Optimizing outcomes from ovarian tissue cryopreservation and transplantation; activation versus preservation

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Submitted on May 14, 2015; resubmitted on July 2, 2015; accepted on July 30, 2015

ABSTRACT: Ovarian tissue cryopreservation and transplantation (OTCP) is gaining increasing traction in the field of fertility preservation as a result of accumulated successes. We now have a decade of experience with the technique, with tens of live births and greater than 90% return of ovarian function in graft recipients. Recently, a novel method of OTCP has been described, termed in vitro activated OTCP which proposes significant changes to the standard protocol. This method aims to stimulate activation of dormant follicles within the grafts prior to transplantation and ensure that mature oocytes can be generated in the immediate short term after transplantation. By contrast, conventional OTCP seeks to maintain dormancy and thus preserve the follicle reserve in the graft with the aim of maximizing graft lifespan. This opinion paper will compare the two methods of OTCP, highlighting their respective advantages and disadvantages, and provide suggestions as to when to apply either one of these methods in a clinical setting.

Key words: ovarian tissue cryopreservation / ovarian tissue transplantation / follicle activation / fertility preservation

Introduction

Ovarian tissue cryopreservation and transplantation (OTCP) is commonly offered as a fertility preservation procedure for patients undergoing gonadotoxic treatments. It has been a decade since the first live births post transplantation of a frozen thawed ovarian graft (Donnez et al., 2004; Meirow et al., 2005), and the last few years have seen the birth of >40 children from auto-transplanted ovarian tissue grafts. While each center has its own specific protocol, OTCP generally involves removal of one ovary or section of the ovary, and preparation of cortical strips of a thickness of 0.3–2 mm (Practice Committee of American Society for Reproductive Medicine, 2014) before slow freezing and storage in liquid nitrogen. Thawing and transplantation may occur years, even decades later, when the patient has recovered from disease and is prepared to start a family. A recent review of OTCP cases reported that in 93% of patients, reimplantation of a single ovarian graft resulted in the restoration of ovarian activity often for years at a time, with the associated return of endocrine function and possibility of pregnancy—both assisted and spontaneous (Donnez et al., 2013).

Recent studies have proposed an adjusted method of OTCP, which is termed ‘in vitro activated’ (IVA) OTCP (Kawamura et al., 2013; Suzuki et al., 2015). This method aims to stimulate activation of dormant follicles within the grafts and ensure that mature oocytes can be generated in the immediate short term after transplantation. The authors suggested that IVA OTCP will be useful for patients with diminished ovarian reserve including those diagnosed with primary ovarian insufficiency, cancer patients after gonadotoxic treatments, and women with aging-associated infertility. In order to assess the validity of these claims and the potential of this new technique, this opinion paper will compare the two methods of OTCP, highlighting their respective advantages and disadvantages, and provide suggestions as to when to apply either one of these methods in a clinical setting.

Differences in technique

The in vitro activation component that sets IVA OTCP apart from conventional OTCP comprises two steps that occur post thawing and prior to graft transplantation. The first step is fragmentation of the tissue grafts...
into cubes of 1–2 mm³. The purpose of this fragmentation is to promote actin polymerization within the graft which has been shown to disrupt the Hippo signaling pathway (also known as the Salvador/Warts/Hippo (SWH) pathway, regulating cell proliferation and organ size), which in turn promotes follicle growth from the secondary to early antral follicle stages (Kawamura et al., 2013). The second step involves in vitro culture of the graft fragments with a phosphatase and tensin homolog (PTEN) inhibitor (bispexrovanadium) and phosphoinositide 3-kinase (PI3K) activator (740YP) for 2 days to stimulate activation of the dormant primordial follicles. The PTEN-PI3K signaling pathway is responsible for regulation of follicle quiescence, and up-regulation of the pathway has been demonstrated to stimulate activation and growth of dormant primordial follicles both in animal models (Adhikari and Liu, 2009) and in human (Li et al., 2010).

An additional difference between the two methods is that IVA OTCP requires the use of vitrification, whereas conventional OTCP mostly uses slow freezing, with a few centers using vitrification. While slow freezing has proven itself clinically, in the form of pregnancies and live births, studies comparing vitrification with slow freezing have conflicting results. Some studies concluded that vitrification results in greater viability of oocytes (Kagawa et al., 2009; Silber et al., 2010) and better preserves growing follicles compared with slow freezing (Amorim et al., 2012), however, other studies found greater follicle preservation in slow freezing (Gandolfi et al., 2006), or no significant difference between the two methods (Isachenko et al., 2009; Rahimi et al., 2010). Since vitrification is not used by most centers for OTCP and since many patients wait more than a decade to reimplant frozen tissue, it will take a while before there are significant clinical outcome data for vitrified ovarian tissue to enable a direct comparison with slow freezing.

