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SHORT COMMUNICATION

A slowly reabsorbed, echogenic surgical thread provides a long-lasting ultrasound-detectable marker of grafted ovarian tissue

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Alberto Revelli, MD PhD was born in Torino, Italy in 1959. He obtained his degree in medicine in 1984, his specialization in obstetrics and gynaecology in 1988 and his PhD in obstetrical and gynaecological sciences in 1996 at the University of Torino, Italy. Since July 1999 he has worked as a researcher in obstetrics and gynaecology at S. Anna Hospital, University of Torino, and in 2006 he was nominated aggregate professor in obstetrics and gynecology in the same University. Since 1999 he had taught reproductive biotechnology and IVF. Presently he is also head of the physiopathology of reproduction and IVF unit at S. Anna Hospital and IVF consultant at LIVET (Italo-Swedish Clinic for Assisted Reproduction) in Torino.

Abstract This communication reports a novel technical solution for the orthotopic transplant of cryostored–thawed ovarian tissue. The described technique was applied to three young women with iatrogenic ovarian failure. An echogenic thread that is reabsorbed after 6 months was used to fasten the thawed ovarian small fragments before grafting them onto the atrophic ovary. This technical solution made it possible to avoid the loss of small tissue pieces during laparoscopic grafting as well as to precisely localize the grafted tissue by transvaginal ultrasound during the following months. The precise localization of the grafted tissue was particularly helpful when its revascularization and functional recovery were followed up using, respectively, colour Doppler and transvaginal follicle growth examination. In conclusion, the use of a slowly reabsorbed, ultrasound-detectable surgical thread as an ultrasound-detectable marker able to improve the localization of the exact site at which ovarian tissue was grafted is proposed.

KEYWORDS: colour Doppler ultrasound, fertility preservation, laparoscopic surgery, ovarian tissue cryopreservation, ovarian tissue grafting, surgical technique

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The maintenance of reproductive competence is a major concern for young women affected by cancer and needing gonadotoxic treatments or at high risk of premature ovarian insufficiency for other causes. Among the current options to preserve the fertility of young females at high risk of premature ovarian insufficiency, ovarian tissue cryopreservation is the only option for prepubertal subjects and is the preferred one for patients who cannot delay the beginning of oncostatic treatments or for whom a hormonal ovarian stimulation to collect oocytes is not recommended. Post-pubertal patients may also undergo retrieval of immature oocytes, in-vitro maturation, IVF and embryo transfer (Chian et al., 2009; Rao et al., 2004), but until now in-vitro maturation has been rarely applied to preserve fertility in oncological patients (Demirtas et al., 2008). Indeed ovarian tissue can be harvested during laparoscopy, a surgical procedure that can be easily organized in a very short time; this represents the best solution for patients who must start their chemotherapy or radiation treatment straightaway. Moreover, ovarian tissue cryopreservation does not require any hormonal stimulation, thus avoiding exposure to high concentrations of serum oestradiol of women with oestrogen-responsive tumours.

Although the entire ovary with its vascular pedicle can be taken away, frozen and cryostored, the better permeability of tissue samples of a small size to cryoprotectants has led to a preferable technique in which repeated biopsies of ovarian cortex are performed and several small fragments of ovarian tissue are used for cryopreservation. Patients who after time achieve complete recovery from the disease, but complain of iatrogenic premature ovarian insufficiency, may have fertility as well as the ovarian endocrine activity restored after receiving back their own thawed ovarian tissue. The ovarian cortex may be grafted either at the orthotopic site (inside the pelvis) or at various heterotopic sites (e.g. the abdominal subcutaneous fat tissue). During the orthotopic transplant, the thawed ovarian tissue is grafted either on the atrophic remnant ovary (Donnez et al., 2006; Meirow et al., 2005; Sanchez-Serrano et al., 2010; Silber et al., 2008, 2010) or, if the ovary had been totally removed, in a peritoneal pouch near the ovarian fossa (Donnez et al., 2006, 2012; Revel et al., 2011). Some authors have transplanted the thawed fragments both on the ovary and on the peritoneum (Andersen et al., 2008; Demeestere et al., 2006, 2010; Donnez et al., 2010; Revelli et al., 2013; Roux et al., 2010), whereas others have grafted it only on the peritoneum even if the athropic ovaries where still available (Dittrich et al., 2012).

