratorial procedures

Collected oocytes were assessed for maturity, and only metaphase II (MII) oocytes were donated for fresh recipients' cycles or for oocyte banking. The oocytes designed for oocyte banking were vitrified using a Kitazato kit (Kitazato, Japan), and the vitrification and warming procedure steps were carried out according to the manufacturer's directions. All oocytes were inseminated by intracytoplasmic sperm injection (ICSI) [11] using husband or donor sperm. Oocytes were assessed for fertilization on the day after insemination, and those showing two pronuclei were cultured further until day 5, as is routine. Blastocysts were morphologically evaluated and a maximum of three blastocysts with the highest grades were selected for transfer to the recipient's uterus. Supernumerary embryos with sufficient developmental and morphologic status were vitrified on either day 5 or day 6.

Endometrial preparation of recipients

Recipients underwent endometrial preparation by initiation on day 2 or 3 of their menstrual cycle, with 4 mg of estradiol valerate (Primogyna, Bayer) for 10–15 days until the endometrial thickness reached ≥ 7 mm, which was monitored by transvaginal ultrasound. Then, 400 to 800 μ g of vaginal micronized progesterone (Utrogestan, Farnocimica) was administered. In summary, all recipients were advised to choose an egg profile from the cell banking data. By chance, if a donor was in the process of institutional approval, it was possible to match the donor egg retrieval with the recipient's endometrial preparation for a fresh transfer, allowing for a comparison.

Study groups

Five hundred and four (504) oocyte donation cycles from 433 patients were performed between 2013 and 2015. Oocyte recipients' cycles were divided into the following two groups according to oocyte origin: fresh ($n = 78$) or vitrified ($n = 426$). All oocytes were fertilized by ICSI using ejaculated sperm, and blastocyst transfer occurred on days 5–6 of embryo development.

Statistical analysis

Patient demographic data were evaluated using descriptive statistics, which included information on means and frequencies. Continuous variables were compared using mean comparison tests (Student's *t* test). Frequencies were compared using Pearson χ^2 test. Regression analyses were used to evaluate

the association between variables. Factors examined in the multivariate models included variables that were significantly different between groups or clinically relevant; and the results were reported as the odds ratios and *p* value. Data analyses were performed using SPSS 22 (IBM SPSS Software, USA) and significance was considered for *p* values ≤ 0.05 .

Results

Donors' demographic profiles according to group are presented in Table 1. Although significant differences were observed between vitrified and fresh donors, both groups were further evaluated in a multivariate analysis to evaluate those that were considered confounding.

For the recipients, the demographic data and clinical outcomes are shown in Table 2. The vitrified group received significantly fewer oocytes to be fertilized. However, the implantation and pregnancy rates were similar, demonstrating the efficiency of the procedure.

To confirm the lack of a difference in the pregnancy rates in cycles using fresh or vitrified oocytes, we built a multiple logistic regression model to evaluate the effect of the use of vitrified oocytes in the pregnancy rates adjusted to possibly confounding variables, such as donor age, donor MII oocytes recovered, recipient's age, number of donated MII, and number of embryos transferred. We observed that the neither oocyte vitrification nor any other variable included in the model was associated with successful pregnancies (Table 3), confirming the equivalence of fresh and vitrified protocols for egg recipients.

Discussion

The successful clinical outcome of any egg donation program requires a receptive endometrium and the transfer of high-quality embryos. As endometrial preparation using exogenous steroids is a well-established process, the availability of good oocytes, and consequently high-quality embryos, is a crucial factor for the efficiency of egg donor programs. The availability of a compatible donor for a fresh cycle donation may be

Table 2 Demographic characteristics of recipients and clinical outcomes

Variable	Fresh group	Vitrified group	<i>p</i>
Age (years)	42.0 $\pm$ 4.3	41.8 $\pm$ 4.8	0.790
BMI (kg/m ²)	23.5 $\pm$ 3.3	24.4 $\pm$ 3.6	0.099
MI I oocytes received	10.1 $\pm$ 2.6	9.2 $\pm$ 2.2	0.005
Fertilization rate (%)	74.5%	77.4%	0.195
Blastocyst rate (%)	48.8%	51.6%	0.329
Number of embryos transferred	2.3 $\pm$ 0.7	2.1 $\pm$ 0.5	0.007
Implantation rate (%)	40.0%	36.4%	0.486
Clinical pregnancy rate	60.9%	59.0%	0.771
Cycle cancellation	3.8%	3.9%	0.969

hampered since it depends on the synchronization of two women, whereas the endometrium or ovarian response is not completely predictable, and it is therefore not possible to certify an adequate amount of good oocytes. Because the endometrium and ovarian response are variable and one cannot predict the number of oocytes retrieved in a fresh cycle, it can lead to a recipient's cycle cancellation. Conversely, those limitations may be solved with an efficient oocyte-banking program with a superior oocyte selection.

