J Assist Reprod Genet
DOI 10.1007/s10815-014-0270-8

ASSISTED REPRODUCTION TECHNOLOGIES

The efficiency of a donor-recipient program using infertile donors' egg cryo-banking: A Brazilian reality

Rita de Cássia Sávio Figueira · Amanda S. Setti · Daniela P. A. F. Braga · Assumpto Iaconelli Jr · Edson Borges Jr

Received: 27 February 2014 / Accepted: 1 June 2014

Abstract
Purpose To determine whether Brazilian egg donation treatment outcomes with oocytes donated from infertile couples are equivalent to those obtained worldwide with oocytes donated from fertile egg-donors.
Methods In this descriptive study, egg-donation cycles from 259 women, performed from January 2009 to July 2013, were evaluated. Oocytes were obtained from patients undergoing ICSI who decided to donate their surplus oocytes. We described the survival, fertilization, blastocyst, implantation and pregnancy rates obtained in our infertile donor-recipient program. In addition, we described the results obtained in previous published studies.
Results In our egg-donation program we obtained a fertilization rate of 72.9 %, a blastocyst formation rate of 53.2 %, an implantation rate of 31.1 % and the estimated clinical pregnancy rate per warmed oocyte was 5.4 %. The analyzed studies, performed between 2008 and 2013, included varying numbers of egg-donors (range: 20–600), warmed oocytes (range: 123–3826) and survival rates (range: 85.6–92.5 %). Fertilization rates ranged from 74.2 to 87.0 %, blastocyst formation rate ranged from 41.3 % to 68.0 %, implantation rates ranged from 24.7 % to 55.3 % and the clinical pregnancy rate per warmed oocyte ranged from 3.9 % to 9.8 %.
Conclusions New and reassuring information derived from our egg-donation program demonstrates outcomes similar to those reported for other egg donation programs.

Keywords Egg donation · ICSI · Oocyte · Pregnancy · Vitrification

Capsule Our Brazilian experience of freezing surplus eggs from IVF cycles of infertility patients yields satisfactory results that are not disparate from other reported presumed fertile egg donors, related to survival, fertilization, embryo development and pregnancy rates.

Rita de Cássia Sávio Figueira and Amanda Souza Setti contributed equally to this work.

R. d. C. S. Figueira · A. S. Setti · D. P. A. F. Braga · A. Iaconelli Jr · E. Borges Jr (✉)
Fertility – Centro de Fertilização Assistida, Av. Brigadeiro Luis Antonio 4545, São Paulo, SP 01401-002, Brazil
e-mail: science@sapientiae.org.br

R. d. C. S. Figueira · A. S. Setti
Faculdade de Ciências Médicas da Santa Casa de São Paulo, Rua Dr. Cesário Motta Junior 61, São Paulo, SP 01221-020, Brazil

A. S. Setti · D. P. A. F. Braga · A. Iaconelli Jr · E. Borges Jr
Instituto Sapientiae – Centro de Estudos e Pesquisa em Reprodução Humana Assistida, Rua Vieira Maciel 62, São Paulo, SP 04503-040, Brazil

Introduction

One of the most significant achievements in human assisted reproduction technology (ART) is the improvement in cryopreservation technique. With the introduction and refinement of vitrification, a cryopreservation process using high concentrations of cryoprotectant and ultra-rapid cooling, it is now possible to vitrify sperm, eggs and embryos without the formation of ice crystals (pl), with satisfactory post-warm survival rates [3,4]. Concomitant with the advent of successful oocyte cryopreservation, the use of oocyte donation has evolved rapidly as a vital instrument in ART [8].

The use of frozen donor eggs lightens some of the limitations intrinsic to fresh oocyte donation, such as the synchronization between donor and recipient cycles as well as the ability to quarantine oocytes [12]. In addition, the use of cryopreserved oocytes may provide women with more choices in selecting a donor and more flexibility in timing pregnancy and potentially reduce the cost [7,10].

