Characterization of follicles in girls and young women with Turner syndrome who underwent ovarian tissue cryopreservation

Linn Salto Mamsen, Ph.D.,[a] Karol Charkiewicz, Ph.D., M.B.A.,[a,b] Richard A. Anderson, M.D., Ph.D.,[c] Evelyn E. Telfer, Ph.D.,[d] Marie McLaughlin, Ph.D.,[d] Thomas W. Kelsey, Ph.D.,[e] Stine G. Kristensen, Ph.D.,[a] Debra A. Gook, Ph.D.,[f,g] Erik Ernst, Ph.D.,[h] and Claus Yding Andersen, D.M.Sc.[a]

[a] Laboratory of Reproductive Biology, The Juliane Marie Centre for Women, Children and Reproduction, University Hospital of Copenhagen, Rigshospitalet, Copenhagen, Denmark; [b] Department of Perinatology and Obstetrics, Medical University of Bialystok, Bialystok, Poland; [c] Medical Research Council Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom; [d] Institute of Cell Biology, School of Biological Sciences and Genes and Development Group, School of Biomedical Sciences, University of Edinburgh, Edinburgh, United Kingdom; [e] University of St. Andrews, School of Computer Science, North Haugh, St. Andrews, United Kingdom; [f] Reproductive Services and Melbourne IVF, Royal Women's Hospital, Parkville, Victoria, Australia; [g] Department of Obstetrics and Gynaecology, University of Melbourne, Parkville, Victoria, Australia; and [h] Department of Obstetrics and Gynaecology, Randers Regional Hospital, Randers, Denmark

Objective: To characterize ovarian follicles of girls and young women with Turner syndrome (TS) who underwent ovarian tissue cryopreservation (OTC).
Design: Retrospective case–control study.
Setting: University hospital.
Patient(s): Fifteen girls and young women with TS aged 5–22 years at OTC were included, together with 42 control girls and young women aged 1–25 years who underwent OTC because of cancer.
Intervention(s): None.
Main Outcome Measure(s): Follicle density (follicles/mm^3), morphology, and health were assessed in ovarian cortex biopsies from TS patients and compared with controls. Hormone concentrations were measured in serum and follicle fluids. Immature cumulus oocyte complexes were obtained and matured in vitro.
Result(s): Follicles were found in 60% of the biopsies (9 of 15) from TS ovaries. In 78% of the ovaries (7 of 9) with follicles, the follicle density was within the 95% confidence interval of the control group. There was a high rate of abnormal follicle morphology. Six follicle-specific proteins were expressed similarly in TS and control ovaries. However, apoptosis and zona pellucida protein expression were found to be abnormal in TS. Turner syndrome follicle fluid from small antral follicles had lower concentrations of estrogen and testosterone and higher concentrations of antimüllerian hormone than controls. Thirty-one cumulus oocyte complexes were collected from one patient and cultured for 48 hours in vitro, resulting in five metaphase II oocytes (maturation rate 16%, degeneration rate 19%).
Conclusion(s): The benefits of OTC may be limited to a highly selected group of TS mosaic patients in whom a sizeable pool of normal follicles is present at OTC. (Fertil Steril® 2019;111:1217–25. ©2019 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Turner syndrome, follicle density, ovarian tissue cryopreservation

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Reprint requests: Linn Salto Mamsen, Ph.D., Rigshospitalet, Laboratory of Reproductive Biology, Copenhagen 2100, Denmark (E-mail: linn.salto.mamsen@regionh.dk).

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Turner syndrome (TS) is caused by the absence of one of the two X chromosomes in all cells or a proportion of cells, affecting approximately 1:2,000 Caucasian girls (1). The most common karyotype is 45,X (47%), followed by different mosaicisms, most commonly 45,X/46,XX (17%). The main reproductive effect of TS is primary (or premature) ovarian insufficiency (POI) (2, 3). Although menarche occurs spontaneously in 15%–30% of girls with TS, the prevalence of natural pregnancy is as little as 2%–7% (2–6).

Two previous studies have reported the presence of ovarian follicles in 15 of 47 (7) and in 8 of 9 (8) adolescent patients with TS. Mosaicism and spontaneous menarche were predictive for the presence of follicles, consistent with pregnancy being most prevalent in women with mosaic TS (2, 5), and ovarian tissue cryopreservation (OTC) has been suggested as an option for fertility preservation (7). Predictors for the presence of ovaries without follicles in girls with TS include karyotype 45,X, low serum antimüllerian hormone (AMH), high serum FSH, and absent menarche or puberty (7, 8). Oocyte donation is the only way for TS patients with POI to conceive, but pregnancies carry a substantial risk to mother and fetus (6, 9, 10).

The aim of this study was to characterize the number and morphology of follicles in girls and young women with TS, who underwent OTC. We have also evaluated follicle and oocyte function to assess the potential for future fertility restoration, to evaluate whether to perform OTC in this group of patients.

MATERIALS AND METHODS

Patients and OTC

A total of 15 girls and young women with TS aged 5.0–22.4 years (mean age 15.4 years) were included together with 42 control girls and young women aged 1.5–25.5 years (mean age 15.2 years). The control group members were referred to the Danish program for fertility preservation by OTC because of a cancer diagnosis and have not received any gonadotoxic treatment before OTC. Patients from both groups were only included if an ovarian cortex biopsy was spared for histology in connection with OTC. All patients underwent OTC between the years 2002 and 2017. Follicle densities in the control girls with cancer aged <18 years have previously been published (11). One additional ovarian biopsy from a girl with Fanconi anemia was included for immunohistochemical (IHC) staining. During the preparation of ovarian tissue, two patients presented with visible antral follicles on the ovarian surface, from which follicle fluid from a total of eight small antral follicles (3.4–6.7 mm in diameter) was collected. Additionally, 31 cumulus–oocyte complexes (COCs) located in the surplus medulla tissue (which was not cryopreserved) were obtained from one patient. These COCs derived from much smaller follicles with a diameter of only a few millimeters. Patient characteristics are given in Table 1; there were seven in the Danish cohort, five from United Kingdom, and three from Australia. All controls were age-matched patients having OTC for conditions other than TS in Denmark. The ovarian cortex was prepared as previously described for slow freezing (12, 13) and stored in liquid nitrogen. Additionally, one small piece of cortex ($\leq 2 \times 2 \times 1$ mm) was obtained for histological examination. The OTC schemes were approved by the ethics committee of Copenhagen and Frederiksberg (H-2-2011-044) and Lothian Health (ref. 06/S1103/26). The storage and collection of patient data were approved by the Ministry of Health (J. no. 30-1372) and by the Danish authorities to comply with European Union tissue directives. All participants, or parents for younger patients, gave informed consent in writing.

