

ORIGINAL ARTICLE

Impact of abnormal karyotype on reproductive outcome in premature ovarian insufficiency

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Abstract

Purpose: This study evaluated the reproductive potential of premature ovarian insufficiency (POI) patients with abnormal karyotypes undergoing infertility treatments.

Methods: A retrospective analysis of infertility treatments in POI patients with an abnormal karyotype treatment. Clinical and laboratory data were analyzed.

Results: The study group was forty-nine POI patients. Follicular growth was achieved in 29% (89/307) controlled ovarian stimulation (COS) cycles in 57% (28/49) of patients. Oocyte retrieval was attempted in 47% (23/49) of patients with a proportion of successful oocyte retrieval per oocyte pick-up (OPU) of 59.4% (41/69). The average number of retrieved oocytes was 2.4 ± 2.7 per patient and fertilization rate was 70.7% (29/41). Embryo transfer (ET) performed in eight patients with a total of nine ET attempts, resulting in 33.3% (3/9) of live birth rate per ET. Three patients delivered a healthy baby (6.1% (3/49) of live birth rate per patient). Mosaic Turner syndrome patients had a longer duration of amenorrhea and lower chances of successful follicular growth with OPU in 35.7% (5/14) of patients, whereas 47XXX had shorter duration of amenorrhea and COS with follicle growth with OPU in 83.3% (5/6).

Conclusion: COS might provide an opportunity for POI women with abnormal karyotypes to conceive a biological offspring.

KEYWORDS

chromosomal abnormalities, controlled ovarian hyperstimulation, karyotype, premature ovarian insufficiency, turner syndrome

1 | INTRODUCTION

Premature ovarian insufficiency (POI) can be either primary or secondary, and is generally defined as oligo/amenorrhea due to loss of ovarian function before the age of 40.¹ It is a state of near complete follicular exhaustion, resulting in hypergonadotropic

hypogonadism. POI affects 1% of women and the etiology is heterogeneous, including chromosome/genetic abnormalities, autoimmune disease, and chemo-/radiation therapies. However, even with continuous innovation and improvement of diagnostic modalities, the pathophysiology of the majority of cases remains largely unclear.

Leonti Grin and Bunpei Ishizuka contributed equally to this study.

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Genetic origins, specifically chromosomal abnormalities, have been recognized as one of the common diagnosed causes of POI with a higher prevalence among women with primary absence of menstruation.²⁻⁴ Overall, the prevalence of chromosomal abnormalities in POI varies among reports from different populations, ranging from as low as 2.5%–32%, with an accepted average of 15%.²⁻⁶ The presence of two intact X chromosomes is required for normal ovarian development and function. Among different abnormalities of the X chromosome linked to POI, numerical (e.g., 45 X, and 47,XXX) and structural ones are the most common.^{2,3,7} Other possible abnormalities include: autosomal translocations (Robertsonian or reciprocal), X-isochromosomes, and other rearrangements.

As most POI patients exhibit anovulation due to exhaustion of ovarian follicles, egg donation is the most successful treatment to date, for patients who desire childbirth. Nevertheless, some POI patients still possess residual ovarian follicles,^{8,9} and hence have a potential for pregnancy using their own eggs.¹⁰ Previous studies on reproductive outcomes using different protocols focused on POI patients with normal karyotypes,^{11,12} or did not assess for chromosomal abnormalities.¹³⁻¹⁶ It was recently suggested that women with POI and an abnormal karyotype might have a reduced chance of conceiving using their own eggs, compared to women with POI from non-genetic causes.¹⁷ However, the literature concerning the reproductive outcome of POI with an abnormal karyotype is still scarce. Thus, the probability of pregnancy in POI patients with abnormal karyotypes using their own eggs remains a clinical conundrum. In the present study, we aimed to explore the chances of pregnancy and live birth in POI patients with abnormal karyotypes, undergoing in vitro fertilization (IVF)-embryo transfer (ET) treatments with their own eggs.

