

■ COVER STORY

From The Endocrine Society's Research Affairs Core Committee  
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# Perspectives on Oncofertility:

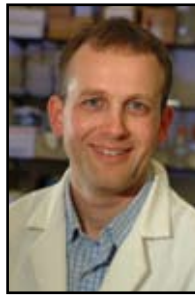
## Preserving Fertility for Young Cancer Patients

### Introduction

Remarkable advances in cancer therapy have improved survival rates for cancer patients, yet the use of life-preserving chemo- and radiation therapy often results in loss of fertility for young adults and children. The ability to easily preserve sperm before cancer treatment gives men options for a family later in life, but young women and girls have fewer fertility preservation options. Rapid referral to reproductive endocrinologists for emergency in vitro fertilization (IVF) is feasible for some but not all young women, and is not appropriate for girls. Recently, however, advances in ovarian tissue cryopreservation and in vitro follicle maturation are brightening the outlook for preserving the fertility of women and girls with cancer at the time of diagnosis. The specific needs of cancer patients at the time of diagnosis and the presentation of best options for their oncology and fertility needs, as well as the new advances in fertility care, led to the creation of a new interdisciplinary collaboration called oncofertility. This program aspires to offer fertility options to all cancer patients when they are diagnosed. This Tri-Point article focuses on the changing fertility outlook for young women who develop cancer.<sup>1</sup>

### From the Basic Scientist Perspective

- Cryopreservation of oocytes and ovarian tissue is being actively investigated to increase fertility preservation options for all female cancer patients.
- The ovary contains numerous immature follicles that are more likely to survive cryopreservation than mature oocytes.
- New 3-D matrices support the unique architecture of the follicle, which is necessary for oocyte growth and maturation in vitro.
- Using an alginate hydrogel follicle culture system, immature mouse follicles can be matured in vitro and fertilized, resulting in live birth of fertile offspring.



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Women diagnosed with cancer have only two established techniques for fertility preservation: protecting the ovaries from radiation (oophorectomy) and emergency IVF.<sup>2</sup> Oophorectomy might protect a woman's fertility<sup>3</sup> but can lower the chance of successful pregnancy.<sup>4</sup> Emergency IVF, in which mature oocytes are collected, fertilized, and the resulting embryo cryopreserved, is also limited in cancer patients; the hormonal stimulation required for obtaining mature oocytes can cause long delays in treatment or might be contraindicated in hormone-sensitive cancers.<sup>2</sup> Women must either have a partner or accept donated sperm to form embryos for preservation.<sup>2</sup> Emergency IVF is also not an option for children diagnosed with cancer before puberty.<sup>4</sup>

Cryopreservation of mature oocytes is another option, but the aqueous content of the egg makes it difficult to freeze them without fracturing and destroying delicate cellular substructures due to the formation of ice crystals, resulting in low oocyte survival and pregnancy rates.<sup>5, 6</sup> As with emer-

gency IVF, oocyte cryopreservation for cancer patients is less than ideal because of the necessity for hormonal stimulation and limited availability to pre-pubertal girls.

### **Ovarian Tissue Cryopreservation**

The experimental technique of ovarian tissue cryopreservation might expand fertility preservation options for women with cancer, including childhood cancer patients. Immature oocytes within the ovarian cortex are small, contain less water, have fewer active proteins, and are therefore much better candidates for cryopreservation than mature oocytes.<sup>7</sup> Cryopreserved ovarian tissue could be used for autotransplantation<sup>8</sup> or in vitro maturation of follicles to produce mature oocytes.<sup>9</sup> The latter approach is considered more desirable because it eliminates the possibility of reintroducing cancer cells with transplantation and provides a way to harvest more mature oocytes.

