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ARTICLE

Effects of two vitrification protocols on the developmental potential of human mature oocytes

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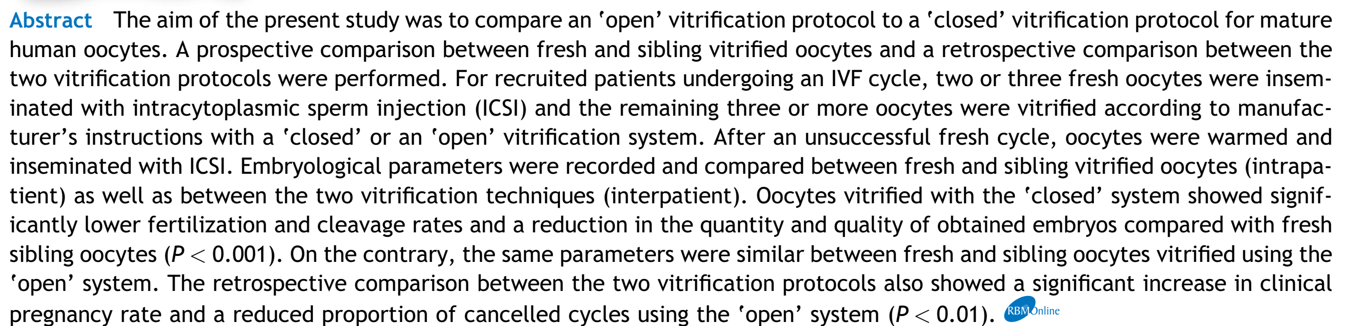

Abstract The aim of the present study was to compare an 'open' vitrification protocol to a 'closed' vitrification protocol for mature human oocytes. A prospective comparison between fresh and sibling vitrified oocytes and a retrospective comparison between the two vitrification protocols were performed. For recruited patients undergoing an IVF cycle, two or three fresh oocytes were inseminated with intracytoplasmic sperm injection (ICSI) and the remaining three or more oocytes were vitrified according to manufacturer's instructions with a 'closed' or an 'open' vitrification system. After an unsuccessful fresh cycle, oocytes were warmed and inseminated with ICSI. Embryological parameters were recorded and compared between fresh and sibling vitrified oocytes (intrapatient) as well as between the two vitrification techniques (interpatient). Oocytes vitrified with the 'closed' system showed significantly lower fertilization and cleavage rates and a reduction in the quantity and quality of obtained embryos compared with fresh sibling oocytes ($P < 0.001$). On the contrary, the same parameters were similar between fresh and sibling oocytes vitrified using the 'open' system. The retrospective comparison between the two vitrification protocols also showed a significant increase in clinical pregnancy rate and a reduced proportion of cancelled cycles using the 'open' system ($P < 0.01$). RBMOnline

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KEYWORDS: CryoTip, CryoTop, oocyte cryopreservation, vitrification

Introduction

Oocyte cryopreservation has gained an increasing role in assisted reproductive technologies over the last decades. Some authors report oocyte cryopreservation as a main strategy for fertility preservation (Noyes et al., 2010; Porcu et al., 2008) or oocyte donation programmes (Cobo et al., 2010; Nagy et al., 2009) but its principal application deals

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with supernumerary oocytes during IVF procedures, especially where particular legal or ethical contests are in force (Boldt et al., 2003; Porcu et al., 2000). Several strategies are available for oocyte cryopreservation, mainly classifiable as slow-freezing or vitrification protocols (Jain and Paulson, 2006; Varghese et al., 2009). Vitrification has been reported to be a promising technique and its application is under validation in several clinics. Both strategies gained recent developments, which significantly improved results with respect to earlier applications (Bianchi et al., 2007; Cobo et al., 2008; De Santis et al., 2007; Fabbri et al., 2001; Fadini et al., 2009; Kuwayama et al., 2005b; Nagy et al., 2009; Quintans et al., 2002; Rienzi et al., 2010). Recent data suggest that pregnancies and infants conceived following oocyte vitrification are not associated with increased risk of adverse obstetric and perinatal outcomes (Chian et al., 2008; 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 cryopreservation cocktails. However, significant differences can be observed among protocols, especially regarding devices and sample storage systems.