2 | MATERIALS AND METHODS

2.1 | Patients

We retrospectively analyzed the clinical outcome of controlled ovarian stimulation (COS) cycles based on medical records in women diagnosed with POI referred for infertility treatment to Rose Ladies clinic from January 2014 to March 2019. The study was approved by the Biomedical Ethics Committee of Rose Ladies clinic, Tokyo, Japan (RLC-019). The present clinical trial was registered under number UMIN000040360 and carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Inclusion criteria for the study were the following: serum FSH levels >25 mIU/ml (on two occasions with 1 month apart) before age 40, at least 4 months of amenorrhea/oligomenorrhea (ESHRE guideline, Webber et al. 2016), and abnormal karyotype analysis. Serum FSH, LH, estradiol (E2), and progesterone were measured using an AIA-900 Automated Immunoassay Analyzer (TOSOH BIOSIENCE). With this method, intraassay and interassay coefficients of variation were 3.3% and 3.2% for FSH, 2.3% and 2.7% for LH, 5.3% and <3.7%

for E2, and 5.6% and 4.9% for progesterone, respectively. AMH (Anti-mullerian hormone) was measured using a fully automated Access AMH immunoassay (Beckman Coulter Diagnostics) with intraassay and interassay coefficients of variation were 0.7–2.2% and 0.5%–1.4%, respectively, and detection limit was ≤ 0.02 ng/ml (0.14 pmol/L). Karyotype analysis was performed on G-banded metaphase chromosomes using a standard protocol with heat denaturation followed by Giemsa staining that generated 500–550 band resolutions.

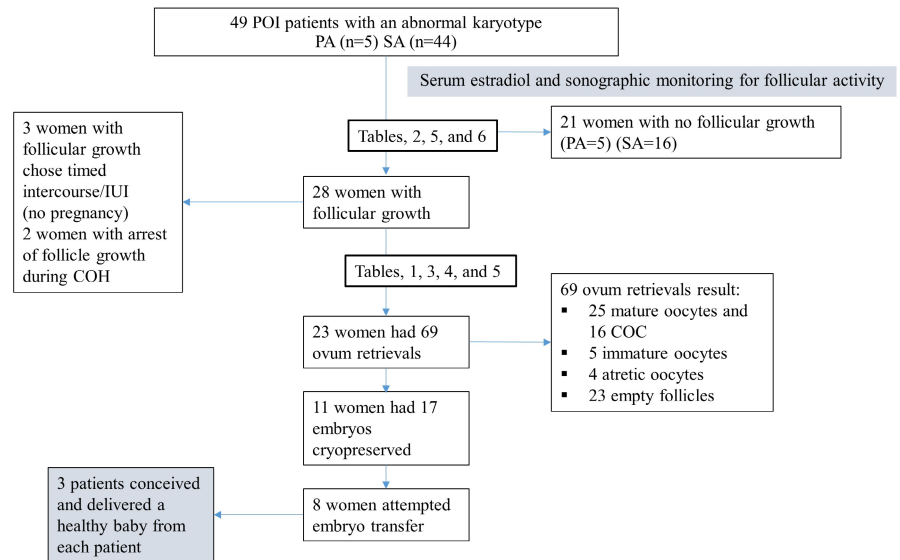
2.2 | Controlled ovarian stimulation and monitoring of follicle growth

We treated patients with 0.625–1.875 mg estrogen (Premarin[®]; Pfizer Inc.) combined with cyclic addition of medroxyprogesterone acetate (7.5 mg, Provera[®]; Pfizer Inc.) or dydrogesterone (30 mg, Duphaston[®]; Abbott Japan Inc.), to suppress elevated endogenous gonadotropins below 10 mIU/ml before exogenous gonadotropin stimulation. In cases refractory to the estrogen–progesterone treatment, GnRH agonist nasal spray 350–1200 micrograms daily (Busrecur[®] nasal solution 0.15%; Fuji Pharma. Co., Ltd.) was added before starting exogenous gonadotropin stimulation.