Tissue Processing

Tissues from Edinburgh (subjects 1, 3, 9, 13, and 14) were fixed in 10% neutral buffered formalin, and tissues from Melbourne (subjects 7, 8, and 11) were fixed in 4% paraformaldehyde, whereas the remaining tissues from Copenhagen were fixed in Bouin's solution. In Edinburgh and Melbourne 5- or 6-μm sections of paraffin-embedded human ovarian cortex were prepared, de-waxed, and stained with hematoxylin and eosin for estimation of follicle density (14). In Copenhagen, 30-μm sections were stained with periodic-acid Schiff and Mayer's reagents for further estimation of follicle density, and 5-μm sections were processed for IHC staining.

Immunohistochemical staining

Sections were deparaffinated in xylene and rehydrated in ethanol, followed by antigen retrieval in either 10 mM sodium citrate, pH 6, or 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 9. Retrieval was not required for zona pellucida (ZP) protein 1 and 2 (ZP1 and ZP2) (15). Endogenous activity was inhibited using 1.5% peroxidase, followed by inhibition of nonspecific binding with 1% bovine serum albumin (Sigma Aldrich). Sections were incubated with primary antibodies overnight at 4° except for ZP protein antibodies, which were incubated at 37°C for 1 hour; details of antibodies and conditions are given in Supplemental Table 1 (available online). Secondary antibody used was rabbit-anti-mouse-HRP (Dako), visualized with 3.3′-diaminobenzidine tetrahydrochloride (DAB+ Substrate Chromogen System, Dako). Both Universal negative control serum (BioCare Medical) and antibody dilution buffer were used in place of primary antibody as negative controls and showed no staining (Supplemental Fig. 1). An Apop Tag Plus Peroxidase In Situ Apoptosis Detection Kit (Millipore) detecting apoptosis TUNEL was also included.

Follicle Density

Two methods were used to estimate the nongrowing follicle density in the ovarian cortex. In Copenhagen, the follicle density was estimated in 30-μm sections, using a mathematical model described by Schmidt et al. (16). In brief, this model was based on the fraction of sections, the mean primordial follicle diameter, and a correction factor (α) to account for the possibility of counting the same follicle more than once. Because the mean diameter of a primordial follicle is 44 μm (17) and the sections were 30 μm, there was a possibility to count the same follicle two or three times (16). In Edinburgh and Melbourne follicle density was measured in 5- to 6-μm

TABLE 1

Clinical data on the TS girls, including age, karyotype, menarche, and serum hormone levels.

Subject no.	Age at cryopreservation (y)	OTC center	Karyotype	Spontaneous menarche	AMH (ng/mL)	FSH (IU/L)	Nongrowing follicles per mm^3 in ovarian cortex
1	5.0	Edinburgh	45,X	NA	0.73	NA	106
2	8.8	Copenhagen	45,X (161/200, 80%) 46,X,r(X) (39/200, 20%)	NA	<0.067	4.4	0
3	13.5	Edinburgh	45,X 46,X,r(X)	Yes (11 y)	0.412	5.5	3
4	13.5	Copenhagen	45,X (7%) 46,XX (93%)	Yes (13 y)	NA	3.1	47
5	14.4	Copenhagen	46,X, del(X) (p11) (10/10, 100%)	NA	<0.040	4.2	0
6	14.4	Copenhagen	46X i(Xq10) (40%) 46,XX (60%)	Yes (14 y)	1.618	4.5	20
7	14.7	Melbourne	45,X (43%) 46 X,add (X) (q28) (56%)	No	<0.4	82.9	0
8	14.8	Melbourne	45,X (8%) 46,XX (92%)	Yes (13 y)	20.2	5.1	519
9	15.4	Edinburgh	45,X 46,X,r(X)	Yes (14 y)	0.297	5.1	3
10	17	Copenhagen	45,X 46,X,i(Xq)	NA	NA	31	0
11	17.4	Melbourne	46X, deletion X(p11.23)	Yes (11 y)	3.2	12	0
12	17.8	Copenhagen	45,X (60%) 46,XX (40%)	NA	NA	NA	138
13	20.7	Edinburgh	45,X 47,XXX	Yes (13 y)	0.365	0.4	1
14	22.3	Edinburgh	45,X 46,XX	Postpubertal	0.06	<0.1	3
15	22.4	Copenhagen	45,X	NA	NA	13	0

Note. Numbers and percentages given in parentheses are of analyzed lymphocytes. i(X) = isochromosome; r(X) = ring X chromosome.

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sections by the method of McLaughlin et al. (14). In brief, all tissue sections were examined for the presence of follicles. To avoid overcounting, follicles were only assessed when the nucleolus was observed. The follicle density was determined by dividing the total number of follicles in the biopsy by the volume of tissue analyzed. To evaluate whether the two methods of data collection were comparable a predictive model was used (14), which combines an age-related normative model for follicle population in the human ovary (18) and an age-related normative model for the volume of the human ovary (19). Comparison of data obtained by the two methods used shows good agreement using the predictive model (14).

Hormone Assays

Follicle fluid was aspirated using a 29-gauge syringe from small antral follicles (<7.0 mm in diameter) during preparation of ovarian tissue for cryopreservation. Estradiol, T, AMH, and inhibin-B concentrations were measured in follicle fluid (after appropriate dilution) using commercially available ELISA kits. Estradiol and T were measured with Nova Tech ELISA assays (DNOV003, DNOV002); AMH and inhibin-B were measured with the UltraSensitive AMH/MIS and inhibin-B ELISA kit (AL-105, AL-107; Ansh Labs) (20).

