Fertility preservation in girls with Turner syndrome: to do or not to do?

In their study, Nadesapillai et al. (1) set out to answer a critical question, namely, "Based on karyotype, puberty status, and hormonal data, which girls with Turner syndrome (TS) could benefit from fertility preservation by ovarian tissue cryopreservation (OTC)?" The question is crucial because signs of a depleted ovarian reserve are often encountered at an early age, so OTC could be a way of preserving the follicle pool of these girls before premature ovarian insufficiency (POI) occurs. Indeed, only a third of girls with TS enter puberty, although the majority experience POI at a young age because of extensive and accelerated follicle apoptosis. Natural conception is infrequent, with only 5.6% of a series of 500 women with TS conceiving spontaneously. Moreover, miscarriage rates are twice as high as in the general population.

Interestingly, a number of studies have demonstrated the presence of follicles in the ovarian cortex in girls with mosaic TS. These investigators, in collaboration with our team, recently showed (2) that small follicles from patients with mosaic TS undergo folliculogenesis after their frozen-thawed ovarian cortex is grafted to nude mice. It was concluded that OTC could be a valid option to preserve fertility in young patients with mosaic TS when sufficient numbers of follicles are present, preferably before the age of 12 years.

Very recently, Dunlop et al. (3) reported the first clinical pregnancy in a patient with TS who conceived naturally after autotransplantation of her cryopreserved ovarian tissue, which had been biopsied at the age of 15 years. Unfortunately, the pregnancy resulted in a miscarriage. Years earlier, Donnez et al. (4) had achieved a successful pregnancy and live birth (LB) in a patient with mosaic TS and POI after allografting ovarian tissue from her monozygotic twin, who still had ovarian function despite her own TS mosaicism. A second pregnancy and LB followed 2 years later.

In the present article, Nadesapillai et al. (1) report a very impressive series of 93 girls with TS who underwent unilateral oophorectomy for fertility preservation and agreed to donate one cortical fragment to research. Buccal swabs were taken to examine an additional cell line for X-chromosome aberrations. Among the 93 participants, whose mean age was 11.9 years (range 3–19 years) at surgery, 25 (27%) had cardiac anomalies.

The participants were divided into four groups: prepubertal girls (aged <12 years with no sign of puberty); spontaneous thelarche (defined as Turner stage 2); spontaneous menarche (with at least one menstruation); and no puberty (girls aged ≥ 12 years showing no sign of spontaneous puberty). In 35% (33/93) of girls, macroscopically normal ovaries were visible at surgery, and follicles were found in 32% (30/93) of girls.

Karyotyping of both lymphocytes and buccal cells revealed that girls with a 46XX cell line in lymphocytes or buccal cells were more likely to exhibit follicles. The same conclusion was reached for prepubertal girls and girls with spontaneous puberty when follicle-stimulating hormone was <10 IU/L and antimüllerian hormone (AMH) was ≥ 0.1 μg/L. Nevertheless, AMH levels should be interpreted with caution when counseling because the investigators themselves reported that follicles were present also in some girls with AMH values <0.1 μg/L.

Previous studies have demonstrated a significant positive correlation between spontaneous puberty and the presence of follicles (62%–86%) in cases of spontaneous menarche. In our opinion, however, it is essential to be aware of the presence of follicles without waiting for spontaneous menarche to occur, because sometimes it never does. Concerning follicle density, girls with TS have a significantly lower ovarian reserve (between 33,000 and 80,000 follicles per ovary) compared with girls of corresponding age without TS (between 250, 000 and 500, 000). The number of follicles in cryopreserved ovarian tissue is a key factor because it is well known that $>50\%$ of primordial follicles are lost after transplantation because of ischemia.

In fact, both the number and quality of follicles in girls with TS are of vital importance. Follicles with abnormal morphological features were described by Nadesapillai et al. (1), Mamsen et al. (5), and in another article from the same group (2). The X-chromosome content of ovarian cells was karyotyped using fluorescence in situ hybridization. Among follicles in girls with numerical aberrations, 92% of oocytes showed normal chromosome content, whereas granulosa and stromal cells were aneuploid mainly (2). As reported above, using a xenograft model, Peek et al. (2) observed that primordial follicles did indeed undergo folliculogenesis. Fragments from 12 patients with TS contained follicles at all stages after xenografting, including secondary and antral follicles (2). Notably, however, follicle density in postpubertal patients was significantly lower than in prepubertal patients, which is why we recommend cryopreserving ovarian tissue before the age of 12 years when possible.

In this study, Nadesapillai et al. (1) attempted to identify which girls with TS could benefit from OTC, and we acknowledge that they achieved their goal. By investigating such a large series, they were able to offer concrete clinical recommendations for fertility preservation and OTC in Turner girls. Their article (1) concluded that karyotyping additional cell lines in girls with the 45X karyotype in lymphocytes was strongly recommended. A combination of karyotyping and measuring AMH and follicle-stimulating hormone levels would allow clinicians involved in fertility preservation to properly counsel girls with TS and their parents and determine which girls with TS could stand a real chance of childbearing after OTC. Importantly, cardiac screening is strongly advocated before fertility preservation counseling.

Of course, some concerns remain:

- Could unilateral oophorectomy further impair hormone production in these girls? Although it is widely known that unilateral oophorectomy will advance the age of menopause in women of reproductive age by only 1–2 years, we are unable to answer this question in girls with TS. We favor unilateral oophorectomy because we know that the follicle pool is already smaller in patients with TS

than in their healthy counterparts, and it is likely that their ovarian reserve will be completely depleted at a very young age.

- Young girls (before 7–8 years of age) with TS are not fully aware of their health status, so making a conscious choice is challenging, despite their parents' participation in the decision-making process.
- Although a recent clinical pregnancy (ending in miscarriage) was described after autotransplantation of cryopreserved ovarian tissue in a girl with TS, there is a lack of evidence of successful outcomes. Only one case of allotransplantation between monozygotic twins, both with TS mocaicism, achieved a LB, knowing that the donor still had well-preserved ovarian function. However, there have been no LBs after autotransplantation of cryopreserved ovarian tissue in patients with TS.
- Prepubertal ovaries contain numerous abnormal follicles, but previous studies have demonstrated that their proportion decreases after xenografting in TS (2) as well as XX patients, as reported in one of our previous articles on prepubertal ovaries.

Although some experts still consider the technique experimental in patients with TS, we have always believed that they could be candidates for OTC as long as there is a reasonable chance of finding primordial follicles in their ovarian tissue. In our opinion, OTC should be performed before puberty (at least before age 12) to avoid depletion of the ovarian reserve as best we can. We also agree with Nadesapillai et al. (1) and Dunlop et al. (3) that young patients should be psychologically prepared for OTC, as they need to make a decision they may not be emotionally or mentally ready for.

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