Fertility preservation in girls with Turner syndrome: limitations, current success and future prospects

In this issue of the *Fertility and Sterility*, Dr. Mamsen and a distinguished group of scientists report a multicenter, retrospective, case-control study exploring the feasibility of ovarian tissue cryopreservation in girls with Turner syndrome (TS) (1). Consistent with previous studies, they find that detectable serum antimüllerian hormone (AMH) levels, normal serum follicle-stimulating hormone (FSH) levels, and mosaic karyotype are predictors of the presence of primordial follicles. In 7 (78%) of 9 cases of ovaries where follicles were found, the follicle densities were within 95% of the controls, their mean was lower than in age-matched controls, and the sample size was too small for a robust comparison. In addition, two different methods of follicle counting were used, which depended on the participating center; hence, the measurements may have had variability. Although the ovarian tissue originated from multiple centers, the control tissues were from one center, which might also have affected the stringency of the comparisons.

The authors also found differences in morphology, some molecular markers, and antral fluid AMH and testosterone levels between the follicles of TS girls and controls. This indicates that the presence of primordial follicles is not an assuring sign by itself as some of them may not be functional or of good quality. Finally, from a limited number of cumulus–oocyte complexes obtained, only a small fraction (16%; 5 of 31) resulted in in vitro matured oocytes, confirming the negative impact of missing X chromosome material on oocyte function. Nevertheless, based on the data from their own and other previously published studies, the authors cautiously concluded that ovarian tissue cryopreservation should be offered to eligible girls with TS as a fertility preservation method.

Although the fertility preservation field initially focused on the needs of adult cancer patients, the emphasis has now shifted to include pediatric patient populations with and without cancer who are at high risk for ovarian insufficiency. As part of that shift, fertility preservation has attracted increasing attention in TS. In TS, the partial or complete absence of an X chromosome results in the accelerated loss of the primordial follicle reserve. In the overwhelming majority of the cases, in the absence of a 46,XX mosaicism, the ovarian reserve will be depleted before the child reaches a double-digit age.

In the United States, there is no universal neonatal screening for TS; as a result, by the time a diagnosis is made and the fertility issues are brought to the forefront, these girls might have already experienced primary ovarian insufficiency. Moreover, in the case of a non-46,XX-mosaic Turner girl, even if follicles are preserved the likelihood of euploid embryo generation will be limited because at least half of the metaphase-2 oocytes will lack an X chromosome. Therefore, in non-mosaic TS, prospects for fertility preservation may be severely limited, even with early intervention. Add to that the higher likelihood of TS-related cardiac and urogenital abnormalities that can complicate and contraindicate pregnancy, girls with full-blown TS face a challenging course in reproduction.

The odds may be more favorable for TS girls with 46,XX mosaicism because they tend to have less-accelerated follicle loss, depending on the level of euploid mosaic presence. In our experience, all TS girls from whom we have successfully cryopreserved oocytes carried 46,XX mosaicism (2). Supporting the authors' observation of increased abnormalities in follicle structures, including the absence of oocytes, we also encountered a higher incidence of empty follicles during oocyte retrievals for cryopreservation from TS teenage girls. On the other hand, every non-mosaic TS girl we encountered had already experienced primary ovarian insufficiency, and we were not able to consider any form of fertility preservation other than maternal oocyte freezing for oocyte donation purposes in those cases.