Western Blot

Western blot analyses were performed according to the manufacturer's instructions (Invitrogen, provided by Thermo Fischer). In brief, follicle fluid proteins were separated on a NuPAGE 4%–12% Bis-Tris mini gel, and the proteins were subsequently blotted to a polyvinylidene difluoride membrane (Thermo Fischer). The membrane was blocked in 5% skimmed milk and incubated with primary AMH antibody (AMH 39/48; Ansh Labs) overnight at 4°C and subsequently with secondary horseradish peroxidase–conjugated goat-anti-mouse antibody (Sigma Aldrich) for 1 hour at room temperature. Signal was detected with Pierce SuperSignal West Femto Substrate (Termo Fisher) and visualized with the DNR MicroChemi 4.2 bio-imaging system. We have previously validated the specificity of the AMH antibody used by blocking with surplus recombinant AMH, whereafter all AMH-related bands disappeared (21).

In Vitro Maturation of COCs

Immature COCs from small antral follicles were collected from the surplus medulla tissue from one patient with mosaic TS and cultured in in vitro maturation medium (Origio) supplemented with FSH and LH and overlaid with liquid paraffin at 37°C in 5% CO_2 humidified incubator for 48 hours, as previously described (22). Cumulus cells were removed after 48 hours, and the developmental stage of the denuded oocytes was evaluated using an inverted microscope and classified as either "GV," with a distinct germinal vesicle, "MI," no germinal vesicle and no polar body, or "MII," one polar body extruded.

Statistics

Data on follicle density from the Danish girls aged <18 years have previously been published (11). An adjusted model including the new data has more normally distributed residuals (84% of a perfect Gaussian distribution) and hence is expected to have lower generalization error when assessing densities from subjects not used to derive the model. Statistical analysis was performed using the GraphPad Prism 6.07 program (GraphPad Software) and Microsoft Excel version 14.6, with linear regression to evaluate follicle density against age and Mann-Whitney *U* test to compare the hormone concentrations in follicular fluid. Significance was defined as $P<.05$.

RESULTS

A total of 15 girls and young women with TS aged 5.0–22.4 year old were included in this study. Three cases were postpubertal (i.e., age > 18 years). Moreover, 11 of the 15 cases were diagnosed with mosaic TS.

Follicle Density

Follicles were found in 60% of the biopsies (9 of 15) from TS ovaries: eight girls with a mosaic karyotype and one 45,X, who was aged only 5 years and the youngest in the series (Table 1). No follicles were found in two patients with menopausal FSH levels (ages 14 and 17 years), in two with mildly elevated FSH levels (aged 17 and 22 years), or in a further two patients (aged 8 and 14 years) with undetectable AMH levels. All patients with follicles had a detectable AMH level and/or FSH < 10 IU/L (except one for whom no hormone data were available).

Although follicle density was below the age-matched mean for most TS patients, it was within the 95% confidence interval (CI) for controls in 78% of the cases (seven of nine); in 22% (two of nine) it was below the 95% CI (Fig. 1). Including both TS patients with and without follicles (n = 15), no correlation between follicle density and age was found ($P>.1$). When follicle densities from the present study were combined with previously published TS data including both patients with and without follicles (n = 23) (8), no correlation between follicle density and age was found ($P>.1$) (Fig. 1). The cortex tissues were fixed in either 4% paraformaldehyde or Bouins solution before hisological examination, and theoretically this difference in fixation may impact the follicle densities; however, the two methods have been previously compared and found to give very comparable results (14).

Follicle Morphology and IHC

Most ovaries (six of nine) showed a high rate of abnormal follicle morphology. The follicle morphology of three subjects is illustrated in Figure 2. The major abnormalities observed in primordial follicles were misshapen, vacuolated oocytes and an incomplete layer of granulosa cells surrounding the oocyte (Fig. 2A), leading to irregular oocyte shape and partial lack of connection to the basal lamina and stromal cells. This also manifested in di-oocytic follicles (Fig. 2B). In many follicles the granulosa cells were swollen and did not have the flattened appearance normally observed in primordial follicles. Nuclear material within some oocytes was diffuse or pale, suggesting an absence of the germinal vesicle membrane (Fig. 2A). Empty and degenerating follicles were often seen (Fig. 2A, B, D). Granulosa cell invasion of the oocyte of primary follicles was occasionally seen (Fig. 2E), together with shrunken granulosa cells and contracted ooplasm (Fig. 2F). In some subjects, normal morphology was detected in the majority of follicles (Fig. 2G, H, I), as in control ovarian tissue.

The presence of six granulosa cell or oocyte-specific proteins (20, 23) was detected by IHC in three TS ovaries (subjects 1, 6, and 12) and one age-matched control (Supplemental Fig. 2). Staining by TUNEL (subject 8) showed some areas with healthy follicles (Supplemental Fig. 3C); however, there were other areas of poor stromal integrity, with most follicles having only an occasional healthy granulosa cell and evidence of apoptosis in the oocyte (Supplemental Fig. 3D). High levels of ZP1 and ZP2 staining were observed within the oocytes and scattered throughout the cortical stromal tissue, which may indicate residual ZP proteins from eliminated follicles and which has been observed previously after xenografting of normal ovarian cortex (Debra Gook, unpublished data, December 1, 2006) (Supplemental Fig. 3A and 3B). Normal very low levels of ZP1 expression were detected in 43% of the primordial follicles (83 of 193), and levels were elevated in 57% (110 of 193), which suggests atresia. Normal ZP2 expression was detected in 23% of the follicles (45 of 199). The proportion of morphologically normal follicles was estimated to be 7% from TUNEL staining.