The ovarian follicle is a unique structure that contains the egg surrounded by and intimately connected to somatic support cells. In vivo and in vitro studies have established the importance of cell-cell communication in the growth and differentiation of the follicle.<sup>10</sup> In traditional 2-D culture systems, somatic cells detach from the oocyte and spread onto the culture surface, disrupting somatic cell-oocyte communication. The inefficiency of oocyte maturation in the 2-D system has been the primary stumbling block to in vitro maturation protocols. In contrast, 3-D culture systems maintain the overall architecture of the follicle and support the vital communication pathways between the egg and the somatic cells, more effectively allowing normal follicle development.<sup>11, 12</sup>

### **How the 3-D Culture System Works**

In our 3-D culture system, ovarian follicles are encapsulated within beads of alginate hydrogel, a linear polysaccharide derived from algae and composed of repeating units of b-D-mannuronic acid and a-L-guluronic acid. Mild ionic cross linking of the guluronic residues, in the presence of calcium, gels the matrix while maintaining cell viability and causing minimal nonspecific protein adsorption and cell adhesion.<sup>13</sup> Immature follicles can be placed in alginate droplets, which after gelation replace the ovarian stroma/matrix and support the 3-D culture of ovarian follicles in vitro. We call this method in follicle maturation (IFM). Alginate was chosen for encapsulation because immature mouse follicles can be encapsulated easily in alginate hydrogel beads. Follicles can be removed from alginate by degrading the gel with an alginate-specific enzyme that has no known interactions with mammalian cells. In addition to mechanical support, alginate is highly porous, thereby allowing soluble factors and hormones to diffuse through the gel between the media and the follicle.

Following encapsulation, follicle growth and oocyte maturation during an 8-12 day culture period mirrored follicle development in vivo, in which follicles form a central fluid-filled antral cavity and the oocyte is surrounded by cumulus cells. Follicles retrieved from the hydrogel were treated with human chorionic gonadotropin, and about 70% of fully

grown oocytes matured to metaphase II after 16 hours. These eggs were fertilized in vitro (~68%,  $n = 99$ ) at rates similar to that achieved with in vivo ovulated mature eggs (~82%,  $n = 65$ ), indicating that the 3-D hydrogel supports normal oocyte development in a manner far superior to any other method developed to date.<sup>11</sup> Transplantation of embryos derived from follicles cultured in the alginate system into CD1 foster mothers resulted in live births of healthy, fertile offspring from 20% of the transferred embryos, a significant improvement over previously reported live birth rates of less than 5%.

In vitro and in vivo tissue engineering approaches for previously intractable biological problems have gained significant momentum in the past 2 decades. Applying tissue-engineering principles to reproductive health and fertility preservation has been promising in that the biological obstacles to in vitro follicle development appear to be surmountable. The success of studies in mice using the hydrogel system presents an opportunity to move the in vitro maturation technology from the bench through biomaterials to the bedside and eventually giving fertility options to patients who have only immature follicles available as starting material.

### **From the Clinical Scientist Perspective**

- Autotransplantation of cryopreserved tissue has led to live births in both nonhuman primate and female cancer patients, but success rates remain low.
- In vitro follicle maturation of immature follicles from cryopreserved ovarian tissue is being investigated as an alternative to autotransplantation.
- As the first step in translating in vitro follicle maturation to the clinic, researchers are currently optimizing the 3-D alginate hydrogel matrix for human follicles and tissue transplant.

As described in the previous section, cryopreservation of intact ovarian tissue for later transplantation or for IFM and IVF appears to be a promising area of research that could extend fertility preservation to both women and girls at the time of cancer diagnosis. Autotransplantation of previously cryopreserved ovarian tissue has led to restoration of graft function<sup>14</sup> and has resulted in two live births,<sup>15, 16</sup> with two other reports of miscarriage.<sup>17, 18</sup> Current research includes evaluating slow-freezing methods and using various cryoprotectants for producing intact follicles that are able to undergo development post thaw.<sup>19</sup> A three-step freezing method with ethylene as the cryoprotectant is in development and has been tested using human oocytes,<sup>20</sup> and preliminary findings have shown nearly 100% survival with exceptional morphology. Concurrent with these efforts, researchers are refining methodologies to successfully develop immature oocytes in frozen-thawed tissue for fertilization, either in vivo, by transplantation back into the individual, or in vitro by developing effective culture systems to support follicle growth and differentiation.

