A retrospective series of homologous intracytoplasmic sperm injection cycle results of 99 women with mosaic Turner syndrome

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Background: Although pregnancy is reported in both classical and mosaic forms of Turner syndrome (TS), both spontaneous and in-vitro-fertilisation (IVF) success rates were found higher in mosaic cases. In this study we analysed homologous intracytoplasmic sperm injection cycle results of infertile patients diagnosed with mosaic TS. *Methods*: Ninety nine female patients who had infertility complaints for 2 years or more and were diagnosed with mosaic TS were included in the study. They were treated according to a standard antagonist protocol. Embryo transfer was performed after pre-implantation genetic diagnosis (PGD) in 53 cases while embryo transfer was performed in remaining 46 cases without PGD. *Results*: While 45,X/46,XX karyotype was found in 55 of 99 cases, 45,X/46,XX/47,XXX karyotype was found in 32 cases. The remaining participants consisted of rare karyotype forms. The total number of patients conceived after the antagonist protocol was 31 (31.3%). While 18 of these cases resulted in term delivery (58%), the remaining 13 cases resulted in miscarriage (41.9%). Pregnancy could not be obtained in only 2 cases whose karyotype were 45,X/47,XXX and 45,X/46,XX/46,XY. Karyotype analysis was performed in only 2 of 18 newborn babies due to suspicious physical findings, but the results were reported as normal. *Discussion*: On the basis of our observations in this largest mosaic TS series, homologous IVF should be considered in infertile patients with Turner syndrome with high-grade mosaicism. PGD should also be recommended to TS patients on IVF treatment.

Keywords

Mosaic Turner syndrome; Homologous IVF; Antagonist protocol; PGD

1. Introduction

Turner syndrome (TS) is characterized by a complete or partial absence of the X chromosome. It occurs one in 2500 live births and is not linked to advanced maternal age [1]. In 60% of cases, the paternal X chromosome is lost during the meisosis. Classical 45,X karyotype is seen in 50% of cases. The remaining half, are karyotypes with an X-isochromosome or mosaicisms [2]. The main feature of TS in terms of reproductive biology is ovarian dysgenesis, characterized by hypergonadotrophic hypogonadism [3]. The developmental defect of the uterus is one of the main problems against fertility [4]. In both classic and mosaic TS cases, the chances of spontaneous pregnancy and giving birth to a healthy baby are very low and are reported as 2% [3]. The rate of TS cases completing pubertal development is about 10%, and in 90% of cases, the entire ovarian reserve disappears before completion in puberty [5]. For this reason, oocyte or ovarian tissue cryopreservation should be planned before completion of puberty in patients diagnosed with TS. However, in a study conducted by the Swedish group in girls with TS, only 26% of the cases showed follicle in cortical biopsy [6]. If the pregnancy is conceived with a frozen oocyte, the risk of spontaneous abortion and fetal chromosomal anomalies are increased. On the other hand, in classical TS cases without frozen oocyte, pregnancy can only be achieved by embryo donation. In donation cycles, the risk of fetal chromosome anomaly is negligible. Another fertility preserving method is ovarian tissue cryopreservation (OTC). Especially in young girls and in cases with TS diagnosed during puberty, performing OTC instead of ovarian stimulation is important for fertility preservation and protects the patient from unethical ovarian stimulation.

The main cause of gonadal dysgenesis in TS cases is haploinsufficiency of the X-chromosome genes that leads to premature ovarian senescence [7]. The presence of dysgenetic gonad is more pronounced in classical cases (45,X) where the X chromosome is completely destroyed. However, in patients with mosaic TS with multiple karyotypes (45,X/46,XX and 45,X/47,XXX), gonadal disgenesis is milder. The presence of the second X chromosome may allow both pubertal and follicular development [8]. Although pregnancy is reported in both classical and mosaic forms, both spontaneous and IVF success rates were found higher in mosaic cases. In

this study, we analysed homologous intracytoplasmic sperm injection cycle results of infertile patients diagnosed with mosaic TS. Although there are isolated case series and reviews of spontaneous and IVF pregnancies in classical and mosaic TS cases [9], there are no publications using homologous intracytoplasmic sperm injection cycle in large patient series. Previous studies in the management of infertility due to TS mostly reported results of oocyte donation [7, 9]. On the contrary, our study will allow obtaining new data by applying homologous intracytoplasmic sperm injection to all TS cases. Thus, it will be possible to compare donation cycles with homologous intracytoplasmic sperm injection cycles. This study is the largest case series in the literature investigating the reproductive outcomes of 99 mosaic TS cases treated with homologous intracytoplasmic sperm injection.