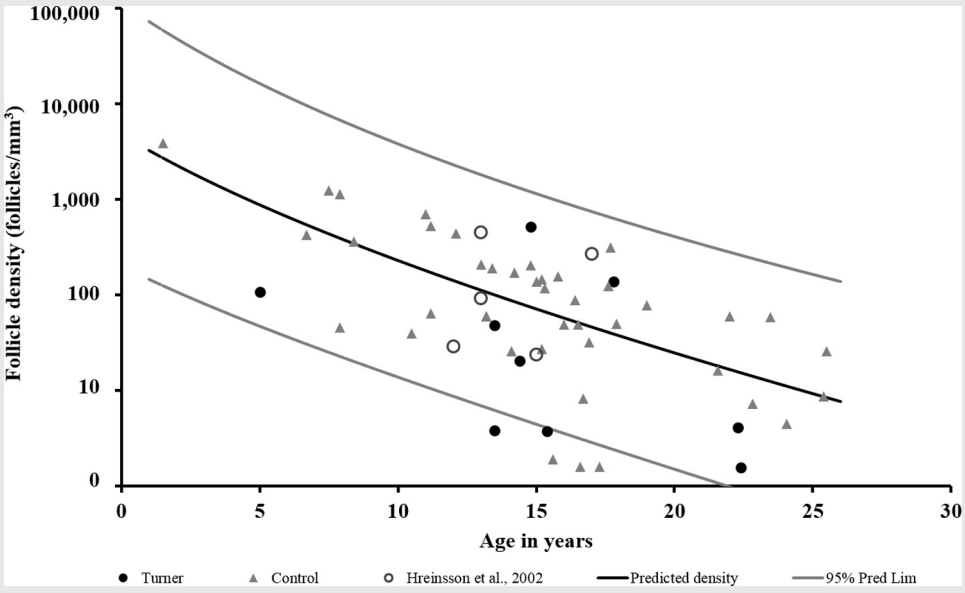
Hormone Concentrations in Follicle Fluid

Hormone concentrations were measured in eight follicles obtained from two girls with mosaic TS (subjects 4 and 12): none of the other girls had small antral follicles that could be aspirated. The mean diameter (±SEM) of the follicles was 5.0 ± 0.4 mm (range, 3.4–6.7 mm), and the mean concentrations in follicle fluids were as follows: E_2 19 ± 9 nmol/L, T 132 ± 23 nmol/L, AMH 2,941 ± 587 ng/mL, and inhibin-B 81 ± 15 ng/ml (Fig. 3A, Supplemental Table 2). The concentrations of estrogen and T in follicular fluid from girls with TS were significantly lower and AMH higher than the concentrations in follicular fluid from size-matched (3.4–5.9 mm) follicles from age-matched controls (24) ($P=.036$, .001, and .005, respectively; Supplemental Table 2). No differences in inhibin-B concentrations between TS and normal follicle fluids were found ($P>.1$). All six follicle fluid samples from subject 12 were analysed with Western blot detecting for AMH and compared with six follicle fluid samples obtained from control size-matched follicles, and no differences in AMH processing were detected (Fig. 3B).

In Vitro Maturation of Oocytes

Cumulus–oocyte complexes (n = 31) were cultured from one mosaic girl (subject 12). After 48 hours of culture, 6 had degenerated and 13 remain at the germinal vesicle stage, whereas 12 had resumed meiosis (7 at metaphase I and 5 at the metaphase II [MII] stage), resulting in a maturation rate

FIGURE 1



Follicle density in normal ovaries (*triangles*) and in girls with TS (*filled circles*) plotted against age. Only TS tissues with follicles detected (9 of 15) are included. Previously published data on follicle density in girls with TS (n = 5) are included (8) (*open circles*).

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of 16% and degeneration rate of 19%. Although this outcome is only from one patient, this is a lower maturation rate and higher degeneration rate than that previously reported by our group for COCs from young women (<20 years) with normal ovaries (55%, 4%, respectively) (22).

