Archives of Reproductive Medicine and Sexual Health

ISSN: 2639-1791

Volume 2, Issue 2, 2019, PP: 09-12

Fertility Preservation in Turner Syndrome Patients-Safety Issues and Problems of the X Chromosomal Content of Granulosa Cells-Will it Hinder Oocyte Development–Still Needs Clarification-A Short Communication

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Turners syndrome (TS) is one of the commonest sex chromosome disorders, that affects roughly 1 in 2500 newborn girls [1]. Biggest problem that is encountered in TS patients is besides short stature, they fail to undergo puberty,in view of increased rate of ovarian follicular atresia that => gonadal insufficiency and infertility. Practically half of the women found to have TS have monosomy X (45X), but mosaicism for a second normal 45XX cell population is present in 15% of women [2]. Roughly 20% of patients having TS go through puberty normally having spontaneous periods but are under the risk of premature ovarian failure (POF) [3]. Ovarian stimulation along with oocyte cryopreservation and cryopreserving ovarian tissue are the methods that can be utilized for fertility preservation.

Discussion of fertility preservation options is done at the earliest for any girl with TS; which might be at the time of diagnosis, despite if diagnosis is reached at the time of infancy or childhood. Serial examination of ovarian reserve markers can be attempted. In girls that are prepubertal having mosaic TS possessing a low percentage of cells with 45X karyotype relative to 46XX and when antimullerian hormone (AMH) levels are not inappropriately low for patients age (i.e. levels in the lower quartile for age [4]), some practitioners use (every 2-3mths) AMH monitoring for making a decision regarding when intervention is required. Once AMH levels start to present a decrease in twice consecutive checkups, fertility preservation should be thought off. With this method, TS girls might get help to mature both physically and psychosocially, and be of more help in being able to use procedures like oocyte cryopreservation instead of being limited to the ovarian tissue cryopreservation option which till now is in experimental stage.

Once the ovarian reserve assessment shows an age –inappropriate reduction in ovarian reserve in girls having mosaic TS, and irrespective of ovarian reserve in the non mosaic TS population Oktay et al. [5] recommended to consider fertility preservation at the earliest age possible.

For those who are sexually and or psychosocially immature or unable to tolerate ovarian stimulation procedures, oocyte cryopreservation needs to be recommended under experimental, Institutional Review Board (IRB)-Approved protocols. Besides obtaining an IRB-Approved consent from the parents, verbal consent for the procedure must be got from the children >9 years of age. The consent form needs to explain properly that at present the success of ovarian cryopreservation and transplantation can't be quantified in girls with TS [5].

In post menarchal girls, who are typically 13 years of age or older and mature enough developmentally to tolerate ovarian stimulation, oocyte cryopreservation should be presented. Though the procedure is not considered experimental in adults, A Human Subjects and Institutional Review Board approved consent is still encouraged for this procedure to be undertaken

in the pediatric population. At least, parents and children need to be given a detailed written consent form that explains the potential limitations along with the absence of TS specific success rates from this procedure. Embryo cryopreservation can be considered under similar situations for the rare females coming with TS that have reached adulthood with enough Ovarian reserve persisting and a committed partner or those who are ready to utilize donor sperms [reviewed in ref 5].

Peek et al tried to study what is the X Chromosomal content of oocytes and granulosa cells of primordial/primary (small) follicles and stromal cells in ovaries of young patients with TS. Most women with TS experience a decrease or complete loss of fertility in view of accelerated loss of gametes. For determining if fertility preservation in this group of women is feasible, a strong requirement of information on the chromosomal content of ovarian follicular and stromal cells. Small follicles (<50μm) and stromal cells were isolated from ovarian tissue of young TS patients and examined for their X Chromosomal content. Besides ovarian cells, various other cell types from the same patient was analysed. Following unilateral ovariectomy, ovarian cortex tissue was obtained from 10 TS patients (aged 2-18 yrs) with numerical abnormalities of the X Chromosome. Ovarian cortex fragments were prepared and cryopreserved. One fragment from each patient was thawed and enzymatically digested to obtain stromal cells and primordial/primary follicles. Stromal cells/granulosa cells and oocytes were analysed by FISH using an X Chromosome specific probe. Extra-ovarian tissue used in control was obtained from subjects undergoing oophorectomy as part of their gender affirming surgery. Ovarian follicles were detected in 5 of the 10 patients studied. A method was developed to determine the X Chromosomal content of meiosis 1 arrested oocytes from small follicles. This showed that 42 of the 46 oocytes (91%) that were analysed had a normal X Chromosomal content. Granulosa cells were largely 45X, but showed different levels of X Chromosome mosaicism between patients and between follicles of the same patient. Despite the presence of a low percentage (10-45%) of 46XX ovarian cortex stromal cells. Normal macroscopic ovarian morphology was observed (figure 1,2,3). The level of mosaicism in lymphocytes, buccal cells or urine derived cells was not predictive of mosaicism in ovarian cells.The limitations of this study was that the results were based on a small number (n=5) of TS patient samples but give proof that the majority of oocytes have a normal X Chromosomal content and that follicles from the same patient can differ with respect to the level of mosaicism of their granulosa cells. The functional consequences of these observations need more investigations. The wider implications of this study are that despite normal ovarian and follicular morphology stromal cells and granulosa cells of small follicles in with TS may display a >level of mosaicism. Moreover the level of mosaicism of ovarian cells cannot be predicted from the analysis of the extraovarian tissue. These findings should be considered by physicians when offering cryopreservation of ovarian tissue as an option for fertility preservation in young TS patients [6].