Ovarian stimulation was performed by using 225–450 IU of urinary FSH (Folyrmon-P injection[®]; Fuji Pharma. Co., Ltd.) combined with GnRH agonist nasal spray 350–1200 micrograms daily (Busrecur[®] nasal solution 0.15%). Follicle growth was monitored weekly by measurement of serum estrogen and gonadotropin levels to detect growing antral follicles. Follicles could be detected by transvaginal ultrasound when endogenous serum estrogen levels with additional >50 pg/ml from the basal levels. When antral follicles were detected, monitoring frequency was increased to every 2–3 days until the follicles reached >16 mm in diameter. In several clinical situations such as a random start of ovarian stimulation in the presence of elevated serum gonadotropin levels, an immediate decrease in endogenous gonadotropin levels was conducted by daily injection of 0.25 mg of a GnRH antagonist, cetrorelix acetate (Cetrotide, Merck, Tokyo, Japan) without causing an endogenous flare-up by GnRH agonist, and ovarian stimulation was performed by simultaneous daily administration of 300–450 IU of urinary FSH. Follicle growth arrest was defined as a stop of growth at 12 mm or less with absence of increase in serum estradiol levels.

When follicle reached 14–18 mm, oocyte maturation was induced by a single injection of 10 000–15 000 IU hCG (Gonatropin; Asuka Pharma). Three patients attempted timed intercourse or intrauterine insemination after hCG triggering due to their personal reasons, whereas all other cases received oocyte pick-up (OPU) from follicles using ultrasound-guided, transvaginal retrieval via a 19 or 20 G needle at 36 h later after hCG administration, Ovarian stimulation was generally terminated if no follicular growth was detected despite 4 weeks of daily gonadotropin injection with the exception when an increase in serum estradiol was detected on the 4th week of stimulation and extended the ovarian stimulation to further try and detect follicle growth.

FIGURE 1 A flow chart of the study. COC, cumulus-oocyte-complex; IUI, intrauterine insemination; PA, primary amenorrhea; SA, secondary amenorrhea



2.3 | In vitro fertilization and embryo transfer (IVF-ET)

After oocyte retrieval, IVF was performed by using conventional IVF or ICSI (intra cytoplasmic sperm injection) before culturing fertilized oocytes in the fertilization medium (Quinn's Advantage® Fertilization HTF Universal Medium; Cooper Surgical, Trumbull, CT, USA) for 16 h. ICSI was performed in ~50% of cases due to mostly male factor. After fertilization, embryos were cultured in cleavage medium (Sequential Cleavage, ORIGIO). Expert embryologists evaluated the quality of deriving embryos at 48 and 72 h after insemination according to the method described by Veeck.¹⁸ In the majority of cases, we cryopreserved the high quality embryos at cleavage stage (day 2–3) by vitrification method using Cryotop® (Kitazato Corporation), whereas poor quality embryos judged at cleavage stage were continued culture up to day 6 in a medium (G-2 PLUS™, Vitrolife). If the embryos were developed to high quality blastocysts based on Gardner's criteria (Gardner et al. 2000), they are vitrified for future ET. Frozen-thawed embryos were transferred following artificial endometrium preparation by the exogenous administration of estrogen (Estrana Tape; Hisamitsu Pharmaceutical). The dosage and duration of estrogen supplementation were based on the ultrasound examination of endometrial thickness in individual patients. After confirmation of >8 mm of endometrial thickness, patients received the treatment of vaginal progesterone tablets (Lutinus tablets®, Ferring Pharma).