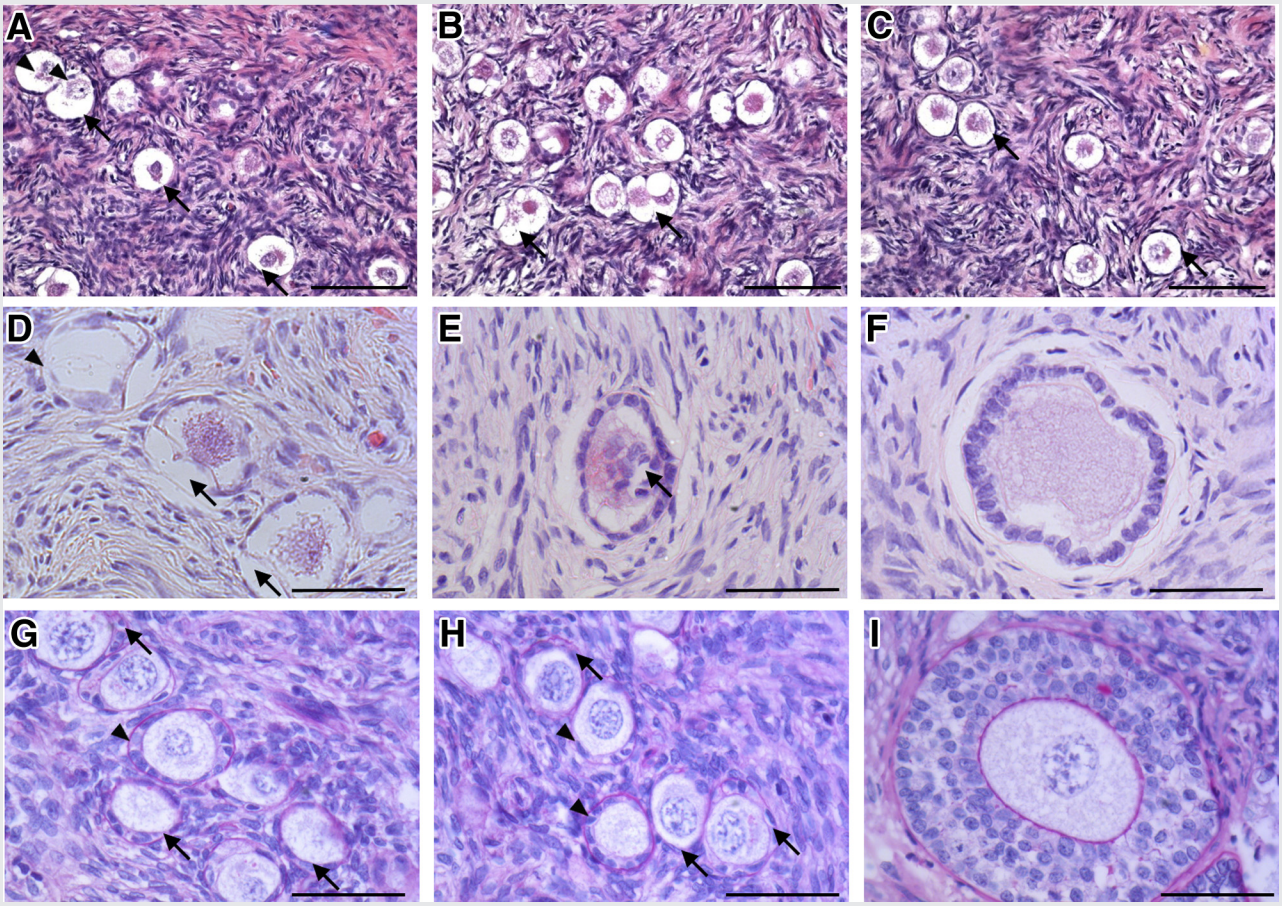
DISCUSSION

To our knowledge, this study is the first to characterize follicles and explore the in vitro maturation potential of oocytes from girls and young women with TS in comparison with age-matched controls. Follicles were detected in the ovarian cortex in 9 of 15 patients with TS, and the presence of follicles was associated with detectable serum AMH and normal FSH levels. Follicle density was within the 95% CI of normal age-matched girls and young women in seven of these nine patients. Turner syndrome patients originated in Denmark, the United Kingdom, and Australia, whereas all control patients were Danish, and we cannot rule out that the evaluated ovarian parameters would have been different in a cohort originating in the United Kingdom or Australia, although the methods used were cross-validated (14). All except one had mosaic TS, confirming observations from Borgström et al. (7) and consistent with women with this karyotype having a higher chance of conceiving (2, 5, 7, 9). Although follicles were detected in the youngest patient included, a 5-year-old with 45X karyotype, primordial follicle morphology was abnormal, with most follicles having an incomplete granulosa cell layer, oocyte vacuoles or collapse, or absent oocyte (empty follicles). The other patient (aged 22 years) with nonmosaic TS had a zero follicle count. These data, albeit based on this very limited patient group, suggest that OTC may not be an appropriate method for fertility preservation in girls and young women with nonmosaic TS, even in the absence of overt POI.

The diversity of follicle morphology between patients further complicated the prediction of who may benefit from OTC. In some cases follicle morphology was similar to normal, whereas in others a high proportion and range of abnormalities were seen. This is supported by increased expression of ZP proteins (normally very low in healthy primordial follicles and elevated in atretic follicles [25]) and DNA fragmentation detected in one TS patient. This may indicate limited potential for later fertility in this TS patient. Empty follicles have been observed in human ovarian cortex cultured in the presence of an inhibitor of mammalian target of rapamycin (mTOR) (26) and were detected in ovaries analyzed in all three centrers, negating any effect of different fixation methods. However, all six glycoproteins related to oocyte growth and follicle health that were evaluated were detected, suggesting that at least a proportion of TS follicles may be normal and functional.

From one 18-year-old girl with TS, immature COCs were aspirated and matured in vitro. This demonstrates that TS oocytes can develop to the MII stage and may possess fertility potential. This is consistent with a case study that reported oocyte retrieval and maturation to MII in a young woman with mosaic TS (27), with 65% of the oocytes obtained having a normal karyotype. Although the karyotype was not assessed here, these findings suggest that immature oocytes can be collected from the medulla tissue in connection with OTC and that these oocytes could be an additional source for fertility preservation in mosaic TS, although the maturation rate seemed lower than with oocytes from normal women.

FIGURE 2



Ovarian morphology in tissue from three girls with TS. **(A–C)** Subject 8. **(A)** Primordial follicles with missing granulosa cells (*arrows*); some follicles had diffuse or pale nuclear material, suggesting absence of germinal vesicle membrane (*arrowheads*); **(B)** fused follicles (*arrows*); **(C)** follicles with normal granulosa cells (*arrows*). **(D–F)** Subject 1. **(D)** Primordial follicles with collapsed oocytes (*arrows*) and empty follicle (*arrowhead*); **(E)** granulosa cell invasion of the oocyte of a primary follicle (*arrow*); **(F)** primary to secondary transition, shrunken granulosa cells and contracted ooplasm. **(G–I)** Subject 12. **(G, H)** Normal morphology in the majority of primordial follicles, with flat granulosa cells (*arrows*) and primary/intermediate follicles with cuboidal granulosa cells (*arrow heads*); **(I)** tertiary follicle. Scale bars = 100 μm in **A-C**; 50 μm in **D-I**.

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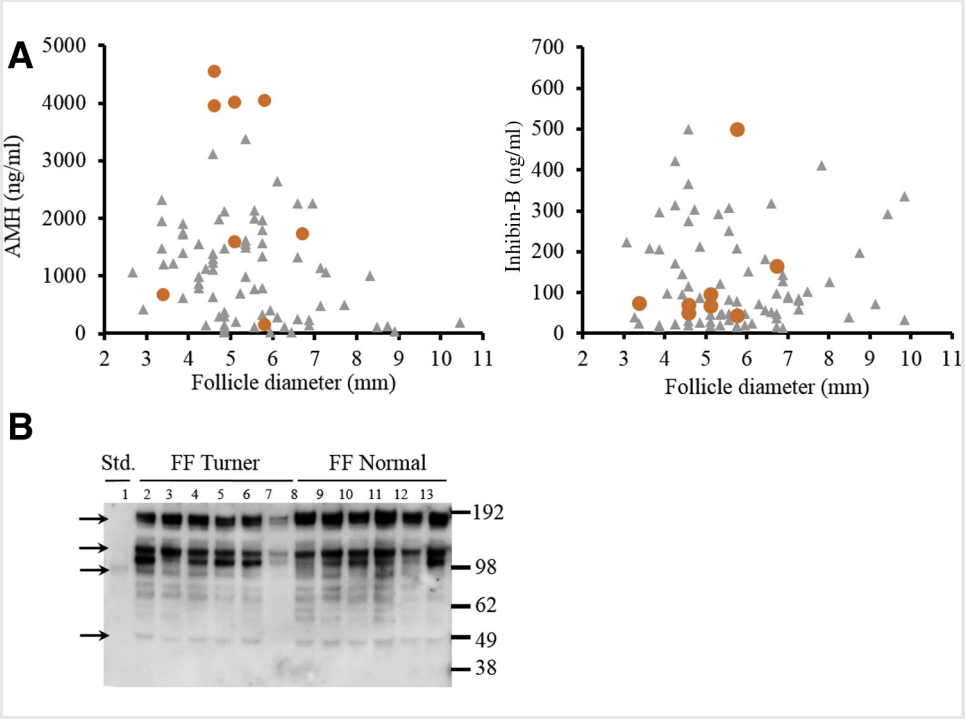
We also identified that hormone concentrations in follicle fluid from small antral follicles from TS ovaries were strikingly different from the concentrations found in size-matched normal follicles, with low concentrations of E_2 and T and markedly higher AMH. Inhibin-B concentrations seemed normal. The low T concentration may reflect abnormal theca cell function and may impact follicle development, including granulosa cell proliferation, which is reflected in the low E_2 concentrations. Antimüllerian hormone is predominantly present during follicle development until follicular selection for dominance (20). There is a strong negative relationship between follicle fluid AMH and E_2 in normal women (28), which seems to be exaggerated in TS. These high AMH concentrations together with very low steroid concentrations in TS follicles may reflect abnormal function of the somatic cells of the follicle, and additionally through impairment of the regulation of folliculogenesis, may contribute to the accelerated follicle loss in TS confirmed here. Western blot was used to evaluate the processing of AMH in TS follicle fluids and non-TS (normal) follicle fluids from size-matched follicles. No difference in AMH processing was detected between TS and normal, suggesting that the processing of AMH in TS patients are similar to normal.