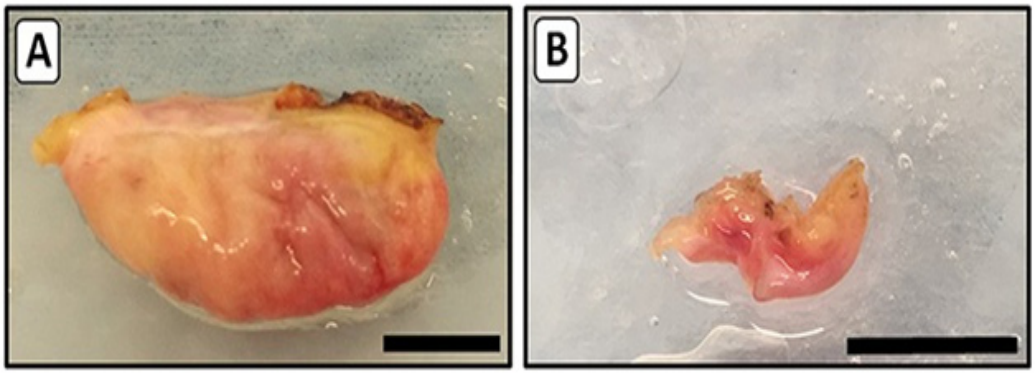

Fig 1. **Courtesy ref no.6-Macroscopic images of ovaries from TS patients.** *After unilateral ovariectomy intact ovaries were transported to the laboratory and photographed. A representative example of an ovary from a mosaic 45,X/46,XX girl (Patient D) with normal morphology and volume is shown in panel A. The small fibrous streak ovary shown in photo B is from a girl with 45,X monosomy (Patient I). The brown discoloration of the tissue is due to electrocauterization during surgery. Bars represent 1 cm.*

