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Outcomes of immature oocytes collected from ovarian tissue for cryopreservation in adult and prepubertal patients

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KEY MESSAGE

Oocyte in-vitro maturation and vitrification procedures after ex-vivo collection from ovarian tissue could be considered as an additional option for fertility preservation.

A B S T R A C T

The efficiency of oocyte in-vitro maturation (IVM) and vitrification procedures after ex-vivo collection from ovarian tissue were assessed according to patient age, number of retrieved oocytes and tissue transport conditions. The combined procedure was performed in 136 patients: 130 adults (mean 27.6 ± 5.6 years) and six prepubertal girls (mean 8.7 ± 2.3 years). A higher mean number of oocytes were collected in girls compared with adults (11.5 ± 8.0 versus 3.8 ± 4.2, respectively, $P < 0.001$) but the percentage of degenerated oocytes was significantly higher in girls (35.5% versus 17.1%, respectively, $P < 0.001$). IVM rates were significantly lower in prepubertal than postpubertal population (10.3% versus 28.1%, $P = 0.002$). In adults, a negative correlation was observed between number of retrieved oocytes and age ($P = 0.002$; $r = -0.271$); the correlation was positive between anti-Müllerian hormone (AMH) and number of collected oocytes ($P = 0.002$; $r = 0.264$). IVM rates were not correlated with AMH levels ($r = 0.06$) or age ($r = -0.033$). At present, nine oocytes and one embryo have been warmed in four patients and one biochemical pregnancy obtained. This suggests the combined procedure could be an additional option for fertility preservation.

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Introduction

New therapeutic strategies for cancer have led to great improvements in cure and survival rates in young cancer patients (Howlader et al., 2011). However, antineoplastic therapies may be associated with significant gonadotoxicity and young cancer survivors usually express a strong desire to preserve childbearing potential (Letourneau et al., 2012; Meirow et al., 2010; Scanlon et al., 2012). Therefore, fertility preservation is an emerging field that offers new hope for women and girls treated for cancer before or during their reproductive years. All oncological guidelines address this issue by recommending that young patients be informed regarding possible fertility risks associated with treatment and that they be referred to a fertility centre if required (Knight et al., 2015; Peccatori *et al.*, 2013). Different approaches for fertility preservation in women diagnosed with cancer have been reported (Chung *et al.*, 2013; Demeestere et al., 2013; Jeruss and Woodruff, 2009). Among them, ovarian tissue cryopreservation (OTC) has emerged as an alternative to the standard oocyte or embryo vitrification option. This experimental procedure has several advantages as it can be rapidly performed without ovarian stimulation, even in prepubertal girls or when chemotherapy has already started. It also offers the possibility of storing a large number of follicles within ovarian fragments that can be later transplanted to restore fertility (Meirow, 2008; Meirow et al., 2014; Rodriguez-Wallberg and Oktay, 2012). Since the first live birth after transplantation of cryopreserved ovarian tissue (Donnez et al., 2004), this technique has led to the birth of over 80 healthy babies and has an estimated success rate of 25–30% (Donnez and Dolmans, 2015; Jensen et al., 2015; Meirow et al., 2016; Van der Ven *et al.*, 2016).

Despite these encouraging results, some advanced cancers or disseminated diseases, such as leukaemia, are considered to pose a high risk of recurrence after ovarian tissue transplantation due to the potential presence of neoplastic cells within the tissue (Bastings et al., 2013). Moreover, progress must still be made to improve the success rate of the procedure. OTC preserves the primordial and primary follicles, while the antral follicles, which contain immature growing oocytes, do not survive the procedure (Gosden, 2002). During the process, the ovarian cortex is dissected and frozen, while the medulla containing all the growing follicles is usually discarded or stored to assess the presence of neoplastic cells. These immature oocytes represent an additional source of gametes after in-vitro maturation (IVM). The vitrified oocytes can be directly used later for IVF and embryo transfer without any risk of disease transmission.