Vitrification can be achieved by direct or indirect contact with liquid nitrogen and has been successfully used in the cryopreservation of oocytes, embryos and blastocysts (Desai et al., 2007; Mukaida et al., 2003; Oktay et al., 2006). With the increasing concerns about liquid nitrogen contamination, closed loading systems that can achieve adequate cooling and warming rates have been investigated (Bielanski et al., 2000; Kuwayama et al., 2005a). Despite the rising applications of closed vitrification devices, there are no extensive published studies on the use of closed vitrification systems for human oocytes.

The primary aim of this study was to compare embryological parameters obtained from fresh and sibling vitrified oocytes using two vitrification protocols based on a 'closed' system (CryoTip; Irvine Scientific, Santa Ana, CA, USA) and an open system (CryoTop; Kitazato, Fujinomiya, Japan) (Kuwayama et al., 2005a).

Materials and methods

The present study was conducted at the Infertility Unit of the Department of Obstetrics and Gynaecology, Fondazione Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy), on patients enrolled between June 2007 and April 2009. Patients undergoing intracytoplasmic sperm injection (ICSI) in the fresh cycle, from which at least three supernumerary oocytes were retrieved, were offered the opportunity to have their oocytes vitrified for subsequent use. During fresh cycles, two or three oocytes were inseminated per patient, according to national law restrictions, clinical conditions and the couples' wishes. In the first phase of the study, from June 2007 to May 2008, oocytes were vitrified using the CryoTip (closed) method, while in the second period, the vitrification procedure was based on the CryoTop (open) protocol. Approval for the application of both vitrification protocols was obtained by the local institutional review board. All participating women gave their informed consent.

A clinical pregnancy was defined as the demonstration of an intrauterine gestational sac with yolk sac by transvaginal ultrasonography.

Oocytes

Fresh oocytes were obtained following ovarian stimulation using a long protocol with a gonadotrophin-releasing hormone (GnRH) analogue or a protocol using a GnRH antagonist as previously reported (Paffoni et al., 2008). Oocyte retrieval was performed via transvaginal aspiration of the follicles 36 ± 0.5 h after human chorionic gonadotrophin (HCG) administration. Oocyte–cumulus complexes were washed in flushing medium and transferred to IVF medium (Quinn's Advantage Protein Plus Fertilization Medium; Sage, Trumbull, CT, USA). After an incubation of 2 ± 0.5 h at 37°C in an atmosphere of 5.5% CO_2 in air, cumulus cells were completely removed from all oocytes by pipetting them through 170 μm internal diameter pipettes (Flexipet; Cook, Bloomington, IN, USA) after a brief exposure to 40 IU/ml of hyaluronidase (Sage) in IVF medium (Sage). A maximum of three metaphase II (MII) fresh oocytes were destined to routine ICSI procedure and subsequent embryo transfer. Patients were included in the study only when a cohort of MII oocytes with uniform characteristics regarding morphology, texture, granularity of the cytoplasm and polar body appearance were available. In these cases, oocytes were included in the study and allocated to ICSI (two or three oocytes) or vitrification (three or more oocytes). Conversely, oocytes with a morphological appearance different from the main cohort were excluded from the study group. Vitrification of supernumerary oocytes was completed within 40 h from HCG administration.

Oocyte vitrification and warming

Two different commercial kits for vitrification were used according to the manufacturers' instructions.

Vitrification Freeze/Thaw Kit (Vit Kit-Freeze/Thaw; Irvine Scientific) for vitrification procedures performed between June 2007 and May 2008 (CryoTip, closed method) and CryoTop Safety Kit (Kitazato) for vitrification procedures performed between June 2008 and April 2009 (CryoTop, open method).

The basal medium for vitrification and warming solutions was modified culture medium M-199 containing HEPES buffer and serum substitute supplement. For both procedures, warmed oocytes were checked for survival and cultured in standard conditions at 37°C (6% CO_2) until the ICSI procedure (2 h). Warmed oocytes were considered as not having survived if lysed, extensively vacuolized or somehow damaged in cytoplasmic or extracytoplasmic structures. Warming cycles were performed 2–6 months after unsuccessful ICSI treatment with fresh oocytes.

CryoTip (closed)

CryoTip vitrification procedures were performed at room temperature, as described elsewhere (Kuwayama et al., 2005b). Up to three oocytes were placed into modified human tubal fluid-HEPES medium (Quinn's Advantage HEPES Medium; Sage) for 1 min. Oocytes were then gradually exposed to an

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