2. Materials and methods

2.1 Patient selection

One hundred twelve female patients who had infertility complaints for 2 years or more and were diagnosed with mosaic TS were included in the study. The subjects who participated in the study were selected among the patients who applied to BAU Medicalpark IVF-Center, and Kayseri Memorial IVF-Center with the complaints of infertility between 2014 and 2020. While some of the patients were referred to our clinic with the diagnosis of TS, some of them were diagnosed as TS in our clinic. Women with classical TS cases were not included in the study. The minimum age of the patients with TS included in the study was 27, the maximum age was 41, and the mean age was 36.1 ± 4.2. TS diagnosis was made according to TS guidelines. TS diagnosis was considered in female participant regardless of age, with characteristic phenotype, growth or pubertal delay, primary/secondary amenorrhea and infertility. Carrying Turner syndrome karyotype was accepted as the basic selection criterion. At least 30-cell karyotype be performed in the presence of clinical suspicion for TS. Women with mosaic TS selected based on a high degree of karyotypic abnormal cells. If the fraction of abnormal karyotype cells exceeded 10%, it was accepted as TS. With this approach, mosaicism can be detected with 95% confidence [10].

It was not known whether some patients had TS in their first IVF trials, because these patients did not have the fenotypic features of TS and only had infertility complaints. For this reason, these patients were initially treated without karyotype analysis. This group of patients was diagnosed with mosaic TS in genetic studies performed due to recurrent implantation failure or poor ovarian reserve. Serum levels of anti-Mullerian Hormone (AMH) were measured in participants with diminished ovarian reserve. Chromosome analysis was performed in the peripheral blood lymphocyte cultures of the patients whose genetic anomaly was considered as a result of fertility history or physical examination. None of the participants had experienced a spontaneous pregnancy. The women were informed about cardiovascular risks related

to Turner syndrome and referred for a cardiology consultation and examination. Since cardiologists suggested that patients with Turner syndrome with any significant cardiovascular abnormalities should avoid IVF/intracytoplasmic sperm injection (ICSI) and pregnancy to prevent related cardiovascular morbidity or mortality the 13 patients with aortic coarctation or bicuspid aortic valve were excluded from the study. Ninety-nine patients with normal ecocardiographic findings and normal blood pressure values underwent IVF/ICSI. On day 3 of the spontaneous cycle, the basal hormonal levels of the patients were measured. Pelvic examination and transvaginal ultrasound findings were assessed as normal genitalia, and the antral follicle count was evaluated. Since women with TS have an increased rate of autoimmune disorders such as Hashimato's thyroiditis, and metabolic disorders such as glucose intolerance the thyroid function tests and glucose screening were performed. Patients with Hashimoto's thyroidism, Addison's disease, and renal anomalies were also excluded. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of medeniyet University Goztepe Training and Research Hospital (approval number: 2013-KAEK-64).

2.2 Karyotype analysis

The couple was referred for cytogenetic examination before the IVF/ICSI cycle. The diagnosis of TS was made by karyotype analysis of peripheral blood which shows the numerical and/or structural abnormalities of the X chromosome. Since mosaicism can be better defined in approximately 30% of non-mosaic patients by increasing the number of cells examined, evaluating additional tissue samples or using fluorescence *in situ* hybridization method we preferred to use the last mentioned method for diagnosis of mosaic forms. In the conventional metaphase analysis performed in the first 100 cells, 45,X karyotype was detected (100%). However, a metaphase analysis performed on 100 cells may not be enough to diagnose a mosaic variant. So using fluorescence *in situ* hybridization (FISH) technique the woman's genetic evaluation was extended, and two types of cell lines found after analyzing 500 lymphocytes. Generally, TS was diagnosed if the fraction of abnormal karyotype cells exceeded 4%, whereas in our study, if the fraction of abnormal karyotype cells exceeded 10%, it was accepted as TS. In this way, the accuracy rate of our definition has been increased. No additional method was used for karyotype analysis. The fraction of normal karyotype cells in women TS was 62.8%. The fraction of abnormal karyotype cells in women with TS was 36.2%. The rate of normal karyotype cells in patients with pregnancy or delivery TS was 82.4%. The majority of cells were XO and XX chromosomal pattern. The second genetic evaluation excluded the presence of a Y chromosome. The male karyotype was revealed to be normal. Couples with karyotype anomalies in their partners were not included in the study. In the karyotype analysis, the total number of pa-