While both methods aim to restore reproductive capability to patients after treatment, a more comprehensive evaluation requires a comparison of the two methods in terms of four key measurements; safety, graft lifespan, endocrine function and fertility outcomes.

**Safety**

The exposure of the ovary to chemical stimulants as part of IVA OTCP represents a significant safety issue. The long-term implications of such exposure on the oocyte and subsequent fetus have not been assessed and thorough investigation into the long-term effects of such treatment will be essential before more widespread clinical use. It might be possible to consider using only one of the two ‘activation’ steps, i.e. fragmentation of the tissue without additional chemical treatment. However, it is unclear whether disruption of the Hippo signaling alone via fragmentation would be sufficient to cause follicle activation. Hippo signaling acts on the later stages of follicle growth (secondary to antral) rather than the dormant follicles, therefore the potential target population is far smaller than that of the dormant follicle reserve which makes up the vast majority of the follicle population.

It is worth considering that culture of the fragments with activating substances may be largely unnecessary since mass activation and growth of primordial follicles has been shown to occur spontaneously in (untreated) grafts immediately following transplantation (Dolm ans et al., 2007; Smitz et al., 2010; Gavish et al., 2014). This spontaneous activation may be due to the removal of growing follicles as part of the preparation of the grafts, and thus the removal of the negative regulation that growing follicles exert on the dormant follicle pool (Dolmans et al., 2007; Roness et al., 2013). It may also reflect the post-menopausal FSH levels in women with low ovarian reserve that provide a supra-physiological stimulation of early follicle growth, since primordial/primary follicles express receptors for FSH (Oktay et al., 1997; Kristensen et al., 2015) until growing follicles start to secrete estradiol and inhibit B to down-regulate pituitary output. Unfortunately, no untreated control group was included in the study to enable a comparison of the degree of added activation provided by each of the additional treatments (Suzuki et al., 2015).

In the case of cancer patients there are additional safety concerns to consider. In many of these patients ovarian tissue is removed and cryopreserved following initiation of one or more cycles of chemotherapy (Shapira et al., 2014). With IVA OTCP the early growing follicles that were exposed to toxic chemotherapy are maintained in the graft, and then stimulated to grow further with the ultimate purpose of utilizing them for fertilization a short time after transplantation. There are animal data that suggest that follicles exposed to chemotherapy treatment at any period post-dormancy—in particular at the early growing stages—and then fertilized carry significant risk of fetal malformation and pregnancy loss (Meirow et al., 2001; Bar-Joseph et al., 2010; Kujo et al., 2010). It is therefore possible that the primary follicles in the grafts which are stimulated to grow via IVA and then fertilized are likely to carry an increased risk for any resulting pregnancies, and thus it would seem inadvisable to allow IVA OTCP with ovarian tissue removed within a few months of chemotherapy treatments (Chung et al., 2013). In contrast, since conventional OTCP does not artificially accelerate follicle growth, the likelihood that exposed follicles will be fertilized is extremely small as they are far more likely to undergo atresia.

**Graft lifespan**

The key steps added by IVA OTCP to induce follicle activation have the additional—and unwanted—effect of reducing graft lifespan. A recent review of 60 cases of conventional OTCP showed that the mean duration of graft function was 4–5 years (Donnez et al., 2013). Since the long-term functioning of the graft is contingent on the survival of a sufficiently large population of dormant follicles (the number of growing follicles in the graft does not impact on graft lifespan), conventional OTCP seeks to minimize the number of follicles lost to activation immediately following transplantation. As IVA OTCP promotes activation of the dormant follicles, fewer dormant follicles will remain in the graft, thereby reducing the overall graft lifespan. There is clinical evidence that reducing graft size, as in IVA OTCP, reduces graft lifespan; in one of the earliest published cases of OTCP, strips of thawed ovarian tissue were transplanted to the left ovary while small fragments were injected into the right ovary, and only the ovarian strips placed in the left ovary resumed function (Meirow et al., 2005). This is supported by animal studies which have directly compared the impact of graft dimensions and found that reducing graft physical dimensions (i.e. thickness) has adverse effects on the graft follicle pool due to increased activation, resulting in accelerated loss of dormant follicles (Gavish et al., 2014), which would likely lead to rapid exhaustion of the graft follicle population. The further step of culturing the grafts with activating agents would only increase the loss of follicles to activation and growth, reducing the remaining reserve and further decreasing graft lifespan.