The ever-increasing demand for egg-donors has led to the development of a new phenomenon: commercial "egg banks"(CEBs), entities that provide cryopreserved oocytes to

Published online: 22 June 2014

egg donation recipients [8]. Quaas et al. [8] have identified 3 operational models of CEB in the United States: (i) servicing a single in vitro fertilization (IVF) center; (ii) affiliated with a network of IVF centers; and (iii) shipping cryopreserved oocytes to any IVF facility. The CEBs aid the more efficient sharing of eggs between multiple recipients, improving the cost effectiveness through better use of resources [6]. In addition, the establishment of these banks considerably simplifies the logistics and means by which oocytes can be donated [1].

In Brazil, egg donation may not have profitable or commercial purposes. Therefore, in Brazilian context, surplus oocytes are obtained from a population of patients that are undergoing IVF treatments. Thus, the oocytes available for donation are originated from infertile couples. In May 2013, the voluntary donation of gametes was approved by the Brazilian Federal Medical Council meaning that fertile women can make altruistic egg donations. However, to this moment we have not seen a widespread use of that practice.

In the current study, we asked whether Brazilian egg-donation treatment outcomes obtained with cryopreserved oocytes donated from infertile patients are equivalent to those obtained worldwide with cryopreserved oocytes donated from fertile egg-donors.

Materials and methods

In this descriptive study, metaphase II (MII) oocytes were obtained from patients undergoing intracytoplasmic sperm injection (ICSI) cycle, in a private assisted fertilization center, who decided to donate their surplus oocytes. All patients were tested for syphilis, hepatitis B and C, HIV types 1 and 2, and HTLV types 1 and 2. Additionally, tests for the detection of Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis and Neisseria gonorrhoeae were also performed. Patients were allowed to donate their surplus oocytes if negative results were obtained in all tests. A written informed consent, in which patients agreed to donate their surplus oocytes and share the outcomes of their own cycles for research purposes, was obtained.

Controlled ovarian stimulation

In our service, ovarian stimulation was achieved by the administration of recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonist, as previously described [9]. Oocyte retrieval was performed using transvaginal ultrasound 35 h after the administration of recombinant hCG (rhCG; Ovidrel, Serono).

Endometrial preparation

In egg recipients, two units (100 mcg each) of 17 β-estradiol transdermal patches (Estradot, Noven Pharmaceuticals Inc, S.A., Miami, FL, USA) were applied, beginning on day 1 to day 5 of the cycle. Patches were replaced every 72 h. Ultrasound scans were performed every three days and, in the presence of an endometrial proliferative morphology and thickness of at least 7.5 mm, which were observed approximately on day 14 of the cycle, the administration of progesterone 600 mg was started (Utrogestan, Farmoquímica, Rio de Janeiro, RJ, Brazil) and maintained for 4 days, when embryo transfer was performed. Both E2 and P4 were administered concomitantly for 10 days after embryo transfer. E2 and P4 were suspended in the presence of a negative β-hCG test. In the presence of a positive β-hCG test, E2 and P4 were maintained until 6 and 12 weeks gestation, respectively.

Preparation of oocytes and vitrification/warming process

Retrieved oocytes were maintained in culture media (Global® for fertilization, LifeGlobal, Connecticut, USA) supplemented with 10 % protein supplement (LGPS, LifeGlobal, Connecticut, USA) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, Connecticut, USA) for 2 h prior to cumulus cell removal. Surrounding cumulus cells were removed after exposure to a HEPES buffered medium containing hyaluronidase (80 IU/mL, LifeGlobal, Connecticut, USA). The remaining cumulus cells were then mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

Denuded oocytes were assessed for nuclear status, and those observed to have released the first polar body were considered mature.

Oocytes were vitrified three hours after collection and cryo-stored. Both vitrification and the warming procedure were performed using the Cryotop method (8).

Intracytoplasmic sperm injection

Two hours after warming, ICSI was performed in a microinjection dish that was prepared with 4 μL droplets of buffered medium (Global® w/HEPES, LifeGlobal, Connecticut, USA) and covered with paraffin oil on an inverted microscope stage heated to 37.0±0.5 °C. Approximately 16 h after ICSI, fertilization was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were kept in a 50 μL drop of culture medium (Global®, LifeGlobal, Connecticut, USA) supplemented with 10 % protein supplement, covered with paraffin oil and stored in a humidified atmosphere under 7.5 % CO2 at 37ºC until transfer on day 5 of development.

