Ex vivo recovery of mature eggs from unstimulated ovaries in pediatric fertility preservation: proceed with caution

Cancer treatments such as radiation and chemotherapy, although life-preserving, can have the unintended consequence of targeting the reproductive axis. Even beyond the cancer setting, other conditions or their treatments may also have potential threats to fertility. For example, both sickle cell disease and beta-thalassemia require conditioning treatments for hematopoietic stem cell transplantation, and these treatments have a high risk of causing premature ovarian insufficiency. Thus, there is a critical need for fertility preservation.

For prepubertal girls, the primary fertility preservation option is ovarian tissue cryopreservation wherein the ovarian tissue (either a biopsy or the whole ovary) is removed and processed to thin cortical strips containing primordial follicles or the ovarian reserve. These cortical strips are then cryopreserved and can later be thawed and transplanted back into the individual to restore endocrine function and/or fertility. To prepare the ovarian cortical strips, the tissue is typically bisected, and the inner medullary region is removed. During this process, small antral follicles are disrupted, releasing cumulus-oocyte complexes (COCs) into the media. These COCs, which contain oocytes arrested in the prophase of meiosis I, can be recovered. Ex vivo in vitro maturation (ex vivo IVM) can then be performed in defined media to obtain mature eggs arrested at the metaphase of meiosis II (MII), which can be cryopreserved for future clinical use. Thus, ex vivo IVM is an emerging technology that can be combined with ovarian tissue cryopreservation to maximize the fertility preservation potential of the tissue.

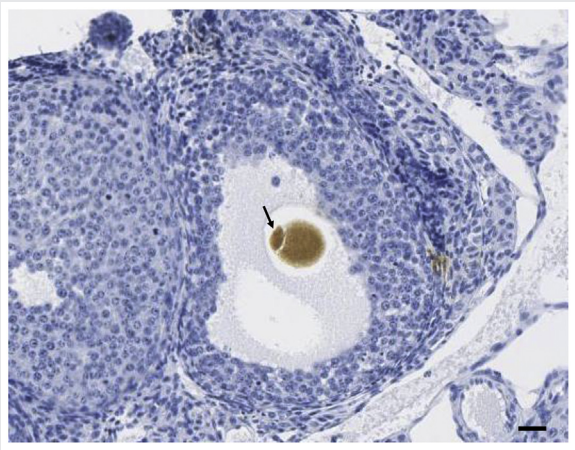
Although live births have been obtained from eggs derived from ex vivo IVM in adults, no study has reported the developmental potential of gametes obtained from a pediatric population and especially from prepubertal girls (1). Nevertheless, multiple research teams have reported their experience in successfully isolating and maturing COCs after ovarian tissue processing for cryopreservation in prepubertal girls (2, 3). When comparing the ex vivo IVM of COCs obtained from adult women relative to prepubertal girls, results showed that COCs obtained from a younger population appeared to have a lower recovery yield and a reduced ability to mature (2).

In their case report, Hanfling et al. (4) joined the existing literature in reporting that they were also able to recover gametes from small antral follicles in media from ovarian tissue processing in prepubertal girls. In this case report, the gametes were recovered from an 8-year-old girl with beta-thalassemia and a 2-year-old girl with sickle cell disease (4). However, unlike most studies that report their results after COC recovery and ex vivo IVM, Hanfling et al. (4) only reported that the gametes were recovered from unstimulated ovaries without any subsequent IVM. Within the ovary, oocytes within follicles are arrested in prophase of meiosis I; during ovulation, a surge of luteinizing hormone stimulates the oocyte to resume meiosis, and it ultimately undergoes meiotic maturation to reach MII. Although Hanfling et al. (4) do not provide any direct empirical evidence (i.e., transmitted light microscopy images showing polar body extrusion or fluorescence microscopy images showing a meiotic spindle), they claimed that 1 in 5 and 1 in 9 of the gametes recovered from the individuals with sickle cell disease and beta-thalassemia, respectively, were mature MII eggs at the time of collection. This phenomenon has been reported previously where 6 of 705 gametes collected ex vivo during the ovarian tissue cryopreservation procedure were already at the MII stage on isolation (2).