### **Recent Research Successes, Challenges**

Monkey ovary cortical strips have been transplanted in



vivo into a variety of sites with re-initiation of antral follicle growth, oocyte maturation, and, following intracytoplasmic sperm injection and embryo transfer, live birth.<sup>21</sup> Ongoing studies are assessing factors that have an impact on graft survival and the retention of follicular reserves that will sustain follicle growth and production of healthy, mature oocytes. In 2004, the first human baby was born after orthotopic transplantation of frozen-thawed ovarian tissue in a woman with Hodgkin's lymphoma.<sup>15</sup> Since then, there have been several case reports demonstrating that autotransplantation of frozen-thawed ovarian cortical strips can yield human oocytes capable of fertilization and early development,<sup>22</sup> pregnancy, and offspring.<sup>16, 17</sup> Nevertheless, current techniques cause substantial loss of follicles and carry a significant risk of reintroducing cancer cells into the "cured" patient.<sup>23</sup> Thus, current methods of ovarian tissue autotransplantation, especially in young girls, raise serious concerns about long-term patient safety.<sup>24</sup>

In vitro follicle maturation offers an alternative to autotransplantation by eliminating the risk of reintroducing cancer cells. As described in the previous section, however, the difficulty of maturing isolated oocytes without their supporting follicle and the failure of small follicles to develop in a 2-D environment have hindered development of in vitro follicle maturation strategies. The 2-D culture system limitations might be even greater in primates, which have larger follicles with a longer growth phase. Primate and human primordial follicles maintained in situ in organ culture (1-mm slices) will begin to grow and can reach the secondary stage, but many follicles are lost. Whereas ovarian slices retain the follicle's general architecture, the poor diffusion properties and the tissue's rigid structure likely limit follicle growth and development.

#### **Hopes from 3-D System and IFM**

The 3-D alginate hydrogel culture system and IFM might offer new opportunities for investigating follicle growth and maturation in primates and, ultimately, for restoring fertility in female patients following ovarian tissue cryopreserva-

tion. Compared to mice, longer culturing intervals will be required in primates to obtain mature preovulatory follicles, and follicle survival could depend on the phase of the menstrual cycle when ovaries are collected. Nonetheless, initial results provide the first evidence for long-term culture of pre-antral primate follicles to the antral stage. Future studies will continue to optimize the alginate hydrogel culture system in terms of gel matrix composition and media components, with the goal of producing mature oocytes that are competent for fertilization, culminating in transplantation of embryos and the birth of live, healthy, fertile offspring. Coupled with advances in ovarian tissue cryopreservation, in vitro maturation might provide an option to women who want to preserve fertility without the undue risks associated with autotransplantation.

#### **From the Clinician Perspective**

- Advances in cancer treatment have led to increased survival rates and a new emphasis on long-term quality-of-life issues.
- The fertility effects of cancer therapies should be communicated to patients.
- Oophorectomy and emergency IVF are the most developed steps. Options for fertility preservation are not yet universally offered to women or prepubertal girls with cancer.
- Advances in ovarian cryopreservation, autotransplantation, and in vitro follicle maturation are expanding fertility preservation options for young women with cancer.
- Interdisciplinary collaboration between medical oncologists and reproductive endocrinologists will facilitate fertility preservation decision-making at the time of cancer diagnosis.

In 2006, approximately 680,000 women in the United States were diagnosed with cancer,<sup>25</sup> and the American Cancer Society estimates that the likelihood of developing cancer in females aged less than 40 years is 1 in 50.<sup>26</sup> Yet, remarkable advances in cancer treatment have brought significant improvements in cancer survival rates: for individuals younger than 45 years, the overall survival rate is nearly 80%.<sup>27</sup> The survival rates achieved with modern therapy suggest that a large proportion of the future adult population will be cancer survivors and significant effort should be made to improve their quality of life.