A pregnancy test was performed 10 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed when the fetal heartbeat was detected.

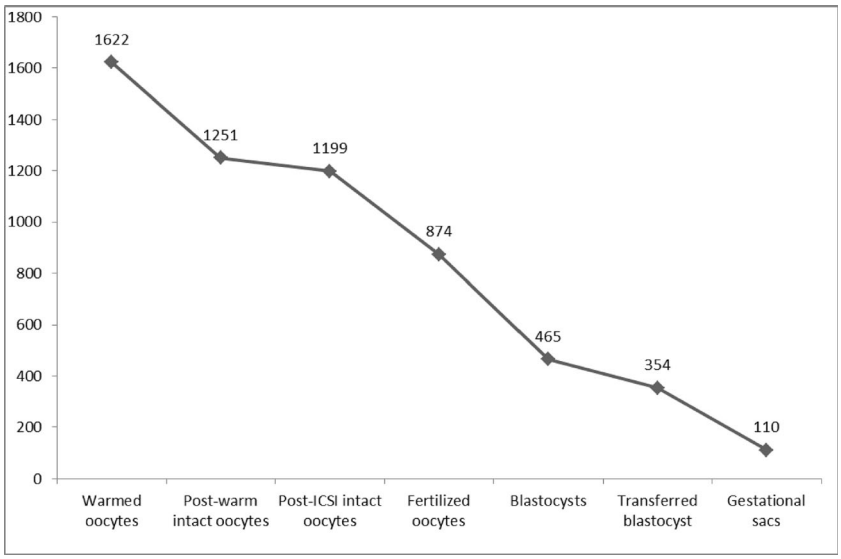

Fig. 1 Oocyte donation cycles' outcomes in our egg-donation program

Data analysis

We described the survival, fertilization, blastocyst, implantation and pregnancy rates obtained in our infertile donor-recipient program. In addition, we described the results obtained in previously published studies. To that end, we used the critical review of the literature performed by Edgar and Gook et al. [4], including articles published in English from January 2001 to April 2011, with particular emphasis on studies which include data on survival rates and ICSI outcomes after donor oocyte vitrification. We also performed a search in Pubmed to identify if any more articles were published from April 2011 to July 2013. The key words used were: human; oocyte; vitrification; cryopreservation and freezing.

As this study is a critical analysis describing the outcomes from conventional oocyte donation and the Brazilian oocyte donation cycles, no statistical analysis was performed.

Results

From January 2009 to July 2013 we have performed 259 egg donation cycles. Donated oocytes were obtained from 200 patients with a mean age±SD of 30.4±3.4 (range: 21–35 y-old). The distribution of infertility factors between egg-donors was: Polycystic ovarian syndrome (26.5 %, 53), tubal factor (20.0 %, 40/200), unexplained infertility (15.5 %, 31/200), endometriosis (15.0 %, 30/200), male factor (12.5 %, 25/200), and tubal and peritoneal factors (10.5 %, 21/200).

A total of 1622 donated oocytes were vitrified and warmed and 1251 survived (77.1 %).

Semen characteristics from egg recipients' male partners were as follows: sample volume: 3.3±1.7 ml, sperm count per ml: 66.2±56.5 x 10^6, total sperm motility 58.4±17.0 %, progressive sperm motility: 48.3±16.9 %, and normal sperm morphology: 3.0±1.6 %.

After sperm injection, 52 oocytes degenerated. We obtained a fertilization rate of 72.9 % (874/1199) with warm

Table 1 Study design, vitrification method and donors' characteristics in the analyzed studies and in our egg-donation program

Study	Year	Method	Design	Donor age range	Mean age
Cobo et al.	2008	Cryotop	Prospective randomized	18–35	26.7±3.6
Nagy et al.	2009	Cryotop	Prospective	<35	-
Cobo et al.	2010	Cryotop	Prospective randomized	<35	26.7±3.9
Garcia et al.	2011	Cryotop	Prospective	18–34	-
Trokoudes et al.	2011	Cryotop	Retrospective	22–35	29.3±3.6
Stoop et al.	2012	CBS high security	Prospective	<35	26.4±4.8
Solé et al.	2013	Cryotop	Prospective	18–34	26.1±4.3
Our experience	2009–2013	Cryotop	Retrospective	21–35	30.4±3.4