Transvaginal ultrasound-guided ET was performed on day 2 or 3 of progesterone treatments using a K-Soft 500 Embryo Transfer Catheter® (Cook, Ireland Ltd.). Serum β -hCG concentration was determined at 14 days after ET. If it is positive (>5 mIU/ml), clinical pregnancy was diagnosed if fetal heart beats were detected by transvaginal ultrasound at 6 weeks later. From the day of ET, all patients had luteal support with daily vaginal tablets of progesterone (Lutinus tablets®) together with weekly progesterone injection (Progeston depot® 125 mg; Fuji Pharma Co, Ltd.). Patients with a positive pregnancy test continued the luteal support until the 12th week of pregnancy. The clinical and

laboratory data of all patients included in the study were retrospectively analyzed.

2.4 | Data analyses

Main outcome of measure in this study was the follicle growth resulting in OPU. Secondary outcomes were the proportions of successful oocyte retrieval per OPU, number of embryo transfer per patient, clinical pregnancy, and live birth per ET. Further secondary outcomes were as follows: the comparison of clinical and laboratory parameters among cycles with successful OPU and empty follicle, the comparison of patient characteristics (especially type of karyotype abnormality), and basal laboratory parameters among patients with successful and unsuccessful follicle growth.

2.5 | Statistical analysis

Statistical analysis was carried out by using Student's *t*-test for variables followed normal distribution as appropriate. Continuous variables that were not normally distributed were compared by the Mann–Whitney *U* or Kruskal–Wallis test, as appropriate. Association between categorical variables (different abnormal karyotypes) was assessed with chi-square test. All tests were considered significant at $p < 0.05$. Data were presented as mean \pm standard deviation or median (range) when appropriate.

3 | RESULTS

3.1 | Characteristics of enrolled POI patients with karyotype abnormality

In 49 POI patients with abnormal karyotype, five patients were primary amenorrhea (PA), whereas 44 patients showed secondary amenorrhea (SA) as described in Figure 1. All women in the study group

	Oocyte (+)	Oocyte (-)	p-value
Patients (n)	n = 18	n = 5	
OPU cycles (n)	n = 47	n = 22	
Age at first visit (years)	32 (27–44)	32 (19–41)	0.78
BMI (Kg/m ²)	21.5 (17.7–27)	22.5 (20–23)	0.6
Menarche (years)	13.5 (10–14)	12.5 (11–13)	0.53
Age at amenorrhea (years)	30 (16–38)	28 (15–37)	0.44
Duration of amenorrhea (years)	4.8 (1.5–14.8)	4.2 (2–6.5)	0.77
AMH (ng/ml)	<0.02	<0.02	0.32
B-FSH (mIU/L)	50.7 (26–140.7)	49.5 (39.1–100)	0.38
B-LH (mIU/L)	23.6 (8.2–53.8)	22.5 (6.5–34)	0.45
B-E2 (pg/ml)	15.3 (9–25.5)	14.4 (7–29.9)	0.22
Chromosomal abnormality			
Numerical	7/18 (39%)	2/5 (40%)	0.37
Structural	6/18 (33%)	2/5 (40%)	0.44
Other	5/18 (28%)	1/5 (20%)	0.72

Note: Values reported as, median (range) or number. FSH, LH and estradiol levels were calculated using a mean value of two measurements. The Mann-Whitney *U* was used for statistical analysis. Abbreviations: AMH, anti mullerian hormone; B-E2, baseline estradiol; B-FSH, baseline follicle stimulating hormone; B-LH, baseline luteinizing hormone; BMI, body mass index; OPU, ovum pick up.

