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Review

In-vitro regulation of primordial follicle activation: challenges for fertility preservation strategies

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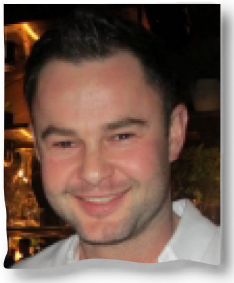

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KEY MESSAGE
There are significant challenges in the development of ovarian tissue culture systems for fertility preservation. One of these is the precocious activation of primordial follicles when ovarian tissue is placed into culture. This review proposes novel strategies to improve the outcomes following culture of primordial follicles.

A B S T R A C T

Ovarian tissue is increasingly being collected from cancer patients and cryopreserved for fertility preservation. While the only available option to restore fertility is autologous transplantation, this treatment is not appropriate for all patients due to the risk of reintroducing cancer cells and causing disease recurrence. Harnessing the full reproductive potential of this tissue to restore fertility requires the development of culture systems that support oocyte development from the primordial follicle stage. While this has been achieved in the mouse, the goal of obtaining oocytes of sufficient quality to support embryo development has not been reached in higher mammals despite decades of effort. *In vivo*, primordial follicles gradually exit the resting pool, whereas when primordial follicles are placed into culture, global activation of these follicles occurs. Therefore, the addition of a factor(s) that can regulate primordial follicle activation *in vitro* may be beneficial to the development of culture systems for ovarian tissue from cancer patients. Several factors have been observed to inhibit follicle activation, including anti-Müllerian hormone, stromal-derived factor 1 and members of the c-Jun-N-terminal kinase pathway. This review summarizes the findings from studies of these factors and discusses their potential integration into ovarian tissue culture strategies for fertility preservation.

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Introduction

Fertility preservation encompasses a number of clinical approaches and laboratory technologies (**Figure 1**), many of which are still considered experimental (De Vos et al., 2014; Wang et al., 2016). Ovarian stimulation and supernumerary oocyte collection, as per a full IVF cycle, offers cancer patients the greatest chance of preserving their fertility (Wang et al., 2016). However, a full IVF cycle is not appropriate for pre-pubertal girls, women without a partner, women with oestrogen-sensitive cancers, or those who have insufficient time for a full IVF cycle (Chian et al., 2013; Wang et al., 2016).

Ovarian tissue is increasingly being collected from cancer patients and cryopreserved for fertility preservation. So far, 86 live births have been reported following ovarian tissue cryopreservation and transplantation (Jensen et al., 2016). This approach is a very encouraging development; however, the risks of reintroducing malignant cells into the patient are unclear (Dolmans et al., 2013). An alternative fertility preservation option to autologous transplantation of ovarian tissue is to grow and mature oocytes completely *in vitro*. Although immature oocytes can be recovered from antral follicles and matured *in vitro* (IVM), it has been proposed that an alternative approach to obtain a homogeneous population of good-quality oocytes would consist of in-vitro culture (IVC) systems that support activation of primordial follicles within cortical strips, isolation and culture of pre-antral follicles, followed by oocyte collection, IVM and IVF (De Vos et al., 2014; Donnez and Dolmans, 2013; Telfer and Zelinski, 2013).

The development of IVC systems for growing oocytes from the primordial follicle stage through to metaphase II (MII) for IVF would be an important breakthrough in reproductive medicine, especially for the increasing number of women who are surviving cancer but learning that they face infertility due to the effects of their treatment. However, progress has been slow in developing IVC systems in humans and large animal species. This is primarily because of the enormous biological complexity, and our incomplete knowledge, of the developmental checkpoints that regulate follicle and oocyte development (Campbell et al., 2004; De Vos et al., 2014; Mermillod et al., 2008; Scaramuzzi et al., 2011). Furthermore, while conventional assisted reproductive technologies such as IVF are undoubtedly essential for fertility preservation, their most critical rate-limiting factor is the limited number of good-quality mature oocytes that can be recovered from a single female (Smitz et al., 2010).