Our main findings demonstrated that clinical pregnancy rates were similar between vitrified and fresh groups. Although the retrospective data demonstrated some demographic differences between donors, we applied a multiple regression model to adjust these possible confounders and confirmed that those differences, especially for the vitrified group, did not influence recipient's outcomes. Although more donated oocytes were obtained in the fresh cycle, the clinical pregnancy rates were similar between fresh and vitrified cycles.

Since 2013, the American Society for Reproductive Medicine published a guideline stating that oocyte vitrification and warming should no longer be considered experimental, which allowed the organization of egg banks [2]. Previous reports have confirmed the efficiency of oocyte cryopreservation as it has found no difference in malformations between children born after vitrification of oocytes and those born through IVF [12, 13].

The experience of our egg donation program demonstrated the equivalence in clinical pregnancy rates between fresh and cryopreserved oocytes. Our data corroborate with other studies showing similar blastocyst developmental rate between fresh and vitrified sibling oocytes [4, 7], and similar implantation and clinical pregnancy rates using fresh or vitrified oocytes in an ovum donation program [9, 10], similar to our outcomes, which confirm the effectiveness of oocyte vitrification in an egg donor bank. In contrast, a study reviewing approximately 11,000 oocyte donation cycles with fresh or cryopreserved oocytes showed lower implantation rates when cryopreserved oocytes were used. The authors explained that these differences could be attributed to the smaller starting numbers of oocytes

Table 1 Demographic characteristics of donors

Variable	Fresh group	Vitrified group	<i>p</i>
Donors			
Age (years)	26.0 $\pm$ 3.5	24.4 $\pm$ 3.9	< 0.001
BMI (kg/m ²)	24.4 $\pm$ 4.3	24.2 $\pm$ 4.1	0.840
Antral follicle count	19.4 $\pm$ 7.1	21.2 $\pm$ 9.2	0.182
Basal FSH (UI/mL)	5.1 $\pm$ 1.8	5.8 $\pm$ 2.8	0.044
MI I oocytes collected	13.7 $\pm$ 7.1	21.5 $\pm$ 7.6	< 0.001

Table 3 Multiple logistic regression model to evaluate the association of vitrified oocytes protocol in the pregnancy rates of recipients, adjusted for confounders

	Coefficient	SE coefficient	<i>p</i> value	Odds ratio	95% Confidence interval
Constant	0.260	1.350			
Vitrified oocytes	−0.353	0.317	0.262	0.703	(0.378;1.307)
Donor age	−0.007	0.026	0.791	0.993	(0.943;1.046)
Number of donor MII oocytes recovered	0.020	0.014	0.140	1.020	(0.993;1.048)
Recipient's age	0.000	0.021	0.988	1.000	(0.960;1.041)
Number of MII oocytes donated	0.035	0.046	0.442	1.035	(0.946;1.132)
Number of embryos transferred	−0.084	0.188	0.656	0.920	(0.637;1.328)

or alternatively lower oocyte quality due to cryopreservation and thawing [14]. Indeed, the oocyte cryopreservation process is extremely sensitive to the IVF laboratory environment, especially the technical skill of the embryologist. Our results showed instead that oocytes vitrified cycles are as good as, if not, more efficient, than fresh egg donation cycles. Cryopreservation of the oocytes allows the grading of MII ova thawing. It reflected in a lower number of MII oocytes used in the cryopreserved group, yet the clinical pregnancy rates were similar. With very young donors, it is likely only a few oocytes are necessary to guarantee outcomes, regardless of whether oocytes are sourced from fresh or vitrified cycles.

Moreover, fresh oocyte donation is restricted by several difficulties compared to egg-banked programs, such as donor availability, the need to synchronize donor and recipient schedules, the requirement of a good recipient endometrium, and the inability to quarantine oocytes, which makes patient management difficult. One of the advantages of an egg-banking program is its impact on the management of patients as synchronization of donor and recipient's cycles can be performed long after retrieval of the oocytes. This allows a recipient to choose ova that are already banked where she knows ahead of time how many ova are available. The recipient also does not have the added burden of a potential cancelation of a retrieval cycle due to poor stimulation. Additionally, egg banks permit the more accurate and safer screening of donors and reduce costs per IVF cycle because several recipients can share oocytes from one donor and also eliminate the recipient's cycle cancelations due to low donor oocytes recovery.

The strong demand for oocyte donation is accompanied by the limited number of oocyte donors in our country for a variety of religious, ethical, regulatory, and financial reasons; thus, oocyte donation is not widely available. In addition, paid egg donation is not permitted in Brazil. This state of affairs has become a major problem for fertility specialists, but oocyte cryobanking helps to improve this situation. Additionally, oocyte cryobanking has a potential use in many other situations such as medical or social fertility preservation, ethical objections for embryo cryopreservation, and unavailable male gametes on the day of oocyte pick-up. Furthermore, donor egg banks contribute to these applications.

In summary, our findings suggest that vitrified oocytes result in similar pregnancy rates when compared to fresh oocytes with blastocyst transfer in an egg donation program. Allocating fewer ova to each recipient is a better use of valuable resources, as opposed to the traditional allocation of all ova from one donor to just one recipient. It may also allow for a quarantine time which may make the process safer.

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