	Follicle growth (+)	Follicle growth (-)	p-value
Patients (n)	n = 28	n = 16	
Age at first visit (years)	32 (19–44)	35 (26–44)	0.18
BMI (Kg/m ²)	21.9 (17.7–27)	20.2 (14–27.8)	0.27
Menarche (years)	13 (10–16)	11.5 (10–16)	0.54
Duration of amenorrhea (years)	4.2 (0.5–15)	9.6 (1–24)	0.007
Stimulation length (days)	16 (3–49)	19 (2–51)	0.35
AMH (ng/ml)	<0.02	<0.02	-
B-FSH (mIU/ml)	51.1 (26–144)	73.7 (38–118.1)	0.028
B-LH (mIU/ml)	24.4 (6.2–53.8)	31 (13–63.6)	0.057
B-E2 (pg/ml)	23.2 (10–43.3)	14.5 (12–44.2)	0.04
Chromosomal abnormality			
Numerical	11/28 (39%)	8/16 (50%)	0.27
Structural	10/28 (36%)	7/16 (44%)	0.44
Other	7/28 (25%)	1/16 (6%)	0.1
Numerical abnormalities			
Turner/Turner mosaic	6/11 (55%)	7/8 (87.5%)	0.19
Non-Turner	5/11 (45%)	1/8 (12.5%)	0.09

Note: Values reported as, median (range) or number. FSH, LH and estradiol levels were calculated using a mean value of two measurements. The Mann-Whitney *U* was used for statistical analysis. Abbreviations: BMI-body mass index, AMH-anti mullerian hormone, B-FSH-baseline follicle stimulating hormone, B-LH-baseline luteinizing hormone, B-E2-baseline estradiol.

had no POI family history, except for one patient whose mother diagnosed with POI due to mosaic for Turner syndrome (TS). The median age of all enrolled patients was 34 (range, 19–44) years. In patients with secondary amenorrhea, age at menarche was 12.5 (10–16) years.

Amenorrhea period before commencing COS was, median (range), 5.8 (0.5–24) years. Majority of patients showed undetectable AMH levels. Basic characteristics were comparable between patients with primary and secondary amenorrhea as summarized in [Table 1](#).

TABLE 1 Comparison of clinical and laboratory parameters between POI women with at least one oocyte-positive cycle vs. without oocyte-positive

TABLE 2 Characteristics of POI patients with secondary amenorrhea and abnormal karyotype with or without a detectable follicular activity after ovarian stimulation

TABLE 3 Comparison of clinical and laboratory parameters between OPU cycles with successful oocyte retrieval and empty follicle in POI patients with abnormal karyotype

	Oocyte (+)	Empty follicle	p- value
OPU cycles (n)	n = 47	n = 22	
Age at first visit (years)	32 (27–44)	32 (19–41)	0.78
BMI (Kg/m ²)	21.5 (17.7–27)	22.5 (20–23)	0.6
Menarche (years)	13.5 (10–14)	12.5 (11–13)	0.53
Duration of amenorrhea (years)	4.8 (1.5–14.8)	4.2 (2–6.5)	0.77
AMH (ng/ml)	<0.02	<0.02	0.32
B-FSH (mIU/L)	50.7 (26–140.7)	49.5 (39.1–100)	0.38
B-LH (mIU/L)	23.6 (8.2–53.8)	22.5 (6.5–34)	0.45
B-E2 (pg/ml)	15.3 (9–25.5)	14.4 (7–29.9)	0.22
COS parameters			
Average stimulation (days)	16.5 (3–49)	19 (2–51)	0.35
Peak estradiol (pg/ml)	326.7 (144–521.1)	267.2 (119–779)	0.27
Peak LH under COS (IU/L)	4.5 (0.3–16.3)	6.5 (2.2–17.2)	0.002

Note: Values reported as, median (range) or number. FSH, LH and estradiol levels were calculated using a mean value of two measurements. The Mann–Whitney U was used for statistical analysis.

Abbreviations: BMI-body mass index, AMH-anti mullerian hormone, B-FSH-baseline follicle stimulating hormone, B-LH-baseline luteinizing hormone, B-E2-baseline estradiol. COS- controlled ovarian stimulation.

3.2 | Comparison of characteristics, clinical, and laboratory parameters between patients with and without detectable follicular activity and between OPU result with successful oocyte retrieval and empty follicle

As shown in Table 2, the main difference in patient characteristics between women with or without detectable follicular activity was in the median duration of amenorrhea before commencing COS (4.2 vs. 9.6 years, $p = 0.007$, respectively). Furthermore, the median FSH levels were significantly higher and the median E2 level was significantly lower in the group of without detectable follicular activity than those in with detectable follicular activity. Other parameters were similar between the groups with or without detectable follicular activity.