Table 2 Oocyte donation and survival rates in the analyzed studies and in our egg-donation program

Study	Year	Recipients (n)	Warmed oocytes (n)	Survival rate (%)
Cobo et al.	2008	30	231	224/231 (86.9)
Nagy et al.	2009	19	153	134/153 (89.0)
Cobo et al.	2010	600	3826	3039/3826 (92.5)
Garcia et al.	2011	119	283	253/283 (89.4)
Trokoudes et al.	2011	77	210	192/210 (91.4)
Stoop et al.	2012	20	123	111/123 (90.2)
Solé et al.	2013	198	-	- (85.6)
Our experience	2009–2013	259	1622	1251/1622 (77.1)

oocytes. Our blastocyst formation rate was 53.2 % (465/874). A total of 231 patients underwent embryo transfer (89.2 %) and the mean±SD number of transferred embryos was 1.56± 0.6. We obtained an implantation rate of 31.1 % (110/354) with blastocyst transfers (Fig. 1). The pregnancy rate per transferred cycle was 37.7 % (87/231), the miscarriage rate was 16.1 % (14/87) and the multiple pregnancy rate was 21.8 % (18/87, 13 twins and 5 triplets). Finally, the estimated clinical pregnancy rate per warmed oocyte was 5.4 % (87/1622) in our egg donation program.

We have analyzed seven studies dealing with oocyte vitrification for donation [1,2,5,6,10–12], two of them were prospective randomized, four were prospective and one was a retrospective study (Table 1). All the studies utilized the same concentration of cryoprotectant that is used in our program (15 % EG, 15 % DMSO, 0.5 M sucrose). Apart from one study [11], all the others used the same method of vitrification that is used in our program, the Cryotop (Table 1). The donor's mean age and range for each study are shown in Table 1.

The analyzed studies, performed between 2008 and 2013, included varying numbers of female donors (range: 20–600), warmed oocytes (range: 123–3826) and survival rates (range: 85.6–92.5 %) (Table 2). Fertilization rates ranged from 74.2 to 87.0 % in the published studies. Only three studies reported their blastocyst formation rate (range: 41.3 %–68.0 %). Four studies reported implantation rates ranging from 24.7 % to 39.9 % with cleavage embryo transfers; and three studies reported implantation rates ranging from 40.8 % to 55.3 % with blastocyst transfers. Finally, the estimated clinical pregnancy rate per warmed oocyte ranged from 3.9 % to 9.8 % (Table 3).

Discussion

Considering the Brazilian context of female gamete donation, we aimed to describe our practice and the results obtained in our model of egg-donation. The present study demonstrates that oocyte cryopreservation can be considered as a tool to provide highly successful outcomes even in an egg infertile-couple donor program. In addition to the high success rates, oocyte cryo-banking can help to eliminate some of the obstacles associated with the current fresh embryo transfer policy [6].

Our CEB is linked to a single ART center, at which all the procedures related to oocyte donation and ICSI are undertaken. The advantage of this model is the familiarity with the

Table 3 Oocyte donation cycles' outcomes in the analyzed studies and in our egg-donation program

Study	Year	Fertilization rate (%)	Blastocyst rate (%)	Implantation rate (%)		Clinical pregnancy/warmed oocyte
				Cleavage stage	Blastocyst stage	
Cobo et al.	2008	76.3	48.7	-	40.8	15/231 (6.5)
Nagy et al.	2009	87.0	68.0	-	55.3	15/153 (9.8)
Cobo et al.	2010	74.2	-	39.9	-	148/3826 (3.9)
Garcia et al.	2011	76.1	41.3	-	43.9	21/283 (7.4)
Trokoudes et al.	2011	84.4	-	24.7	-	20/210 (9.5)
Stoop et al.	2012	77.5	-	33.3	-	10/123 (8.1)
Solé et al.	2013	78.2	-	34.0	-	53/990 (5.4)
Our experience	2009–2013	72.9	53.2	-	31.1	87/1622 (5.4)

embryology unit; however, it may limit access to the CEB to a smaller pool of potential recipients and oocytes available [8].