tients diagnosed with mosaic TS was 53. In 46 patients the diagnosis of TS was made as a result of further research due to recurrent implantation failure or decreased ovarian reserve. Therefore, embryo transfer was performed in only 53 cases by making preimplantation genetic diagnosis (PGD).

2.3 Controlled ovarian stimulation protocol

Women with mosaic TS were treated according to a standard antagonist protocol with individually dosed recombinant follicle stimulating hormone (FSH) starting on day 2–3 of the menstrual cycle. Gonadotrophin-releasing hormone antagonist was started on the 5th or 6th day of stimulation. When at least three follicles reached 16–17 mm in diameter, maturation of follicles was induced with recombinant human chorionic gonadotrophin (hCG) (Ovitrelle, Merck-Serono, 250 mg, Modugno, BA, Italy). Oocyte collection was performed 36 hours after hCG application. Ovarian follicles were aspirated using a single-lumen, 17-gauge needle (Cook Medical, Bloomington, IN, USA) guided by transvaginal ultrasonography. Embryo quality was determined in both groups according to Gardner and Schoolcraft blastocyst grading system. Two top quality or Grade 1–2 embryos were transferred to both PGD group and non-PGD patients. While fresh embryo transfer (ET) was applied to some of the patients in the PGD group, frozen ET was applied to some of them. Fresh ET was applied to all patients in the non-PGD group. Embryos were frozen because the results were delayed in patients who had PGD on the fifth day. In the PGD group, while 5AA embryo transfer was performed in 16 of 20 patients who underwent day 5 ET, 4AA embryo transfer was performed in four. Grade 1 embryo was given to 25 of 33 patients who underwent day 3 ET in the PGD group, while grade 2 embryos were given to the remaining eight patients. In the group without PGD, 5AA embryos were given to 6 of 9 patients who underwent day 5 ET, while 4AA embryos were given to the remaining 3 patients. Grade I embryos were given to 30 of 37 patients who underwent day 3 ET and grade II embryos to 7 of them.

2.4 Statistical analysis

The Statistical Package for Social Sciences, version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Demographic and other individual group parameters were assessed with one-sample Kolmogorov–Smirnov Z test and were found to be not normally distributed. Hence, statistical comparisons between PGD and non-PGD groups were performed by nonparametric Mann-Whitney U test. Data are presented as mean $\pm$ SD. For all comparisons, statistical significance was defined by $p < 0.05$.

3. Results

The mean age of the patients was 36.1 ± 4.2. The average age of patients with TS who conceived and/or gave birth was found to be 31.4 ± 0.3. Although there was no control group in this study, the total rFSH dose, the duration of rFSH usage, the total number of oocytes and metaphase II (MII)

oocytes obtained were similar to the results of healthy population in the same age group. Likewise, in terms of endometrial thickness and E2 levels on the day of hCG, the results of patients with mosaic TS were similar to those of healthy individuals. Serum levels of fasting blood glucose, thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and FT4 levels were found normal. Day 3 basal FSH, luteinizing hormone (LH) and E2 levels of all participants were in the normal ranges. The demographic characteristics and IVF/ICSI results of the cases are shown in detail in Table 1. AMH values were present in 23 of the women in the PGD group and in 30 of the women in the non-PGD group. The difference between two groups in terms of AMH was not significant.

Table 1. Demographic and cycle characteristics of 99 women with mosaic TS.