As shown in Table 3, the median peak LH under COS was significantly higher in the group of empty follicle than that in retrieved oocyte group (4.5 vs. 6.5 IU/ml, $p = 0.002$, respectively).

There was no difference in other parameters between the groups with successful oocyte retrieval and empty follicle.

3.3 | Laboratory outcome of IVF-ET in POI patients with secondary amenorrhea and a karyotype abnormality

During the study period, the median number of COS attempts per patient was eight (range, 1–30) and follicle growth was achieved in 57% (28/49) of patients with a median duration of treatment of 1.5 years (range, 0.5–4.8). Of note, no follicle growth was achieved

in five patients with primary amenorrhea. We could perform OPU in 47% (23/49) of patients and retrieved at least one oocyte in 37% (18/49) with an average retrieval of 2.4 ± 2.7 oocytes per patient. During 69 OPU attempts in 23 patients, a total of 50 oocytes were retrieved: 25 mature oocytes, 5 immature oocytes, 4 atretic oocytes, and 16 cumulus-oocyte complex (COC). Twenty-five mature oocytes were fertilized using ICSI (fertilization rate: 64%, 16/25), whereas 16 COC were fertilized using conventional IVF (fertilization rate: 81%, 13/16).

After fertilization, a total 24 embryos were developed and reached to cleavage stage with median grade 3 (G3) (range, G2–4). All embryos exhibiting good-fair morphology (G1–3) were cryopreserved at day 2 or 3, whereas cleavage stage embryos with low grade (G4) at day 2 or 3 were allowed to develop to blastocyst stage and if not developed were discarded. As a consequence, 11 out of 49 patients (22%) could have cryopreserved 17 embryos. All immature oocytes were degenerated after in vitro maturation attempt.

For ET, a single embryo of the best available quality was transferred per each ET under hormonal replacement therapy. Three clinical pregnancies were achieved when using a 4CC blastocyst (patient 5, 32 years of age), a G3 at 2-cell stage (patient 11, 27 years of age), and a G3 at 4-cell stage (patient 13, 30 years of age). All three pregnancies resulted in each single live birth in total, eight patients attempted nine embryo transfers (two times of ET in one patient). Clinical pregnancy per patient was 6.1% (3/49), and clinical pregnancy and live birth rates per ET was 33.3% (3/9). Clinical outcomes of IVF-ET in individual POI patient with abnormal karyotype who achieved follicle growth after COS and attempted oocyte retrieval are shown in Table 4 and without detectable follicular activity in Table 5.

TABLE 4 Clinical outcome of IVF-ET in POI patients with abnormal karyotype who achieved follicle growth after ovarian stimulation and attempted oocyte retrieval

