Reproductive BioMedicine Online (2013) 26, 595–602

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ARTICLE

Open versus closed oocyte vitrification system: a prospective randomized sibling-oocyte study

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Abstract Vitrification has been successfully applied in the cryopreservation of oocytes and embryos. It can be achieved either by direct (open system) or indirect (closed system) contact with liquid nitrogen. Unlike embryo vitrification, few reports have been published regarding oocyte vitrification in closed systems. In order to validate the effectiveness of a closed and aseptic vitrification approach for oocyte cryopreservation, a prospective, randomized study was performed. Sibling oocytes donated from the same donor were randomly and equally assigned into closed or open vitrification groups. A total of 75 vitrification–warming cycles were performed in each group. Apart from the survival rate (82.9% versus 91.0%, $P < 0.05$), no statistically significant differences were observed in pregnancy (β-human chorionic gonadotrophin positive) (42.7% versus 33.3%), clinical pregnancy (36.0% versus 28.0%), implantation (13.8% versus 10.1%), ongoing pregnancy (33.3% versus 24.0%) and live birth (36.0% versus 24.0%) rates between the closed and open groups, and 27 and 18 healthy babies were born, respectively. This study shows that the replacement of the open vitrification system by a closed system has no impact on clinical pregnancy and implantation rates. Therefore, the closed vitrification system provides an aseptic alternative to the open method for oocyte vitrification.

KEYWORDS: aseptic technique, closed carrier, cryopreservation, oocyte donation, oocyte vitrification, Vitrisafe

Introduction

Although the first pregnancies resulting from frozen oocytes were achieved in 1986 (al Hasani et al., 1987; Chen, 1986; Van Uem et al., 1987), cryopreservation of oocytes had many challenges to overcome in order to be established as a routine technique in IVF cycles. Slow freezing, the standard procedure for oocyte cryopreservation in IVF centres,

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.02.014

had very limited applications (preservation of female fertility, absence of sperm) due to lower pregnancy rates compared with fresh oocyte cycles (Gook and Edgar, 2007).

To overcome the drawbacks of the slow freezing procedure, a new method of oocyte cryopreservation, vitrification, has been developed that was efficient and simple with higher survival, embryonic (Cao et al., 2009; Smith et al., 2010) and pregnancy rates (Rezazadeh Valojerdi et al., 2009; Smith et al., 2010) than slow freezing. Nowadays, due to high success rates, vitrification of oocytes has replaced slow freezing in many IVF centres for women who want to preserve their fertility for medical or social reasons (Cobo et al., 2008; Homburg et al., 2009; Noyes et al., 2010; Porcu et al., 2008) or couples who do not want cryopreserve their embryos due to legal or ethical restrictions (Boldt et al., 2003; Kazem et al., 1995; Ragni et al., 2005) and it has became a valuable tool for egg donor banks (Cobo et al., 2010a; Nagy et al., 2009; Trokoudes et al., 2011). The birth of healthy infants has widely established vitrification as the technique of choice for oocyte cryopreservation (Chian et al., 2008; Cobo et al., 2010b; Noyes et al., 2009).

All together, vitrification procedures represent a heterogeneous group of methods sharing some principal characteristics, such as very high rates of sample cooling and elevated molarity in cryoprotectants. However, significant differences can be observed among protocols, especially regarding devices and sample storage systems.

Vitrification can be achieved by direct (open system; Desai et al., 2007; Kuwayama et al., 2005a; Mukaida et al., 2003; Son et al., 2003; Takahashi et al., 2005) or indirect (closed system; Vanderzwalmen et al., 2009, 2010; Van Landuyt et al., 2011) contact with liquid nitrogen and has been successfully used in the cryopreservation of embryos and oocytes (reviewed by Cobo et al., 2011). With the increasing concerns about liquid nitrogen contamination (Bielanski et al., 2000, 2003), closed loading systems that can achieve adequate cooling and warming rates have been investigated (Kuwayama et al., 2005a; Stachecki et al., 2008; Vanderzwalmen et al., 2009). Despite the rising applications of closed vitrification devices, an undeniable scepticism is evident towards the use of closed carriers for vitrifying oocytes. Unlike embryo vitrification, few reports have been published regarding oocyte vitrification in closed systems (Paffoni et al., 2011; Stoop et al., 2012).

The open vitrification protocols use very high cooling rates in combination with a high concentration of cryoprotectants. As a result, ice crystal formation is successfully avoided (Vajta and Nagy, 2006). The closed vitrification protocols require the loading of samples into devices that are sealed before the vitrification process in order to avoid direct contact between oocytes and liquid nitrogen. The thermal isolation of samples in this way slows the cooling rate. The key to overcome this shortcoming is to find the optimal balance between the speed of cooling and warming and the necessary concentration of cryoprotectant for each step of exposure in order to reach the vitrified state without inducing osmotic-swelling stress.

In order to validate the effectiveness of a closed and aseptic vitrification approach for oocyte cryopreservation, a prospective randomized study was set up comparing the open and the closed vitrification techniques in a population of oocyte recipients sharing sibling oocytes donated from the same donor. Oocyte fertilization rates after intracytoplasmic sperm injection (ICSI) and implantation, pregnancy and live birth rates per transfer cycle were evaluated as primary outcomes.

Materials and methods

A prospective, randomized study was performed. Sibling oocytes donated from the same donor were randomly and equally assigned into the closed group (oocytes vitrified in a closed system) or the open group (oocytes vitrified in an open system). Later on, these oocytes were warmed and donated to recipients who were randomly allocated to receive vitrified oocytes either from the closed or open group. All cases included in the study were part of the satellite oocyte donation programme. Therefore, the recruitment and stimulation of the donors as well as the retrieval and vitrification of oocytes were performed in the central IVF unit, while the vitrified oocytes were warmed, fertilized, cultured and transferred in the satellite IVF unit in another city. The study was approved by the Institutional Review Board (Ref. no. 2/2007, granted 19 January 2007) and informed consent was obtained from all women.

From February 2007 to December 2010, 78 oocyte donors were evaluated at the Iakentro IVF centre. A detailed medical history was taken. The oocyte donors were $\leq$32 years old, had body mass index $\leq$30 kg/m^2, regular menstrual cycles of 25–35 days, two normal ovaries based on transvaginal scan findings, no polycystic ovaries, no endometriosis, no gynaecological or medical disorders and agreed to donate their oocytes for treatment anonymously and altruistically. Oocyte donors were already of known fertility and good ovarian response. Blood sample was collected for karyotype and screening for previous viral infection (hepatitis B and C, human immunodeficiency virus, syphilis) thalassaemia and cystic fibrosis. A single attempt was included for each donor. When lower than expected ovarian response observed and less than 10 oocytes were retrieved, the donors (n = 3) were not included in the statistical analysis.

A total of 150 recipients matched with their donors were included in the study. All recipients were $\leq$50 years old without history of endometriosis. Women with a previous history of failed oocyte donation cycle were excluded from the study. The recipients and their partners underwent blood screening similar to the donors, while a hysterosalpingogram and a diagnostic hysteroscopy had eliminated cases presenting hydrosalpinx or intrauterine pathology. The recipients had a mock transfer in a cycle previous to IVF and if difficulty was encountered a cervical dilatation was performed (Prapas et al., 2004). Recipients were randomized into two groups that received either oocytes vitrified in a closed system (n = 75) or oocytes vitrified in an open system (n = 75), according to a computer-generated allocation sequence prepared by a statistician. All patients entered the study once.