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COMMENTARY

Human ovarian tissue cryopreservation: quality of follicles as a criteria of effectiveness

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Abstract Experiments comparing vitrification and conventional freezing of mammalian ovarian tissue show that vitrification can also guarantee the storage of viable follicles after warming, but conventional freezing is more effective. The central goal of cryotechnology is the preservation of intact follicles. This article presents a critical opinion about the normality of follicles after vitrification of human ovarian tissue and microbial contamination as a result of direct contact of this tissue with liquid nitrogen at vitrification.

KEYWORDS: contamination, follicles, freezing, ovarian tissue, vitrification

Cryopreservation of ovarian tissue can be performed using one of two methods: conventional (slow) freezing and cryopreservation by direct plunging into liquid nitrogen (so-called vitrification or rapid freezing). Comparative investigations of vitrification and conventional freezing performed on mammalian ovarian tissue are limited, and authors present different conclusions.

Such investigations were performed on human, bovine and porcine ovarian tissues and it was concluded that conventional freezing is the method of choice for the cryopreservation of ovarian fragments, resulting in a much better preservation of all types of follicles than vitrification (Gandolfi et al., 2006).

Recently, results of comparative experiments performed on vitrified and conventionally frozen human ovarian tissue with long-term in-vitro culture of tissue fragments after warming were published (Li et al., 2007). The authors believe that the original methodology of vitrification by direct dropping of vitrification solution into liquid nitrogen is as effective as conventional freezing.

The appearance and quality of follicles allows a prognosis of the possibility for a woman to restore her reproductive function. In fact, vitrification is technologically promising, it is simpler and one cryocycle is less time-consuming and cheaper than the conventional freezing method. However, the central goal of cryotechnology is the preservation of intact follicles. Results have shown that vitrification can guarantee the storage of viable follicles after warming, but conventional freezing is more effective (Isachenko et al., 2007, 2009).

Vitrification of human ovarian tissue using the so-called solid-surface method has been reported (Huang et al., 2008). The authors noted that histological observation performed after cryopreservation and in-vitro culture of tissue shows that the majority of primordial follicles remained intact and that the described methods had no statistically significant destructive effect for primordial follicles. The authors concluded that vitrification is effective, simple and inexpensive. However, analysis of **Figure 1** in Huang et al. (2008) throws doubt on the correctness of the authors' conclusions. The presented vitrified oocytes are far from normal (Paynter et al., 1999). Huang et al. (2008) have presented oocytes with vacuolated (A1, **Figure 1**) and partly destructed (B2, **Figure 1**) cytoplasm and picnotic nucleus (A1, **Figure 1**).

Histological micrographs give evidence of the developmental capacity of ovarian fragments after slow conventional freezing, fast thawing and in-vitro culture in large volumes of medium, under constant agitation (Isachenko

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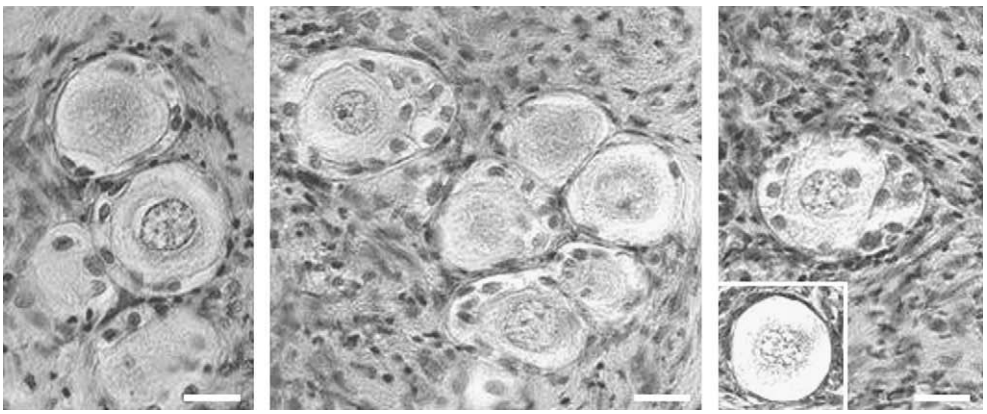

Figure 1 Histological micrographs of follicles from ovarian pieces of three different patients after conventional freezing and culture (Isachenko et al., 2006, 2007, 2008, 2009). Bar = 10 μm.

et al., 2006, 2008). The micrographs indicate whether there is a good possibility of a pregnancy of the patient after transplantation of the tissue: follicles developed quite normally (**Figure 1**).

Vitrification methods that require direct plunging of ovarian tissue into liquid nitrogen and immersion into prewarmed solution for removal of cryoprotectants have been described (Keros et al., 2009; Wang et al., 2008). However, these protocols of vitrification cannot be recommended for the use in medical practice because these protocols presuppose a direct contact with liquid nitrogen, which is a potential source of microbial contamination.

In fact, any technology in reproductive biology and especially in a medical setting must ensure and guarantee the full protection of biological objects from micro-organisms (Charles and Sire, 1971). Liquid nitrogen, which is used for the storage of frozen material, can be a source of contamination by these micro-organisms (Bielanski et al., 2003; Tedder et al., 1995). Filtration or ultraviolet treatment of liquid nitrogen cannot guarantee the absence of contamination of biological material by viruses (Tedder et al., 1995). The two above-mentioned vitrification methodologies with high effectiveness are based on the direct cooling of cells in liquid nitrogen. In contrast, conventional freezing completely avoids direct contact between liquid nitrogen and the tissue.

In conclusion, for the cryopreservation of human ovarian tissue, conventional freezing is more promising than vitrification at present.

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