Recovering healthy mature MII eggs from an unstimulated ovary is initially counterintuitive given that the oocytes do not undergo meiotic maturation until the period of ovulation, which begins at puberty; both of the girls described in this case report were in the prepubertal stage. As the investigators mentioned, the precocious oocyte maturation observed in these two individuals may have been due to the presence of nonmalignant hematologic disorders, conditioning treatments for peripheral blood stem cell transplant, or exposure to hydroxyurea (4). All of these possibilities warrant future investigation. However, another plausible explanation—and perhaps more likely—is that these MII eggs instead come from atretic follicles. Cell death is a common feature of ovarian physiology as follicles that are activated to grow but not selected to ovulate will die by atresia. Granulosa cell death is an early feature of atresia and can be visualized in the pyknotic nuclei. In a healthy follicle, granulosa cells surround the oocyte and regulate key biological processes. For example, granulosa cells produce high levels of cyclic guanosine monophosphate, which diffuses into the oocyte where it inhibits phosphodiesterase 3 activity, keeps cyclic adenosine monophosphate levels elevated, and maintains the oocyte in meiotic arrest. However, when the granulosa cells die, as in the case of atresia, their ability to regulate meiotic arrest in the oocyte is compromised, and the oocytes may spontaneously resume meiosis. In mouse ovaries, mature eggs that have extruded a polar body and are arrested at MII can be observed within the atretic follicles (Fig. 1). Thus, the mature eggs recovered in the study by Hanfling et al. (4) possibly originated from atretic follicles, in which case their clinical value is questionable.

Regardless of the precise origin of these mature eggs, this study underscores that more translational research is needed to better understand the egg quality during the pubertal transition given that gametes at this early developmental age would not normally contribute to physiologic fertility. Compelling data from agricultural species and humans clearly demonstrate elevated aneuploidy in the mature eggs from very young animals and girls, respectively (5). Thus, the clinical use of mature eggs obtained directly from surgically removed ovarian tissue or after ex vivo IVM should be implemented with caution especially in the prepubertal population, and patients and their families should be fully informed of the unknowns of this approach.

FIGURE 1



Spontaneous oocyte meiotic maturation can occur in atretic follicles. A representative image of an atretic antral follicle in an ovarian tissue section from a reproductively young adult mouse. Immunohistochemistry was performed with an antibody that recognizes the oocyte-specific protein MSY2 (*brown*). The tissue section was counterstained with hematoxylin to visualize the nuclei (*blue*). Note that the oocyte within this atretic follicle lacks surrounding cumulus granulosa cells and thus has failed to maintain meiotic arrest. Instead, it has resumed meiosis and has reached the metaphase of meiosis II as evidenced by extrusion of the first polar body (*arrow*). Bar = 50 μm.

Duncan. Reflections. Fertil Steril Rep 2021.

Francesca E. Duncan, Ph.D.
Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

https://doi.org/10.1016/j.xfre.2021.05.003

You can discuss this article with its authors and other readers at
https://www.fertstertdialog.com/posts/xfre-d-21-00085

REFERENCES

1. Shirasawa H, Terada Y. In vitro maturation of human immature oocytes for fertility preservation and research material. Reprod Med Biol 2017;16:258–67.
2. Fasano G, Dechène J, Antonacci R, Biramane J, Vannin AS, Van Langendonckt A, et al. Outcomes of immature oocytes collected from ovarian tissue for cryopreservation in adult and prepubertal patients. Reprod Biomed Online 2017;34:575–82.
3. Yin H, Jiang H, Kristensen SG, Andersen CY. Vitrification of in vitro matured oocytes collected from surplus ovarian medulla tissue resulting from fertility preservation of ovarian cortex tissue. J Assist Reprod Genet 2016;33:741–6.
4. Hanfling SNP, Mayhew A, Robinson E, Graham J, Gomez-Lobo V, Maher JY. Case report: two cases of mature oocytes found in prepubertal girls during ovarian tissue cryopreservation. F&S Reports. 2021;2:296–9.
5. Gruhn JR, Zielinska AP, Shukla V, Blanshard R, Capalbo A, Cimadomo D, et al. Chromosome errors in human eggs shape natural fertility over reproductive life span. Science 2019;365:1466–9.