At present, only a few studies have investigated the feasibility of a combined option that preserves both ovarian tissue and in-vitro matured oocytes (Abir et al., 2016; Fadini et al., 2012; Fasano et al., 2011; Revel et al., 2009). Three live births from in-vitro matured oocytes retrieved during ovarian tissue processing for cryopreservation were recently reported (Prasath et al., 2014; Segers et al., 2015; Uzelac et al., 2015) but the factors involved in the success of the procedure, including oocyte collection, IVM and live birth rates are still unknown.

Previous studies have shown that the combined procedure is feasible in paediatric patients, including prepubertal girls (Abir et al., 2016; Fasano et al., 2011; Revel et al., 2009). However, the viability, developmental competence and fertilization potential of oocytes from paediatric patients must be confirmed as no oocytes have been warmed for fertility restoration yet.

Proper handling of ovarian tissue that originates from an operating room not immediately adjacent to the embryology laboratory constitutes another challenge. Animal studies have confirmed that follicles are subject to acute ischaemia during transport, leading to adverse changes such as a decrease in glucose concentration or increase in apoptotic index in granulosa cells (Ferreira et al., 2001; Pedersen et al., 2004; Sakamoto et al., 2006; Wongsrikeao et al., 2005). To avoid these adverse effects, time of transport and processing before cryopreservation should be as short as possible. However, the effects of timing or temperature during tissue transport on IVM rates have not been carefully investigated. One strategy used to reduce ischaemic damage during ovary transport is to decrease the median transport temperature. Animal studies have suggested that lowering the storage temperature of ovaries during transport is required to maintain the developmental competence of oocytes (Matsushita et al., 2004). However, hardening of the zona pellucida, impaired microtubules and cytoskeleton and damaged cytoplasmic membranes have also been described in oocytes exposed to low temperatures (Bianchi et al., 2014; Lee et al., 2006; Mandelbaum et al., 2004). Only one study reported oocyte maturation rates according to transport time in humans and did not show any negative impact of longer transport times (Yin et al., 2016). Therefore, the impact of the timing of ovarian transport on the developmental competence of oocytes remains uncertain and needs further evaluation.

The present study assessed the efficiency of oocyte IVM and vitrification procedures after ex-vivo collection from ovarian tissue according to the age of the patients, the number of retrieved oocytes, and the timing of tissue transport.

Materials and methods

Ethical approval

The cryopreservation of ovarian tissue, including immature oocyte collection when feasible, was approved by the central Ethical Committee from Erasme Hospital on 16 September 2008 (reference number: P2004/122). All patients or parents were informed about the procedure and provided written informed consent.

Cryopreservation of ovarian tissue

Ovarian tissue biopsies or unilateral oophorectomy were carried out at Erasme Hospital or in one of the participating Belgian centres. Unilateral oophorectomy was usually proposed for prepubertal patients, women who were at major risk of premature ovarian failure, or in cases where it was surgically indicated (Imbert et al., 2014). Tissues were transported in Leibovitz L-15 medium (Life Technologies, Merelbeke, Belgium) at 4°C and processed in the IVF laboratory at between 15 min (on-site) and a maximum of 3 h from removal. Three groups were compared according to the duration of transport (15 min, less than 1 h and between 1 h and 3 h).

Ovarian tissue freezing procedures have been described elsewhere (Demeestere et al., 2003; Imbert et al., 2014). Briefly, the medulla was gently removed and the cortex was cut into small fragments (≈5 × 5 × 1–2 mm). Ovarian fragments were incubated in a cryoprotectant solution containing 1.5 M dimethyl sulfoxide (DMSO) and 0.1 M sucrose for 30 min and then frozen using a slow freezing protocol in a programmable freezing machine (Kryo 360, Planer, UK) before storage in liquid nitrogen.

Cumulus–oocyte–complex (COC) retrieval and IVM procedures have been described elsewhere (Fasano et al., 2011). All visible antral

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