Although it is now well recognized that transplanted frozen/thawed ovarian tissue can restore fertility no one has, to our knowledge, transplanted ovarian tissue to a woman with TS, despite OTC being reported (7–9, 27, 29, 30). Thus, it remains to be demonstrated whether transplanted frozen/thawed ovarian tissue from girls/adolscents with TS has the capacity to restore fertility. The rationale behind OTC in TS patients is different from other medical indications like chemotherapy. In the case of cancer, the ovarian tissue is generally normal before OTC. This contrasts with TS ovarian tissue, which itself has a

FIGURE 3



(A) Concentration of AMH and inhibin-B in follicle fluid (FF) (*red circles*) obtained from two girls with TS aged 13.5 and 17.8 years (subjects 4 and 12) and in age-matched controls (*grey triangles*). Concentrations of AMH in these TS follicles are extremely high, whereas the inhibin-B concentration is similar to that in age-matched controls. **(B)** Detection of AMH in AMH standard (Std.) (lane 1), FF from TS patient subject 12 (lanes 2–7), and in size-matched follicles from different normal women (lanes 8–13). Lane 7 was loaded with less FF than the remaining because the sample was used up, which explains the weaker/no bands seen. Arrows indicate AMH cleavage fragments. The blots show no difference in the composition of AMH forms in TS and normal FF.

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limited life expectancy; auto-grafted TS tissue will therefore also have limited survival, such that the benefit of OTC in TS patients may be limited. However, a key objective of this study was to investigate whether patients with TS might have populations of normal follicles at the time of cryopreservation. This might therefore give at least the possibility of in vivo function after replacement, albeit for a limited duration, when the remaining endogenous pool of follicles was depleted before the patient fulfilled her reproductive wishes. Furthermore, maternal risks in TS, including mortality during pregnancy, are very high, largely owing to cardiovascular risks (9, 10), which has to be taken into account when considering auto-grafting in TS patients. The cardiovascular risks may reflect the underlying connective tissue abnormalities present in TS (31), which may also be relevant to the stromal tissue and somatic cells of the ovary. In vitro maturation (32) and surrogacy may also be an option for TS patients. A recent review suggested that TS patients should be evaluated in early childhood to allow them to benefit from fertility preservation options (33), which is important to these patients and their parents (34).

In conclusion, the present analysis showed that even where follicles are present in girls with mosaic TS, many of these follicles may show abnormalites that are likely to limit their potential for later development and to support fertility. However, normal follicles were also present. Therefore, it seems reasonable to consider OTC for fertility preservation as an option for highly selected adolescent patients with mosaic TS where endocrine assessment does not indicate POI and if other health issues do not preclude pregnancy. Oocytes from TS medulla tissue may also provide an additional fertility option. However, it is important to note that transplantation of frozen/thawed ovarian tissue has not yet been performed in women with TS, and it remains to be seen whether the procedure can restore fertility.

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Caracterización de folículos en niñas y mujeres jóvenes con síndrome de Turner que realizaron criopreservación de tejido ovárico

Objetivo: Caracterizar folículos ováricos de niñas y mujeres jóvenes con síndrome de Turner (ST) que realizaron criopreservación de tejido ovárico (CTO).

Diseño: Estudio retrospectivo de casos y controles.

Ámbito: Hospital universitario.

Paciente(s): Quince niñas y mujeres jóvenes con ST de 5-22 años en la CTO fueron incluidas, junto con 42 niñas y mujeres jóvenes controles de 1-25 años quienes realizaron CTO por cáncer.