#### **Emotional Burden of Infertility**

Subfertility and premature ovarian failure are frequent long-term side effects of cancer therapy in young girls and women,<sup>28, 29</sup> and pregnancy complications have been reported following cancer treatment.<sup>30</sup> For many individuals, the experience of infertility is a serious life crisis,<sup>31</sup> triggering symptoms of depression and anxiety and strong emotional reactions such as anger, grief, frustration, and guilt. Coupled with the stress associated with cancer, infertility presents a significant emotional burden on cancer survivors. Thus, the remarkable improvements in cancer therapy and cancer survival rates have also generated a significant societal need: effective means to preserve the fertility of individuals

undergoing these therapies.

If a woman has a partner at the time of diagnosis and the ability to delay cancer treatment, the preferred method to ensure future viable children is emergency IVF;<sup>2,4,29</sup> however, this is simply not an option for women with hormone-sensitive cancers or for prepubertal patients. Additionally, young patients might be facing loss of fertility with no partner and little financial support, and are being asked to make a bewildering decision about the fate of their embryos or eggs should they not survive the cancer. It is therefore crucial that oncologists view their young patients as cancer survivors and develop a plan *at the time of diagnosis* that takes into account the impact of treatment on fertility while ensuring survival.

### Talking with Patients

As cancer treatment strategies improve survivorship, patients are shifting their focus to understanding and managing the long-term sequelae of their cancer treatment, including its effect on reproductive function. Both the American Society for Reproductive Medicine<sup>32</sup> and the American Society of Clinical Oncologists<sup>33</sup> have published guidelines recommending that doctors talk to patients about the fertility implications of cancer treatment. Yet such conversations have not regularly been a part of the medical oncologist's interactions with patients because their primary focus is on eliminating the cancer by any means necessary. The clinician's ability to advise patients is also severely limited by the dearth of information on how chemotherapeutic drugs undermine fertility. This knowledge gap is due partly to the ever-increasing array of chemotherapeutic options and regimens, the inconsistency with which patients are treated within and between cancer centers, and the need to evaluate ovarian function over an extended period of time. Nevertheless, the breakdown of doctor-patient communication on treatment-related infertility can create a tremendous amount of anxiety for a patient about her choices for treatment, and may lead to significant misunderstandings.

Unfortunately, medical oncologists may be less likely to discuss the impact of treatment on fertility with their female patients, particularly pediatric patients, because they are not aware that any options exist. Overcoming these barriers will require a comprehensive interdisciplinary approach at the intersection of medical oncology and reproductive endocrinology, resulting in a new medical specialty called "oncofertility" that can answer cancer patients' questions about reproductive function and conservation at the time of diagnosis.

### Alternatives Needed



The challenges and stress related to fertility preservation continue to motivate the desire to find alternatives for young female cancer patients. In particular, the promise for female patients with childhood cancer lies in the strides made toward ovarian tissue cryopreservation, ovarian transplantation, and in vitro follicle maturation, all of which could be offered at the time of diagnosis, regardless of patient age. These methods are likely to profoundly affect childhood cancer survivors, for whom no other method of germline conservation is available. The immediacy of a cancer diagnosis means that women

may not be prepared to decide whether to have a child, and ovarian tissue cryopreservation would permit them to rapidly conserve their ovaries, proceed with cancer treatment, and make informed choices about fertility at a later time. If developed, follicle banks with the potential to generate good quality, mature oocytes will give women diagnosed with cancer the hope of having their own babies in the years after cancer recovery. The development and clinical application of these new fertility-preserving techniques depend on the interdisciplinary action of scientists, clinicians, and others within the discipline of oncofertility who can communicate the options to patients at the time of diagnosis and help them weigh their choices. ■

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