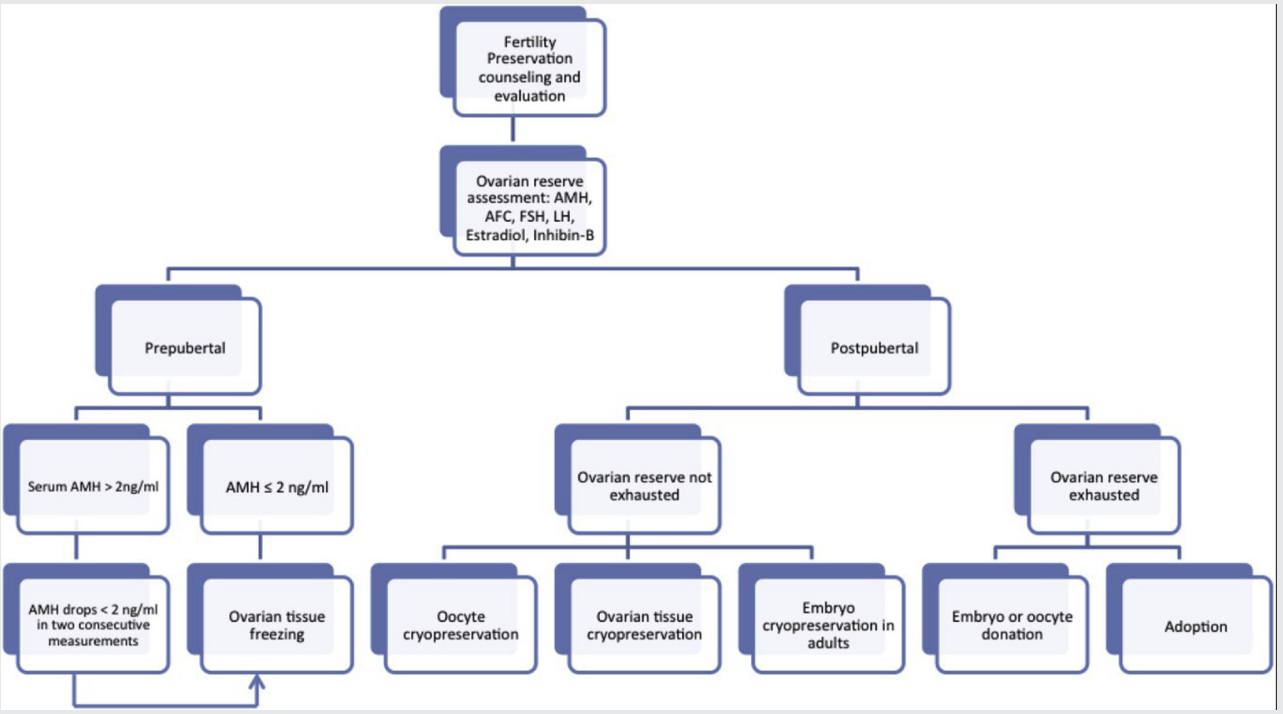
There are current limitations in ovarian tissue freezing and transplantation, and these may be accentuated in the case of girls with TS, given the lower healthy ovarian follicle reserve in their ovaries. There is a significant loss of primordial follicle reserve during the initial ischemic period of ovarian autotransplantation, which necessitates a large initial ovarian reserve in the cryopreserved tissue. As a result, most live births with ovarian tissue transplantation (OTT) are from women whose ovarian tissue was frozen before the age of 25, when the oocyte quantity and quality are relatively high. Even in 46,XX-mosaic Turner cases, the initial ovarian reserve and quality will be lower than an age-matched control. Hence, one would expect lower success rates and longevity after OTT, but there have been no clinical data to back this assumption.

Based on our experience from oocyte cryopreservation, it is likely that girls with higher degrees of 46,XX mosaicism will have a better chance of success after ovarian cryopreservation and transplantation. Another variable that needs to be considered is in some cases there may be differences in the level of mosaicism in the gonadal tissue compared to the somatic tissue, either in favor or to the detriment of the ovary. The results for other types of mosaicism (46,X,i(Xq); 46,X,del(Xp); 46,XX, 47,XXX) are more variable, and the data are more scant in terms of the prediction of when ovarian failure will occur.