The loss of large numbers of primordial follicles via activation is of particular importance in cases of low reserve. For patients whose grafts contain sub-optimal numbers of primordial follicles, due to previous...
gonadotoxic treatments, the further reduction of an already reduced pool of follicles via activation will significantly impact on the lifespan of the graft. On the other hand, the short-term focus of IVA OTCP might be beneficial where the patient desires immediate outcomes, or needs to optimize her chance of conceiving as fast as possible due to increasing age. This may create a dilemma in the case of patients of advanced reproductive age whose grafts contain a low reserve of follicles, but who also have only a short time-span in which to try and achieve pregnancy. Such patients, and their physicians, may have to prioritize between immediate and long-term outcomes.

Endocrine function

An important outcome of conventional OTCP that has not been considered with IVA OTCP is the ability of the graft to restore ovarian endocrine function. The return of ovarian function provides significant benefit to the patient, beyond the potential for reproduction, since the endocrine function of the ovary plays a more extensive role in women’s health. The monthly variations in sex hormones, such as estradiol and progesterone, are instrumental in avoiding osteoporosis and cardiovascular diseases (Francucci et al., 2008; Rivera et al., 2009) and the fact that more than 90% of the circulating estradiol in the follicular phase of the cycle derives from the one pre-ovulatory follicle illustrates the importance of stockpiling follicles to maintain endocrine function. The recruitment rate of follicles becomes reduced when the collective ovarian reserve is attenuated explaining why women in the perimenopausal window maintain normal circulating levels of sex hormones. This is most likely also the case in women with OTCP where the graft has been shown to be active for years despite low or even unmeasurable levels of anti-Mullerian hormone (Janse et al., 2011). In contrast, in IVA-OTCP follicles are artificially activated and they are not subjected to the normal regulatory pathways for recruitment. Thus, the endocrine capacity of the IVA-OTCP tissue will be exhausted earlier and the endocrine capacity of a fraction of the follicles will not be utilized. The added benefit of prolonged ovarian endocrine function and the urgency of pregnancy for the patient therefore need to be taken into account when deciding the most appropriate path of action for an individual patient.

Fertility outcomes

The most important outcome for any fertility preservation procedure is, obviously, a successful pregnancy, which has been demonstrated with both methods. However, compared with the approach of conventional OTCP, IVA OTCP has a very specific short-term goal; to generate a large number of mature oocytes immediately following transplantation. The aim in conventional OTCP focuses on the longer term; to restore ovarian function for as long as possible enabling multiple ovulations and pregnancies, including—if possible—spontaneous pregnancies, without the necessity for medical intervention. Using conventional OTCP, single grafts have resulted in as many as four pregnancies (two IVF and two spontaneous) in one patient (Meirw et al., 2014), and three consecutive live births in others (Macklon et al., 2014). In more than one case, pregnancy was achieved 5 years after transplantation. By reducing graft lifespan, IVA OTCP is unlikely to be able to offer long-term reproduction post transplantation.

Conclusion

OTCP is a demanding procedure, requiring two surgeries, expert preparation of the tissue, and long-term storage. The efficacy of OTCP is affected by a number of different conditions such as diagnosis, age, amount of tissue transplanted and desire for pregnancy, as recently discussed (Andersen, 2015). IVA OTCP induces the activation of dormant follicles thereby reducing the lifespan and the reproductive potential of what is an extremely precious and limited resource. In our experience we routinely see long-term graft survival, with individual grafts generating repeated pregnancies and live births, as well as maintaining hormonal function over a period of years. It is possible that IVA OTCP may provide added benefit in women undergoing transplantation at older ages, where immediate growth and use of oocytes is advantageous, as long as safety concerns have been considered. However, for the majority of women undergoing OTCP, reducing immediate activation and maximizing the follicle reserve would seem a more effective use of the technique, providing far greater long-term benefits.

Authors’ roles

D.M., H.R., S.G.K. and C.Y.A. all contributed to the conception and writing of this paper.

Funding

No external funding was either sought or obtained for this study.

Conflict of interest

None declared.

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