N.	Age at first visit	Karyotype	COS (n)	OPU (n)	OPU outcome	Embryo quality	ET	CP
1	34	47,XXX	14	2	1-MII	2C-G3(D2)	-	N/A
2	36	46,X,del(X)(q25)	20	2	0		-	N/A
3	40	47,XXX	4	1	0		-	N/A
4	32	45,XX,rob(14;21)(q10;q10)	15	4	3-MII	2C-G2(d2),4C-G3*2(D2)	-	N/A
5	32	47,XXX	18	8	4-MII,1-atretic	4CC*2(BLT),4C-G3(D2),4C-G4(D2)	1	Yes
6	30	45X[19]/47XXX[16]	14	1	1-MII	4C-G4	-	N/A
7	19	46,X,del(X)(p.11.23)	1	1	0		-	N/A
8	29	46,X,del(X)(p11.3)	18	10	2-MII,2-MI,1-atretic	1cell	-	N/A
9	27	46Xdel(X)(q24)	5	2	1-MII,1-MI	OPN	-	N/A
10	35	46XX+1gh	15	2	1-MI	atretic	-	N/A
11	27	45X[47]/47XXX[3]	1	1	2-MII	2C-G3,2C-G3	1	Yes
12	29	46,X,del(X)(q27)	13	3	2-MII,1-GV	4C-G2	1	No
13	30	46XX 15ph+	12	5	3-MII,1-GV	4C-G3,3C-G4	1	Yes
14	31	46,X,del(X)(q22)	8	1	0		-	N/A
15	32	46,X,del(X)(q24→qter)	30	9	8-MII,1-atretic	6C-G3,3C-G4,4C-G3	2	No
16	27	45,X[13]/47,XXX[37]	6	1	0		-	N/A
17	31	46XX,t(2;14)(q21;q11.2)	2	1	1-atretic		-	N/A
18	41	47,XXX	8	2	3-MII	3C-G3	1	No
19	35	46,X,del(X)(q26)	14	1	1 - MII	6C-G3	-	N/A
20	41	45,XX,rob(13;14)(q10;q10)	8	3	2-MII	3PN,OPN	-	N/A
21	35	46X,del(X)(p11.2)	26	5	3-MII	3C-G3	1	No
22	40	47,XXX	16	1	1-MII	3PN		N/A
23	43	45X[15]/47XXX[12]/46XX[3]	16	3	3-MII	3C-G4,4C-G4,4C-G3	1	No

Note: The numbers in COS and OPU columns indicate the number of COS and the number of OPU underwent in each patient, respectively.

Abbreviations: #C-G#, Cells-Grade; BLT, blastocyst; COS, controlled ovarian stimulation attempts; CP, clinical pregnancy; ET-embryo transfer; N/A, not applicable; OPU, oocyte pick-up; PN, pronuclei.

3.4 | Difference of clinical outcomes after ovarian stimulation in POI patients according to karyotype abnormality

Our patients could classify into five groups according to X chromosome numerical and structural abnormalities and autosomal chromosome abnormalities (Tables 4 and 6).

In X chromosome numerical abnormalities, the median age of patients with 47,XXX was 40 (range, 32–41) years with a median duration of amenorrhea of 3.5 (range, 1.5–15) years. This group could have a follicle growth with OPU in 83.3% (5/6) patients. Total number of oocytes retrieved in this group was 10 with one degenerated oocyte and nine (90%) mature oocytes or COCs. After fertilization, embryos were cryopreserved in 50% (3/6) patients. We could attempt ET in two patients (patient 5 and 18), and then one patient conceived and delivered a healthy baby (patient 5). In another type of X chromosome numerical abnormalities, mosaic TS group with 34 (range, 27–44) years of the median age showed a median duration of

amenorrhea of 10 (range, 2–24) years. However, patients with mosaic TS had a lower rate of follicle growth with OPU in 36% (5/14). A total of six oocytes or COCs was retrieved and then one patient (patient 11) could cryopreserve an embryo. After ET, this patient conceived and delivered a healthy baby.

Patient with X chromosome structural abnormalities (mainly deletions of critical regions on Xp or Xq) with a median age of 30.5 (range, 19–36) years showed a median duration of amenorrhea of 4.5 (range, 0.5–7) years. This group could have a follicle growth with OPU in 53% (9/17) patients, which resulted in a total of 23 oocytes with four immature and two degenerated oocytes and 17 (74%) mature oocytes or COCs, although embryo cryopreservation was possible in 23.5% (4/17) patients, no pregnancy was achieved after ET in three patients,

In autosomal rearrangements (Robertsonian, reciprocal, and other balanced rearrangements), the median age of patients was 33 (range, 31–41) years and a median duration of amenorrhea of 2 (range, 0.5–3.5) years. After COS, OPU was possible in 80% (4/5) patients, and a

TABLE 5 Characteristics and clinical outcomes of ovarian stimulation in POI patients according to karyotype abnormality