Recently, Quaas et al. [8] have identified seven CEB's in the United States, possessing 1 to 8 years of experience. The number of recommended oocytes for oocyte donation in these CEB's ranged from 4 to 7, which is in accordance with our CEB, which has 5 years of experience and make use of 6 oocytes per cycle. Moreover, in these CEB's the cost for each donated oocyte was reported as approximately $2,225 ($1,500–$2,500) whereas in our CEB it was around $1,100. The majority of CEBs reported using vitrification.

Undoubtedly, having an efficient oocyte freezing/thawing technology is the most essential condition for a successful egg cryo-banking program. The success of oocyte cryopreservation has improved significantly over the past years, and preliminary data for safety are reassuring [7]. In general, data on the success of oocyte cryopreservation are limited to donor populations and infertile couples with supernumerary oocytes [7]. In our reality, the surplus oocytes come from infertile couples, therefore the design of the present study enabled us to analyze the impact of vitrification on the functionality of the oocyte and its capacity to produce an ongoing embryo and the subsequent pregnancy in an infertile-couple donors' egg cryo-banking.

Recently, a resolution of the Brazilian Federal Council of Medicine (CFM) on assisted reproduction determined that: (1) the egg recipient may cover part of the treatment of the egg donor in egg donation cycles, (2) the egg-donor must have up to 35 years of age, (3) a clinically healthy person can donate gametes provided that the donor has not received money in exchange, and (4) egg-donations cycles must be anonymous (donors and recipients should not have information about each other).

A limitation of this study is related to its design. As this study is a critical analysis describing the outcomes from conventional oocyte donation and the Brazilian oocyte donation cycles, no statistical or power analyses were performed.

In conclusion, this study suggests that vitrification is a safe and efficient method for cryopreservation of donated human oocytes originated from infertile couples, affecting neither the functionality of the oocytes nor their capacity to produce and give rise to embryos and pregnancies. Our Brazilian experience of freezing surplus eggs from IVF cycles of infertility patients yielded satisfactory results that are not disparate from other reported presumed fertile egg donors, related to survival, fertilization, embryo development and pregnancy rates. Moreover, the results obtained in our program confirm the potential of the Cryotop methodology in the establishment of infertile-couple donor egg banks.

References

1. Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 2008;89:1657–64.
2. Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod. 2010;25:2239–46.
3. Cohen J, Grudzinskas G, Johnson M. Welcome to the '100 % club'! Reprod Biomed Online. 2012;24:375–6.
4. Edgar DH, Gook DA. A critical appraisal of cryopreservation (slow cooling versus vitrification) of human oocytes and embryos. Hum Reprod Update. 2012;18:536–54.
5. Garcia JI, Noriega-Portella L, Noriega-Hoces L. Efficacy of oocyte vitrification combined with blastocyst stage transfer in an egg donation program. Hum Reprod. 2011;26:782–90.
6. Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertil Steril. 2009;92:520–6.
7. ASRM and SART practice committees report. Mature oocyte cryopreservation: a guideline. Fertil Steril. 2013;99:37–43.
8. Quaas AM, Melamed A, Chung K, Bendikson KA, Paulson RJ. Egg banking in the United States: current status of commercially available cryopreserved oocytes. Fertil Steril. 2013;99:827–31.
9. Setti AS, Cortezzi SS, Figueira Rde C, Martinhago CD, Braga DP, Iaconelli Jr A, et al. A chromosome 19 locus positively influences the number of retrieved oocytes during stimulated cycles in Brazilian women. J Assist Reprod Genet. 2012;29:443–9.
10. Sole M, Santalo J, Boada M, Clua E, Rodriguez I, Martinez F, et al. How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes. Hum Reprod. 2013;28:2087–92.
11. Stoop D, De Munck N, Jansen E, Platteau P, Van den Abbeel E, Verheyen G, et al. Clinical validation of a closed vitrification system in an oocyte-donation programme. Reprod Biomed Online. 2012;24:180–5.
12. Trokoudes KM, Pavlides C, Zhang X. Comparison outcome of fresh and vitrified donor oocytes in an egg-sharing donation program. Fertil Steril. 2011;95:1996–2000.