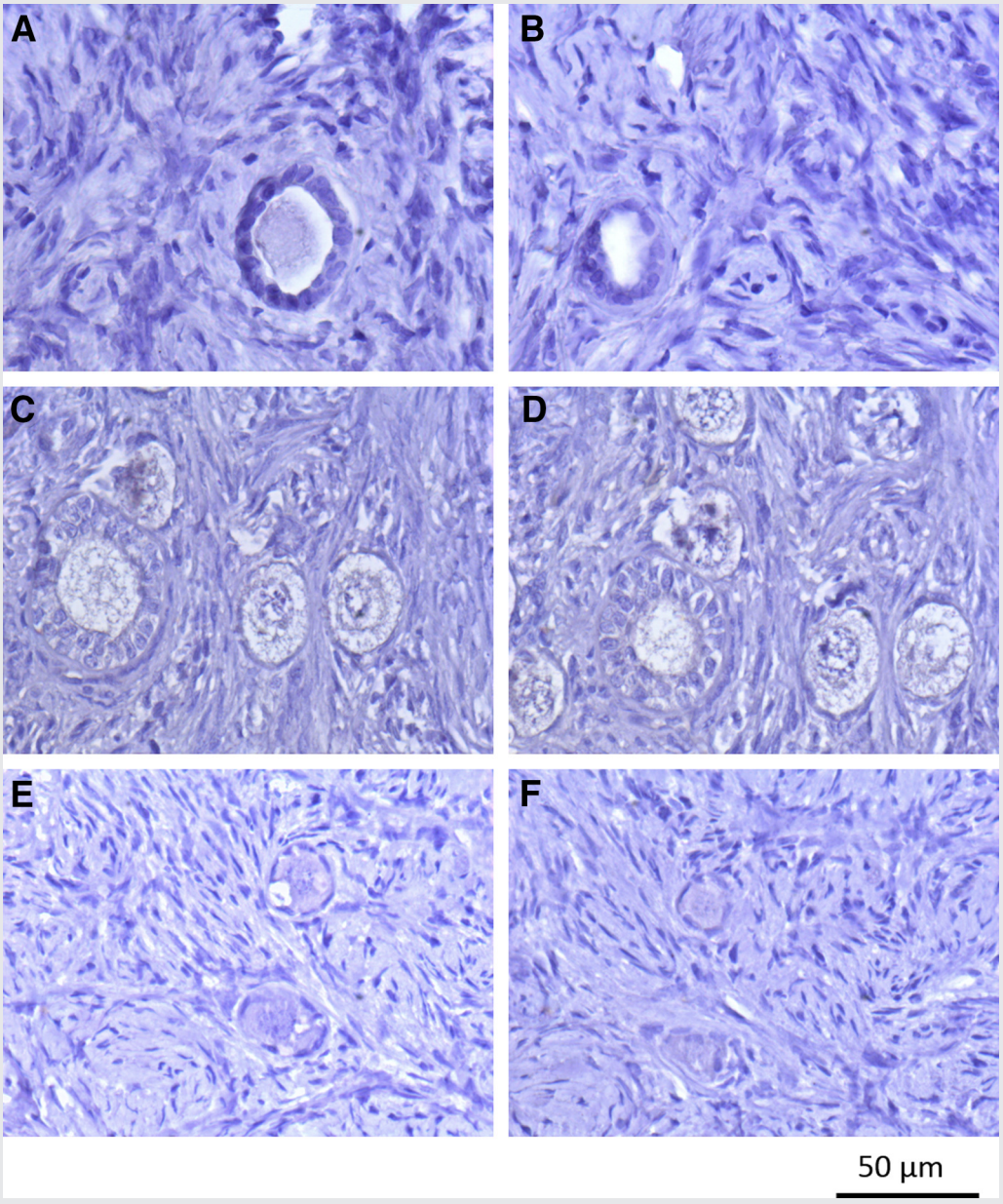
Intervención(es): Ninguna.

Principal(es) medida(s) de resultado: Densidad folicular (folículos/mm^3), morfología, y estado fueron evaluados en las biopsias de corteza ovárica de pacientes con ST y comparadas con controles. Concentraciones hormonales fueron medidas en fluidos sérico y folicular. Complejos cúmulo ovocito inmaduros fueron obtenidos y madurados in vitro.

Resultado(s): Fueron encontrados folículos en 60% de las biopsias (9 de 15) de ovarios con ST. En el 78% de los ovarios (7 de 9) con folículos, la densidad folicular estuvo dentro del intervalo de confianza del 95% del grupo control. Hubo una alta tasa de morfología folicular anormal. Seis proteínas foliculares específicas fueron expresadas de manera similar en ovarios con ST y controles. Sin embargo, apoptosis y la expresión de proteínas de la zona pelúcida fueron halladas anormales en ST. El fluido folicular de folículos antrales pequeños en ST tuvo concentraciones más bajas de estrógeno y T y concentraciones más altas de hormona anti-mülleriana que los controles. Treinta y un complejos cúmulo ovocito fueron recolectados de una paciente y cultivados durante 48 horas in vitro, resultando en cinco ovocitos metafase II (tasa de maduración 16%, tasa de degeneración 9%).

Conclusión(es): Los beneficios de CTO podrían estar limitados a un grupo altamente seleccionado de pacientes mosaico de ST en quienes una cantidad considerable de folículos normales está presente en CTO.