	Mean $\pm$ SD (min–max.)
Age (years)	33.18 ± 4.2 (27–41)
BMI (kg/m^2)	25.47 ± 4.4 (18–32)
Day 3 FSH (IU/L)	7.33 ± 1.0 (6.3–8.1)
Day 3 LH (IU/L)	4.2 ± 4.0 (3.2–6.5)
Day 3 E2 (pg/mL)	41.3 ± 0.2 (36.4–48.6)
Fasting glucose (mg/dL)	87.4 ± 1.2 (76.6–99.5)
Total AFC	8.12 ± 3.3 (7.9–8.8)
Number of IVF/ICSI attempts	3.67 ± 1.9 (0–8.0)
E2 on the day of hCG (pg/mL)	2058.75 ± 1453.3 (182–7326)
Endometrial thickness (mm)	9.85 ± 2.0 (4.5–14)
Duration of rFSH (day)	8.70 ± 1.9 (1–14)
Total dose of rFSH (IU)	2662.54 ± 1333.7 (225–6525)
Infertility duration (years)	8.06 ± 5.2 (2–23)

rFSH, recombinanat FSH; AFC, Antral follicle count; BMI, Body mass index; E2, Estardiol.

In extended genetic evaluation with FISH method we detected different mosaic patterns with a second cell lines (Table 2). The 45,X/46,XX karyotype detected in 55 of 99 cases were recorded as the most frequent mosaic form. The mosaic form we detected in the second frequency was 45,X/46,XX/47,XXX karyotype seen in 32 cases. The remaining 12 cases consisted of rare karyotype forms. Of the 55 cases with 45,X/46,XX karyotypes, 14 of them became pregnant after IVF/ICSI. Nine of the 14 pregnant women resulted in term delivery, while 5 cases resulted in miscarriage. Of the 32 cases with 45,X/46,XX/47,XXX karyotypes, 12 of them became pregnant after the antagonist protocol. While 7 of 12 pregnancies resulted in term delivery, 5 cases resulted in miscarriage. In high grade mosaic Turner forms, healthy and term pregnancy rates were quite low. While only 2 of the 6 cases with 45,X/46,XX/47,XXX/48,XXXX karyotype were pregnant, one of the 2 cases resulted with term delivery while one case resulted with abortion. In one case with 45,X/47,XXX karyotype, pregnancy could not be achieved. Of the 99 mosaic TS cases, the total number of patients conceived after the antagonist protocol was 31 (31.3%).

Table 2. IVF/ICSI outcome of 99 women with mosaic TS according to their karyotypes.

Patients	Maternal karyotype	Pregnancies	Live births	Miscarriages
55	45,X/46,XX	14	9	5
32	45,X/46,XX/47,XXX	12	7	5
1	45,X/46,Xdel(X)	**1**	**0**	**1**
1	45,X/46,XX/48,XXXX	**1**	**0**	**1**
2	45,X/46,XX/47,XXX/49XXXXX	**1**	**1**	**0**
1	45,X/47,XXX	**0**	**0**	**0**
6	45,X/46,XX/47,XXX/48,XXXX	**2**	**1**	**1**
1	45,X/46,XX/46,XY	**0**	**0**	**0**
Total: 99		31	18	13

Table 3. Reproductive outcomes of TS cases according to whether PGD is performed or not.

	PGD (n = 53)	Non-PGD (n = 46)	p value
AMH (ng/mL)	2.12 ± 1.43	2.06 ± 0.12	0.67
Pregnancies, n (%)	19 (35.8%)	12 (26.0%)	0.001*
Live births n (%)	11 (20.7%)	7 (15.2%)	0.44
Miscarriages n (%)	5 (10.8%)	8 (15.0%)	0.02
Oocytes (mean ± SD)	10.11 ± 3.54	9.89 ± 1.99	0.06
MII oocytes (mean ± SD)	8.89 ± 3.09	7.13 ± 1.02	0.54
2PN zygotes (mean ± SD)	6.33 ± 0.31	6.01 ± 0.31	0.09
Embryos formed (mean ± SD)	3.90 ± 1.11	3.02 ± 1.09	0.33
Good quality embryos (mean ± SD)	2.12 ± 1.87	2.63 ± 0.22	0.48
Embryos transferred (mean ± SD)	2	2	0.11
Quality of embryos transferred	4AA/5AA or Grade 1–2	4AA/5AA or Grade 1–2	NA
Day 3 ET, n	33	37	NA
Day 5 ET, n	20	9	NA
Fresh/Frozen ET, n/n	33/20	46/0	NA

*, depicts $p < 0.05$; NA, not applicable; 4AA/5AA, good or top quality embryo; AMH, Anti mullerian hormone; PGD, preimplantation genetic diagnosis; ET, Embryo transfer; MII, metaphase II; 2PN, pronucleus.

