Optimizing the outcomes of ovarian tissue transplantation

In young women suffering from a pathology requiring gonadotoxic treatment, such as chemotherapy by alkylating agents or pelvic radiotherapy, the success of ovarian tissue transplantation is related to the ovarian tissue quality, whether the condition is benign or cancerous.

There are several steps in the preparation of the ovarian biopsies before the freezing process. Regarding the biopsy thickness, it has been shown that a 1–1.5-mm-thick piece of ovarian tissue is optimal because primordial follicles are usually not observed further than 0.8 mm from the mesothelium.

In this issue of *Fertility and Sterility*, Herraiz et al. compared outcomes of three decortication methods, including scratching with scalpel blade, cutting with microsurgical scissors, and separation with slicer, on stromal and follicular viability (1). Interestingly, no previous comparative studies have been performed even though the removal of the remaining medulla is a crucial step to improve the cryoprotectant agent infiltration into the ovarian cortex during the freezing process.

In their in vitro experiments, although there was no significant difference in the percentage of morphologically normal follicles between the different groups, the authors observed that slicer and microsurgical scissors induced an inhibition in the Hippo pathway, ending in massive follicular activation or burnout.

It is important to avoid this phenomenon observed during ovarian tissue cryopreservation and transplantation. Indeed, this massive activation leads to an important depletion of the follicular reserve, because once the follicles are activated, they progress to atresia because they are not surviving to the cryopreservation, thawing, and transplantation. Therefore, because avoiding follicular burnout is considered to be a key factor in the success of ovarian tissue transplantation, ovarian tissue preparation by blade seems to be promising, because this technique does not disrupt the Hippo pathway.

Until now, the follicular burnout has usually been associated with the ovarian tissue fragmentation itself, the ischemia due to avascular transplantation, and the decrease in the level of anti-müllerian hormone after the graft, and not to the method of decortication. However, it seems that activation is also increased when ovarian strips are thinner.

Regarding the stromal fibrosis, Herraiz et al. demonstrated that decortication with the slicer is associated with a reduced fibrotic area compared with the two other techniques, independently from the type of freezing (slow freezing vs. vitrification).

Finally, considering advantages and disadvantages of scratching with scalpel blade, cutting with microsurgical scissors, and separation with slicer, the authors suggested that decortication by a blade should be a good candidate technique.

Even if nowadays the success of ovarian tissue transplantation seems similar to that obtained with oocyte vitrification, the result of assisted reproduction techniques after grafting is still lower than that in the general population undergoing IVF. This is linked to the low ovarian reserve resulting from the follicle loss observed during cryopreservation and after transplantation (2).

Follicle loss after xenotransplantation of fresh and frozen-thawed human ovarian biopsies, already described in 2000, was confirmed in the study by Herraiz et al., but the authors did not find any correlation with the type of decortication (1).

Even if the authors suggested that ovarian decortication before fragmentation could play a role in optimizing tissue quality before ovarian transplantation, we were surprised to note that in in vivo nonhuman studies, there was no difference in terms of follicular density after xenotransplantation whatever the technique of decortication (1).

Even if the type of ovarian tissue preparation seems to play a role in the success of ovarian tissue transplantation, we have to keep in mind that there are probably other factors also involved, such as the transport and freezing media as well as the inevitable posttransplantation hypoxia.

In 2016, Henry et al. demonstrated that supplementation of transport and freezing media with antiapoptotic drugs improves ovarian cortex survival (3). Ovarian tissues treated with antiapoptotic drugs before and during cryopreservation showed higher primordial follicle density and quality after 2 or 6 days of culture. However, these results do not imply that it will improve engraftment or maintenance of primordial follicle health after transplantation.

Moreover, to palliate the post-grafting hypoxia affecting follicular recruitment and responsible for follicle loss of >60% during the first few days after grafting, Henry et al. analyzed the effect of encapsulation of ovarian tissue with the use of vascular endothelial growth factor 165 in a collagen matrix during mice xenografting (4). They demonstrated that it produced a more rapid onset of functional vessel formation and earlier revascularization of the transplant. Improved angiogenesis in ovarian grafts was observed as soon as 3 days after transplantation. This rapid development of mature blood vessels in ovarian grafts is important for limiting ischemic injury and depletion of a high rate of follicular reserve during transplantation.

Another method aiming to try to avoid the post-transplantation hypoxia has been recently proposed. Indeed, in 2018, Manavella et al. described a method of boosting revascularization in xenografted human ovarian tissue with the use of adipose tissue–derived stem cells (ASCs). They observed a mean survival rate of 62% 7 days after transplantation (5). Early increase in vascularization and oxygenation in relation to the addition of ASCs could explain the reduced follicle loss.

In conclusion, the success of ovarian tissue transplantation is multifactorial. Several strategies have already been described to improve the quality of grafted ovarian tissue and the clinical outcomes of ovarian tissue transplantation. In this goal, every step of ovarian tissue cryopreservation has been studied, from ovarian tissue sampling and decortication to transport and freezing procedure, and the surgical

technique of grafting also plays a crucial role to improve tissue survival.

Nevertheless, what is still needed is research to determine which type of procedure, slow freezing versus vitrification, is more appropriate in the ovarian tissue transplantation. Indeed, while slow freezing is currently the conventional method of ovarian tissue freezing, many teams are studying vitrification, which presents the advantage, in addition to the freezing speed, of not requiring a programmable freezer to control the temperature drop.

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https://doi.org/10.1016/j.fertnstert.2019.11.022

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