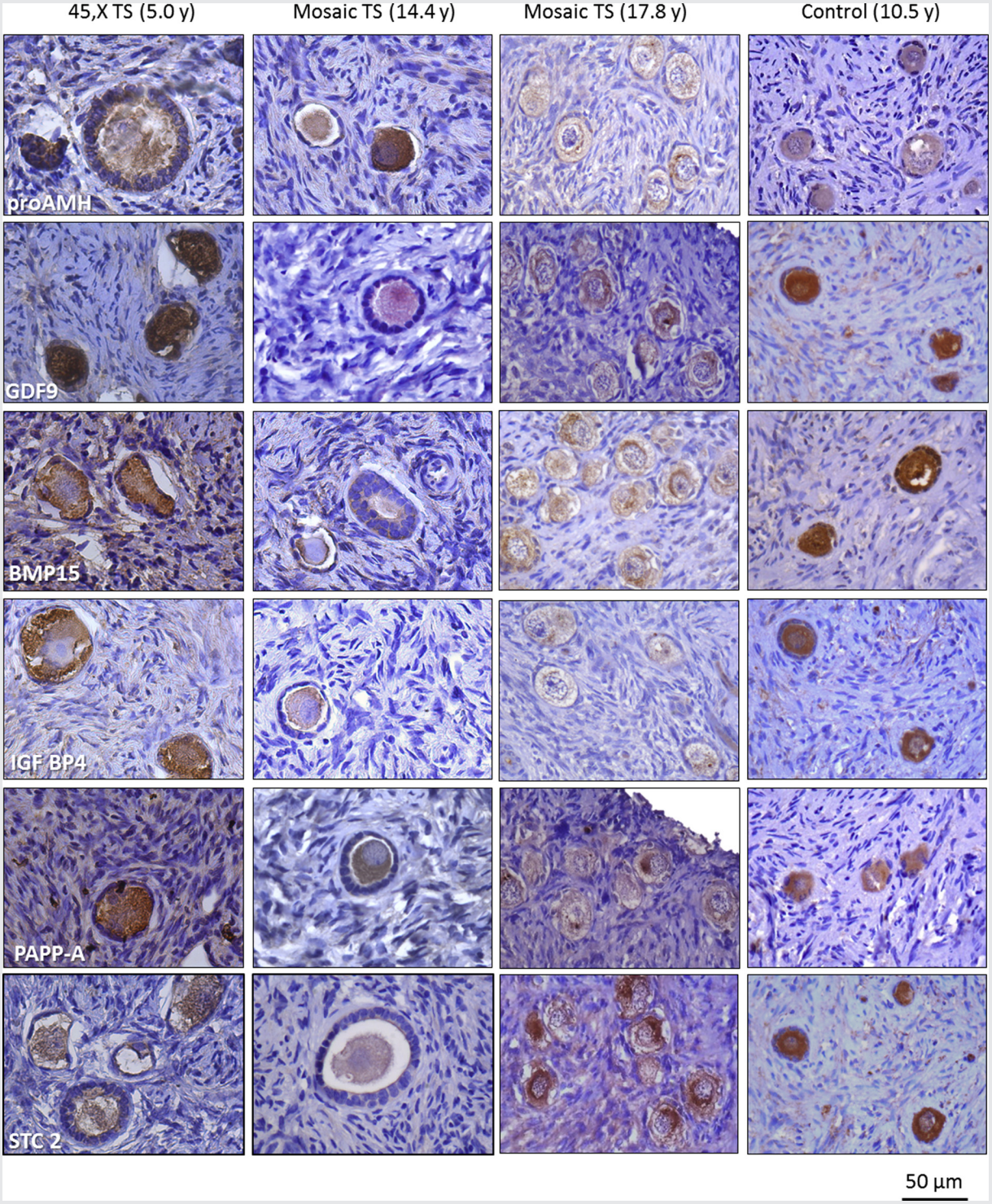
SUPPLEMENTAL FIGURE 1



Negative controls. **(A, B)** TS mosaic, 14.4 years; **(C, D)** TS mosaic, 17.8 years; **(E, F)** control, 10.5 years. **(A, C, E)** Primary antibody preplaced with antibody dilution buffer (1% bovine serum albumin in phosphate-buffered saline). **(B, D, F)** Primary antibody preplaced with Universal Negative Control serum.

Mamsen. Ovarian follicles and Turner syndrome. Fertil Steril 2019.

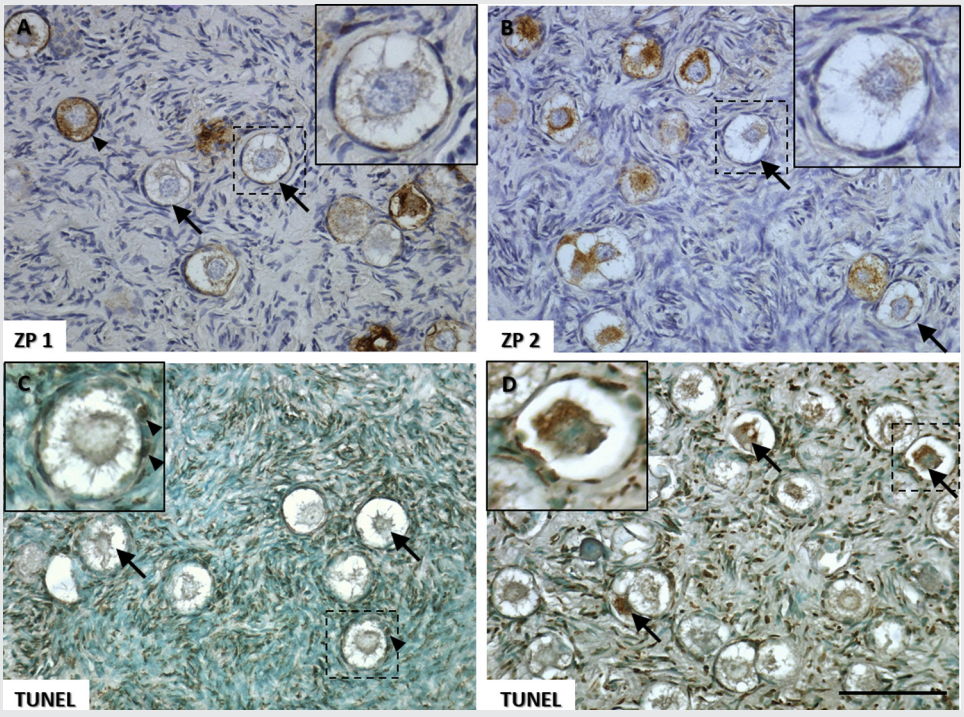
SUPPLEMENTAL FIGURE 2



Expression of six proteins important for follicular growth: pro-region of antimüllerian hormone (proAMH), growth/differentiation factor 9 (GDF9), bone morphogenetic protein 15 (BMP15), insulin-like growth factor-binding protein 4 (IGF BPB4), pregnancy-associated plasma protein A (PAPP-A), and stanniocalcin 2 (STC2) in ovarian cortical tissue from three TS patients aged 5.0, 14.4, and 17.8 years and one control aged 10.5 years.

Mamsen. Ovarian follicles and Turner syndrome. Fertil Steril 2019.

SUPPLEMENTAL FIGURE 3



Expression of zona pellucida proteins (ZP1 and ZP2) and apoptotic marker (TUNEL) in a TS ovary aged 14.8 years (subject 8). **(A)** ZP1 protein was detected in a pattern resembling normal follicles (*arrows*), and in some follicles aberrant staining was observed (*arrowhead*). **(B)** Low expression of ZP2 was observed in healthy-looking primordial follicles (*arrows*), whereas an apparent increased staining was observed in other follicles. **(C)** Stromal cells, oocytes (*arrows*), and granulosa cells in this area were predominantly TUNEL negative (green staining), though TUNEL was observed in some granulosa cells (*arrowhead*). **(D)** Single-stranded DNA (TUNEL positive) was in other areas observed in some oocytes and granulosa cells (*arrows*). Cells of the stromal tissue also had evidence of single-strand DNA (weak brown staining). Dotted boxes indicate enlarged areas. Scale bars, 100 μm and 50 μm on magnifications.

Mamsen. Ovarian follicles and Turner syndrome. Fertil Steril 2019.