While 18 of these cases resulted in term delivery (58%), 13 cases resulted in miscarriage (41.9%). Pregnancy could not be obtained in 2 cases whose karyotype were 45,X/47,XXX and 45,X/46,XX/46,XY respectively. Embryo transfer was cancelled in 8 of 53 cases because of karyotype anomalies in PGD. Karyotype analysis was performed in only 2 of 18 newborn babies due to suspicious physical findings, but the results were reported as normal. Karyotype analysis was not performed for the remaining 16 babies. Karyotype distributions and obstetrics outcomes of 99 cases with mosaic TS are shown in Table 2.

It was possible to perform cytogenetic analysis of conceptus material in 6 of 13 cases who had miscarriage. In the remaining 7 cases, cytogenetic analysis could not be performed in the conceptus. Some of these cases had a miscarriage at home and some did not accept cytogenetic analysis. In the cytogenetic analysis performed in the conceptus material of six cases, polyploidy, trisomy and monosomy X were detected in 3 cases, while no anomaly was detected in the remaining 3 cases.

Fresh ET was applied to 33 cases in PGD group and frozen ET was applied to 20 cases. 3rd day ET was applied to 33 cases whose PGD results were found on the same day. On the other hand, 5th day ET was applied to cases whose PGD results could not be obtained on the same day. While 37 of the non-PGD cases were applied fresh ET for the 3rd day, the remaining 9 cases were applied to the 5th day fresh ET. There was no significant difference between the groups in terms of total oocyte count, 2 pronucleus (2PN) embryo count and good quality embryo counts. When the reproductive parameters of the cases were evaluated as those with and without PGD, it was found that the number of pregnancies in the PGD group was significantly higher ($p < 0.001$). Miscarriage rates in PGD group were significantly lower than non-PGD cases ($p < 0.02$). LBR was found to be significantly higher in PGD group than non-PGD cases ($p < 0.01$, Table 3).

4. Discussion

The majority of patients with Turner syndrome are infertile due to ovarian dysgenesis due to accelerated follicle loss that begins in intrauterine life and continues in the early postnatal period [5]. Spontaneous pregnancy is very rare in classical TS cases and most of these cases can conceive only with oocyte donation [11]. In mosaic-type TS cases, spontaneous pregnancy chance is higher than classical forms, but most of these patients need assisted conception techniques to conceive. Although there is a high chance of obtaining pregnancy using homologous intracytoplasmic sperm injection in

mosaic TS cases, most pregnancies result in miscarriage, still-born, or congenital defects [8].

This study is the largest mosaic TS case series ever reported in the literature. The most common form of mosaicism we detected in this study was 45,X/46,XX (55.5%) which includes both the cell line with the normal and abnormal cytogenetic structure. Our results are compatible with the literature data in terms of the frequency of mosaic karyotype [12]. However, although reported rarely in cases with TS in the literature, 45,X/46,XX/47,XXX were the second frequent karyotype in our study. In classical TS cases (45,X), spontaneous or IVF/ICSI pregnancy was reported only as a case report. However, in mosaic TS cases, spontaneous or IVF/ICSI pregnancy was reported to be significantly higher than the classical form. In the initial studies, it was reported that only healthy pregnancy can occur in mosaic TS cases with 45,X/46,XX karyotype. However, in later studies, healthy and term pregnancies have been reported in cases with other mosaic karyotype forms. In accordance with this Bouchlariotou et al. [7] reported that 33-year-old woman with a mosaic Turner's syndrome karyotype 45,X/47,XXX who conceived spontaneously and had two successful pregnancies. Likewise, in our study, 12 of 32 cases with 45,X/46,XX/47,XXX karyotypes became pregnant and 7 of them resulted in healthy and term delivery. Abortion rate in this group was 41.6%. However, unlike the cases of Syber et al. [2], our patients had an extra cell line in their karyotype (46,XX). In our only case with 45,X/47,XXX karyotype, pregnancy could not be achieved. On the other hand, of 55 cases with 45,X/46,XX karyotypes, 14 of them became pregnant. While 9 of 14 pregnancies resulted in term delivery, 5 cases resulted in miscarriage. Total pregnancy rates were higher in group with 45,X/46,XX/47,XXX karyotype compared to 45,X/46,XX cases (37.5% vs. 25.4%). However, term pregnancy rates were higher in patients with 45,X/46,XX karyotype compared to 45,X/46,XX/47,XXX cases (64.2% vs. 58.3%). Miscarriage rates were higher in 45,X/46,XX/47,XXX groups compared to 45,X/46,XX cases (41.6% vs. 35.7%). It is not surprising that the total pregnancy rates are high in 45,X/46,XX/47,XXX cases. We can list the possible reasons for this height as follows. Although ovarian insufficiency is reported in 45,X/46,XX/47,XXX cases, spontaneous menarche and pregnancy rates are higher than other mosaic forms [13]. In cases with 45,X/46,XX/47,XXX karyotypes, spontaneous menarche and pregnancy rates are around 84% and 69%, respectively, which explains higher pregnancy rates than 45,X/46,XX cases [2]. Spontaneous menarche rate of around 20% in 45,X/46,XX cases may be the underlying reason for the total pregnancy rates being lower in this group of patients.