Recently a plethora of research has been carried out aimed at elucidating the factors that regulate folliculogenesis. The majority of the research has focused on the terminal stages of follicle development, with fewer studies having pursued the regulation of primordial follicle activation (Reddy et al., 2010). Consequently, our knowledge of the mechanisms regulating primordial follicle development is limited. For the development of an optimal IVC system, it would be preferable if regulatory mechanisms for follicle development could be maintained *in vitro*. However, when ovarian cortex tissue is placed into culture, uncontrolled and precocious activation of the primordial follicle pool occurs. This is in stark contrast to conditions *in vivo* where the primordial follicle pool is maintained in a resting state and primordial follicles are gradually activated and enter a protracted period of development. Precocious activation may lead to uncoordinated growth of oocytes and granulosa cells (Smitz and Cortvrindt, 2002). We propose that the rapid induction of primordial follicle activation and uncontrolled development of growing follicles *in vitro* is a principal cause of the reduction in oocyte developmental competence *in vitro*. If IVC of extremely valuable ovarian tissue is to be properly integrated into the human assisted reproductive technologies toolbox, it will be essential to control follicular development *in vitro* to obtain a population of good-quality oocytes (Telfer and Zelinski, 2013). Employing factors that prevent precocious activation of primordial follicles may prove useful, similar to the principle that has been used to avoid the spontaneous resumption of meiosis in IVM (Gilchrist et al., 2016). This review explores the factors regulating primordial follicle activation and pre-antral follicle development, and speculate how some of these factors could be integrated into fertility preservation strategies to produce fully competent oocytes.

In-vitro grown follicles and oocytes

An attractive approach to obtaining a relatively large source of homogeneous oocytes after ovarian tissue cryopreservation is the

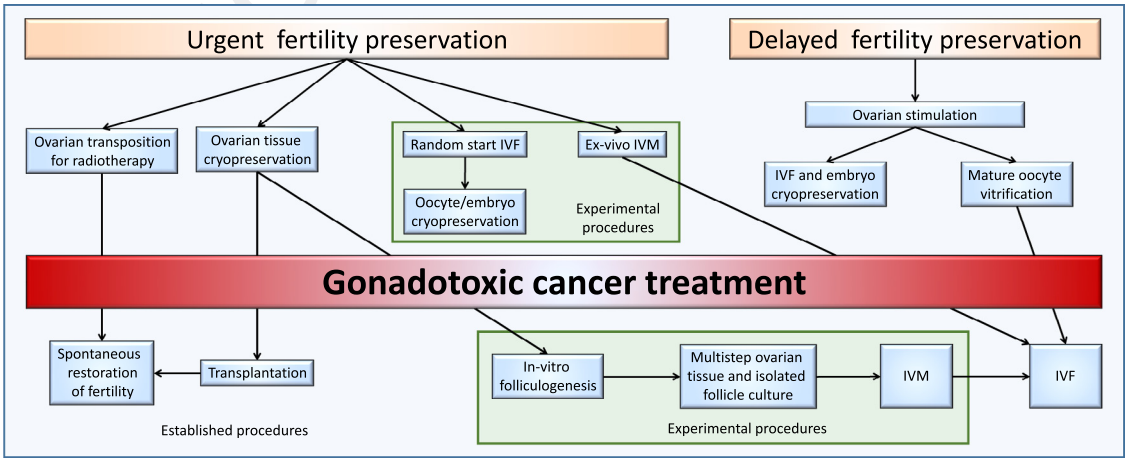

Figure 1 – Fertility preservation strategies for females before cancer treatment. Treatments are tailored to patients depending on the patient's age, the possible delay in treatment and sensitivity of malignancy to hormone treatment. Experimental procedures are enclosed in green boxes. IVM = in-vitro maturation.

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