The orthotopic transplant may be performed using either laparotomy (Meirow et al., 2005; Revel et al., 2011), laparoscopy (Demeestere et al., 2006, 2010; Dittrich et al., 2012; Donnez et al., 2004, 2006, 2010, 2012; Revelli et al., 2013; Roux et al., 2010) or a combination of both (Andersen et al., 2008; Sanchez-Serrano et al., 2010; Silber et al., 2008). The major problem of transplanting thawed ovarian tissue is the massive follicular loss linked to ischaemia: ovarian tissue, in fact, is grafted without any reconstruction of the vascular supply. In order to limit ischaemic damage, ovarian fragments must be placed on a tissue where an active neo-angiogenesis is taking place and must be maintained in strict contact to it using a surgical suture.

Ovarian cortex orthotopic transplantation has proven to be effective both for the restoration of endocrine function and for fertility, being a technique that has resulted in around 25 live births and some ongoing pregnancies to date. Donnez et al. (2004) were the first to describe a live birth after ovarian tissue cryopreservation and orthotopic transplantation; they suggested a two-step surgical approach, including a first laparoscopy to decorticate atrophic ovaries, prepare the peritoneal window and induce neo-angiogenesis and another laparoscopy some days later to graft the thawed tissue. This two-step procedure was adopted by other groups (Demeestere et al., 2006, 2010; Revelli et al., 2013; Roux et al., 2010) with some variants: Demeestere et al. (2006) associated an heterotopic abdominal subcutaneous transplant to promote a quicker recovery of endocrine function, whereas Roux et al. (2010) placed some thawed fragments on the ovaries and the peritoneum even during the first, preparatory laparoscopy in order to better stimulate neo-angiogenesis.

Most authors have transplanted thin ovarian strips (Meirow et al., 2005; Oktay et al., 2001; Roux et al., 2010) or small tissue pieces (Andersen et al., 2008; Demeestere et al., 2006; Donnez et al., 2004; Piver et al., 2009; Revelli et al., 2013): both strategies were shown to restore endocrine function and ovulation. Cutting the ovarian tissue into small fragments allows an optimal perfusion by cryoprotectants, but on the other hand it may cause the loss of part of the tissue during laparoscopic grafting. To minimize the risk of tissue loss in the peritoneal cavity, the small tissue pieces must be fastened together before being placed at the grafting site; then, they must be fixed to it in order to promote a valid and quick revascularization with limited ischaemic damage. Different techniques have been proposed to fix the ovarian tissue to the grafting site: Oktay et al. (2001) included the tissue fragments in a Surgicell matrix, Meirow et al. (2005) injected some very small tissue fragments under the ovarian cortex, Andersen et al. (2008) inserted the fragments into two pockets created at both sides of the atrophic ovary, Sanchez-Serrano et al. (2010) used a fibrin glue to keep the fragments attached to the atrophic ovaries and Donnez et al. (2012) covered the grafted tissue with an Interceed membrane.

The current group performed three transplantations of thawed ovarian tissue, resulting in the restoration of endocrine function and ovulation in all patients and a spontaneous pregnancy, with a healthy live birth, in one of them (Revelli et al., 2013). In the authors' opinion, it is of pivotal importance to guarantee a stable adhesion of the thawed tissue to the grafting site and also to be able to detect with precision whether and when revascularization of the grafted tissue occurs. In this study centre's experience, a precocious (in the first 2 weeks after grafting) invasion of the grafted tissue by newly formed blood vessels, in fact, is highly predictive of a prompt functional recovery with ovulation cycles; to correctly assess revascularization by colour Doppler, the site where the tissue has been grafted must be localized with high precision.

In the three cases, the thawed ovarian tissue was laparoscopically transplanted at the orthotopic site using the two-step surgical technique (Donnez et al., 2004). The novel technical solution that was introduced consists of an echogenic suture thread that persists for 6 months before being