Although 45,X/46,XX karyotype is found in the form of mosaic TS with 15%, 45,X/47,XXX karyotype is 3–4% [13]. The formation mechanisms of both karyotypes are different from each other. The emergence of the 45,X/47,XXX karyotype can be attributed to post-zygotic non-dysjunction that

occurs in the normal disomic cell lines. Since approximately 20% of mosaic cases with 45,X/46,XX karyotype and 84% of cases with 45,X/46,XX/47,XXX in completed their pubertal development and experienced spontaneous menstruation, we can think that ovarian reserves are preserved to some extent. Mechanisms such as postconceptional errors, meiotic non-disjunction and anafase lag suggested in the formation of this form allow the fetuses to be born alive and to maintain the necessary ovarian reserve for spontaneous or IVF pregnancy [8, 14]. In the light of these data, we can argue that the excess of mosaic cell lines does not have a negative effect on fertility. Conversely, low mosaic cell lines do not have a positive effect on fertility. For these reasons, we can say that different karyotypes detected in mosaic TS samples do not have a predictive value on pregnancy formation.

It is recommended that PGD should be performed in all cases because the risk of miscarriage, stillbirths and malformed babies is increased in most of the TS patients who underwent homologous intracytoplasmic sperm injection or conceived spontaneously [2, 8]. Chorion villous sampling, or amniocentesis should be recommended for patients who do not apply PGD but become pregnant. In the present study, PGD was performed in 53 patients diagnosed with mosiac TS before IVF/ICSI. Embryo transfer was cancelled because chromosomal aberration was detected in eight cases. However, despite PGD, miscarrage was found in 41.9% of the cases. If the pregnancy is achived with autologous oocytes, the risk of spontaneous abortion appears to be increased regardless of PGD. For this reason, increased abortion rates in patients with mosaic TS could have been due to implantation failure related to the stimulation protocol or a low endometrial receptivity instead of abnormal karyotype. Since the patients with mosaic TS usually have a milder phenotype and the characteristic stigmatas of TS may not be seen we did not perform karyotype analysis before IVF/ICSI in 46 of the cases. Therefore, we did not perform PGD because we did not know if the patients had TS. Therefore, we may have transferred embryos with chromosomal aberrations to patients and this may have caused an increase in abortion rates.

(A)ctivity and muscle tone (P)ulse (heart rate) (G)rimace response (medically known as "reflex irritability") (A)ppearance (skin coloration) (R)espiration (breathing rate and effort) (APGAR) scores and cord blood acid/base analyzes of newborn babies of TS patients were found to be similar to healthy newborns [15, 16]. In 2 out of 18 babies born healthy, karyotype analysis was performed due to suspicious phenotype appearance and results were reported normally. Danish researchers detected chromosome anomalies in 6 of 26 babies born from mothers with mosaic TS [17]. Similarly, a woman with a mosaic TS karyotype delivered 6 term births and only one baby was diagnosed with TS [18]. In our patients, only 2 babies from 18 term babies underwent a chromosome analysis and were found to be normal. Chromosome analysis was not performed on the other 16 babies. However, it is not very accurate to diagnose

a normal and healthy baby by looking at the phenotype of babies born from TS mothers. Because, in many cases with mosaic TS, the characteristic features of TS may not be found phenotypically [7].