A SYSTEMATIC APPROACH TO FERTILITY PRESERVATION IN GIRLS WITH TURNER SYNDROME

Given these concerns with ovarian cryopreservation in TS, we developed an algorithm to enable early intervention before the exhaustion of ovarian reserve and deferral to an age when oocyte cryopreservation becomes feasible (3) (Fig. 1). Based on this algorithm, we perform an initial ovarian reserve assessment including the measurement of serum antimüllerian hormone (AMH). In prepubertal girls, if the ovarian reserve appears to be age-appropriate and the serum AMH levels are higher than 2 ng/mL (lower quartile for girls aged 5 to 13 years of age), we monitor serum AMH periodically. If the AMH levels do not show significant decline, we plan

FIGURE 1



A proposed algorithmic approach to decision making for fertility preservation in females diagnosed with Turner syndrome. Reprinted from *Journal of Pediatric and Adolescent Gynecology*, "Fertility Preservation in Women with Turner Syndrome: A Comprehensive Review and Practical Guidelines," volume 29, Issue 5, 2016, with permission from Elsevier.

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on oocyte freezing at an appropriate age. If AMH levels show accelerated decline before there are any signs of puberty and we cannot practically perform oocyte freezing, we then consider ovarian tissue harvesting for cryopreservation combined with puncture of small antral follicles to obtain germinal vesicle oocytes for in vitro maturation and supplemental oocyte freezing. In postpubertal girls, we primarily recommend oocyte cryopreservation regardless of the AMH levels because the risk of follicle loss is extremely high. If the ovarian reserve is sufficiently high, we may also consider ovarian tissue freezing as a supplement to oocyte freezing should the parents/patient specifically desire(s) potential preservation of gonadal function and natural fertility.

IMPROVED PROSPECTS FOR OVARIAN TISSUE FREEZING IN TS GIRLS AND OTHERS WITH LIMITED OVARIAN RESERVE?

Despite its limitations, ovarian tissue freezing and transplantation may have improved prospects in the future. Since the performance of the first successful orthotopic ovarian transplantation in 1999 (4), surgical methods for post-transplantation follicle survival and success have improved. One recent approach takes advantage of precision of robotic surgery combined with a neovascularizing human extracellular matrix scaffold (AlloDerm; Allegan) (5). The initial published and extended results are highly encouraging, with 100% endocrine restoration or euploid embryo generation and/or live births, albeit still based on a relatively small number of cases.

Another approach that has been tested in human ovarian xenograft models is spingosine-1-phosphate (S1P), a ceramide-induced death pathway inhibitor that was found to have vasculogenic properties. Continuous infusion of S1P in human ovarian xenografts resulted in accelerated neovascularization, reduced tissue ischemia, and preservation of primordial follicle density at levels similar to pretransplant measurements (6). Although S1P has never been tested in a clinical setting, a synthetic analog currently in use for the treatment of multiple sclerosis may be considered in future clinical trials.

The aforementioned developments are important because if we can improve post-OTT primordial follicle survival with these and similar approaches, we may increase the feasibility of ovarian tissue freeing not only for TS girls but also in other clinical scenarios where initial ovarian reserve is low. This may also enable us to use smaller amounts of tissue from younger patients and to increase the prospects for elective ovarian tissue cryopreservation without significantly compromising the remaining ovarian reserve.

ULTIMATE SOLUTION TO ACCELERATED OVARIAN AGING IN TS GIRLS

Perhaps the biggest discoveries and solutions to premature ovarian reserve depletion in TS will come from our improved

understanding of the mechanisms of accelerated primordial follicle loss, which is triggered by the loss of X chromosome material. If these mechanisms are understood, we may be able to develop targeted treatments to retard or prevent accelerated ovarian aging in girls with TS. Perhaps the most intriguing aspect of the report by Dr. Mamsen and her distinguished group of collaborators (1) is their provision of morphological and molecular clues for the pursuit of mechanistic research on ovarian follicle loss in Turner syndrome. We should follow those clues and study them more comprehensively to decipher the underlying pathophysiology that has eluded our efforts thus far. Until then, fertility preservation it is.

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