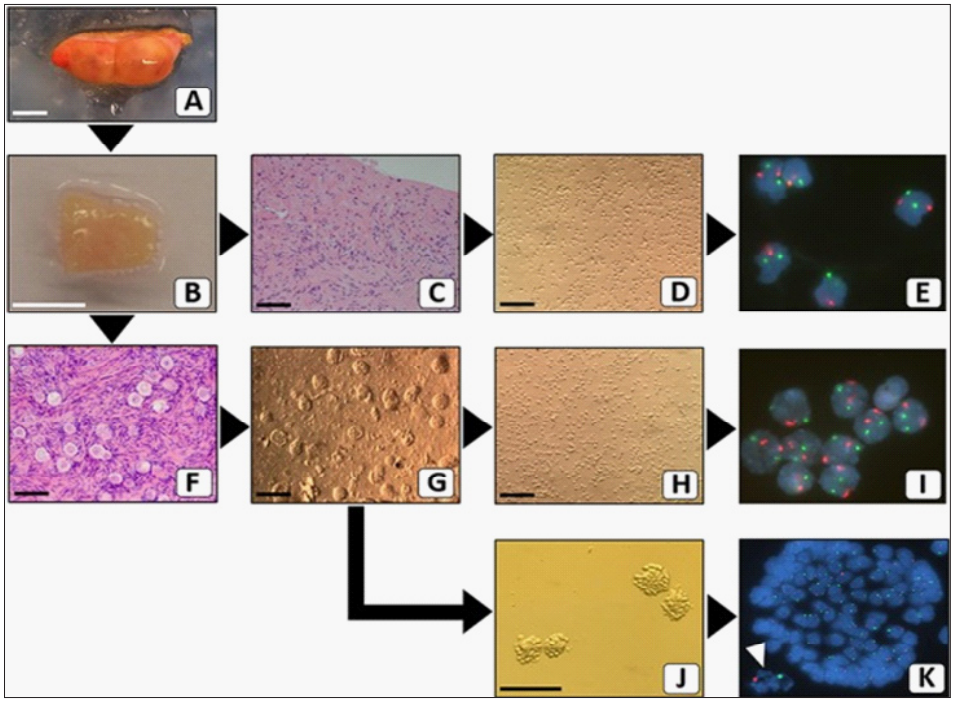

Fig 2. Courtesy ref no-6-Flow scheme for separating ovarian cortex cellular constituents prior to FISH analysis. *After the surgical removal of the intact ovary (A), cortical fragments were prepared. Part of one representative cortex fragment (B) was analysed by standard haematoxylin–eosin staining for the presence of follicles. When no follicles were present (C), the remaining part of the fragment was used to make a suspension of stromal cells (D), for interphase FISH with chromosome X (green) and chromosome 18 (red)-specific probes (E). When follicles were present (F), the remaining part of the cortex fragment was used to make a cell suspension (G) from which small follicles were manually picked up. These isolated follicles were subjected to further digestion (J) and subsequently analysed by FISH. The white arrowhead points to the signals from the oocyte (K). Part of the remaining cell suspension (H) was used for FISH analysis of stromal cells (I). Bars represent 1 cm (A and B) or 100 µm (C, D, F–H and J). Original magnification of FISH signals was ×630 (E, I and K).*

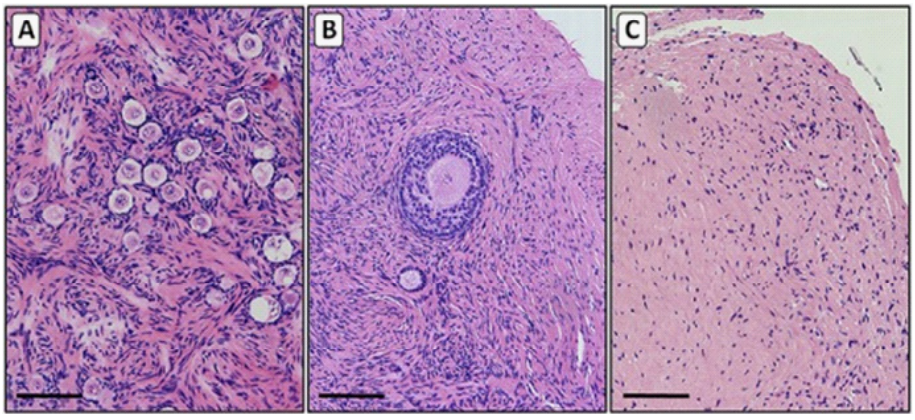

Fig 3. Courtesy ref no.-6 -Histological sections of ovarian cortex from patients with TS. *Haematoxylin–eosin stained 4-µm sections were prepared from cortical tissue from ovaries of mosaic (panels A and B) and 45, X monosomy TS patients (panel C). In addition to the variable number of small follicles in the tissue of the mosaic patients (panel A), a low number (<2%) of secondary follicles were observed (panel B). The ovarian tissue of the monosomic 45, X patients contained no follicles and showed a fibrous texture with relatively low number of cells (panel C). Bars represent 100 µm.*

There are some doubts regarding controlled ovarian hyperstimulation in women who present with TS would give enough number of high quality oocytes for subsequent use later and the safety of the procedure with preexisting medical risks associated with a TS patients. Talauliker et al. who have been running a clinic dedicated to TS reported their results from a cohort of 833 women in their clinic. Of the 833 TS patients in this retrospective study 19.7% had menstrual cyclicity and proof of ovarian activity. Ovarian reserve assessment, that including AMH measurements was suggested to 55 women having regular periods and showing an interest in fertility preservation. Of these 7 women had showed interest in oocyte cryopreservation. All of them were medically fit with normal ECHO study and had baseline ovarian reserve tests like serum FSH, AMH, LH, E2 along with antral follicle count (AFC) using transvaginal sonography (TVS). Ovarian stimulation utilized were a daily subcutaneous (s/c) injection of human menopausal gonadotropins (HMG) in combination with gonadotropin releasing hormone (Gn RH) antagonist and dose of HMG varying from 225 to 450 units based on the ovarian reserve, with antagonist started on day 6 of stimulation. Following 9-10 of stimulation, monitored by TVS and E2 measuements oocyte retrieval was done 36 hrs following HCG. Oocytes morphology was analyzed, and oocytes cryopreserved by vitrification technique [7]. The oocyte retrieval rates (mean+_SD,9+_3.16) in women with TS were comparable to the published data from healthy women. The oocyte yield was higher than expected based on the low anti mullerian hormone levels. There was no correlation between baseline AMH or AFC levels and the number of oocytes retrieved. Thus they concluded that oocyte cryopreservation after ovarian stimulation appears to be safe and successful in women with mosaic TS who wish to consider fertility preservation [8].

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Citation: **Kulvinder Kochar Kaur, Gautam Allahbadia, Mandeep Singh.** *Fertility Preservation in Turner Syndrome Patients-Safety Issues and Problems of the X Chromosomal Content of Granulosa Cells-Will it Hinder Oocyte Development–Still Needs Clarification-A Short Communication. Archives of Reproductive Medicine and Sexual Health. 2019; 2 (2): 09-12.*