There is little evidence that patients with Turner syndrome have a worse prognosis after homologous IVF compared with the general population of poor ovarian reserve. The most important feature of TS in terms of reproductive biology is the infertility problem encountered in most cases. Most classical TS cases have no ovaries. In mosaic forms, the patient may have both ovaries or one ovary, or both ovaries may be in the form of streak gonad. Ovaries, which initially look normal, can turn into streak gonade over time, leading to early menopause. For this reason, having pubertal development of mosaic cases increases the chances of pregnancy but does not fully guarantee future fertility. Therefore, it should be kept in mind that patients with mosaic TS should rush to get pregnant or have either oocyte cryopreservation or OTC. In our study, the total term pregnancy rate resulting from 99 mosaic TS cases was 18 (58%) and the ratio of abortus was 13 (41.9%). When the literature is reviewed, healthy and term pregnancy rates of TS patients were reported as 38%. In our series, term pregnancy rates were found to be 20% higher than the rates reported by Tarani *et al.* [9]. The possible reason for this elevation in birth rate may be due to the fact that PGD was performed before IVF/ICSI in most of the TS patients and that the diseased embryos were not transferred. Another possible reason may be the women with classic TS were not included in the study. Furthermore, the determination of the high rate of normal karyotype cells in cases with TS who conceived is a data supporting our high birth rates. Cell fraction rates with normal karyotype were not specified in 38% birth rates reported in donation cycles applied in cases with TS.

In our study, the total oocyte and MII oocyte counts collected from patients with TS were found to be higher compared to other studies in the literature. Although the average age of our participants was above 36 years, the high number of oocyte collection during oocyte pick-up (OPU) may seem paradoxical. One of the possible reasons for this mismatch may be due to oocyte pooling in some of the patients with TS. A second possibility may be that the fraction of normal karyotype cells in the ovary is very high in this cohort, which may explain this discrepancy to literature. Finding 46,XX as the second karyotype in almost all TS cases supports this view. In the karyotype analysis performed on lymphocytes, buccal cells, and urine cells of a 14-year-old girl, 45,X was detected and ovarian tissue preservation was planned. In the FISH analysis of the patient's ovarian tissue, more than one different karyotype was detected, unlike peripheral karyotype. The authors argued that karyotyping of extraovarian cells was not predictive of the karyotype of ovarian cells in the same patient. This case report also contributes to the explanation of the high number of follicles in our patients [19]. Furthermore, the determination of the rate of normal karyotype

cells as approximately 80% in cases with TS who conceived is a data supporting our suggestion. On the other hand, low level mosaicism detected for monosomy X in some cases may not be a true mosaicism. Although all these possibilities contribute to explaining the high oocyte numbers, it may not be sufficient to make a clear interpretation and should be clarified with further studies.

Infertile women with classical or mosaic TS are usually counseled to consider heterologous IVF/ICSI because of the homologous IVF/ICSI increases the risk of aneuploid embryo [20, 21]. When our findings and literature data are evaluated together we can suggest that it may be worth trying homologous IVF/ICSI should be tried even in women with high grade mosaic TS before oocyte donation. The presence of 46,XX or 47,XXX cell lines or both in the karyotype analysis should not scare the clinician, but should direct the patient to IVF/ICSI considering that pregnancy rates increase significantly compared to classical forms. This study has two important differences compared to previous studies. First, this study is the mosaic TS study with the largest number of participants in the literature. Our second finding was that the number of oocytes retrieved, clinical pregnancy and live birth rates were significantly higher than previous studies using donation and homologous oocytes.

On the basis of our observations, homologous IVF should be considered in patients with Turner syndrome regardless of cell lines in their karyotype analysis. If the conditions are suitable for PGD in patients with TS who have undergone IVF/ICSI, it should be recommended to patients.

Author contributions

Ethics approval and consent to participate

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Conflict of interest

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