	47,XXX	mosaic TS	XS	AR	PVC
	n = 6	n = 14	n = 17	n = 5	n = 2
Age at first visit (years)	40 (32–41)	34 (27–44)	30.5 (19–36)	33 (31–41)	32.5 (30–35)
BMI (Kg/m ²)	22 (19–23.4)	20.5 (18–27)	22 (18–23)	21 (17–24)	20.5 (18–23)
Menarche (years)	12 (10–14)	13 (11–16)	11.5 (10–14)	13 (11–14)	13
Duration of amenorrhea (years)	3.5 (1.5–15)	10 (2–24)	4.5 (0.5–7)	2.0 (0.5–3.5)	2.3 (2–2.5)
AMH (ng/ml)	<0.02	<0.02	<0.02	<0.02	<0.02
B-FSH (mIU/L)	51 (43–58)	70.5 (28.8–118)	47.3 (26–144)	66 (36.4–88)	84 (69–100)
B-LH (mIU/L)	29.5 (23–31)	26.8 (13.7–63.6)	19 (8–53.8)	25.1 (17.2–31)	25 (17–34)
B-E2 (pg/ml)	21 (12.5–43)	14 (10–43.2)	16 (6–23)	10 (5–15)	17 (15–19)
COS outcomes					
Patients with OPU, % (n)	83 (5/6)	36 (5/14)	53 (9/17)	80 (4/5)	100 (2/2)
OPU per patient (n)	2 (0–8)	1 (0–3)	2 (0–10)	2 (0–4)	3.5 (2–5)
Oocyte per patient (n)	1 (0–5)	1 (0–3)	2 (0–9)	1 (0–3)	2.5 (1–4)
Total oocytes (n)	10	6	23	6	5
Total embryos (n)	6	6	6	3	3
Total ET (n)	2	2	4	0	1
Pregnancy (n)	1	1	-	-	1
Live birth (n)	1	1	-	-	1

Note: Values reported as, median (range) or number. The Kruskal–Wallis test was used for statistical analysis.

Abbreviations: AMH, anti mullerian hormone; AR, autosomal rearrangements; B-E2, baseline estradiol; B-FSH, baseline follicle stimulating hormone; B-LH, baseline luteinizing hormone; BMI, body mass index; COS, controlled ovarian stimulation; ET, embryo transfer; OPU, oocyte pick-up; PVC, polymorphic variants of chromosomes; TS, turner syndrome; XS, X chromosome structural abnormalities.

total of six oocytes aspirated with one degenerated oocyte and five (83%) mature oocytes or COCs. Three embryos could be cryopreserved in 25% (1/4) patients and are currently awaiting for transfer.

We also found two patients with polymorphic variants of chromosomes (Sahin et al. 2008). The first patient (46,XX,+15ph) was at 30 years-old with 2.5 years of amenorrhea. After 5 times of OPU attempts, she obtained a total of four oocytes with 75% (3/4) mature oocytes and 1 GV resulting in 2 cryopreserved embryos. After ET, she conceived and delivered a healthy baby. The second patient (46XX+1gh) was at 35 years-old with 2 years of amenorrhea. After 2 times of OPU attempts one oocyte could be retrieved, but due to an abnormal fertilization (3 pronuclei) and poor quality embryo (4 cell stage-grade 4), ET was not performed.

4 | DISCUSSION

In this study, we found more than half of POI patients with abnormal karyotypes could respond to ovarian stimulation with successful follicular growth, followed by oocyte retrieval.

Previous studies showed successful ovulation induction rates in patients with normal and abnormal karyotypes, ranging between 0%–46%, with clinical parameters varying among the reports.^{11,13,19–22} In our hospital, the success of ovulation induction under ovarian stimulation in patients with normal karyotype was 48% per patient during six years of observation.²³ Although the method for data collection

was not identical, the success of ovulation induction was quite similar between POI patients with normal and abnormal karyotypes. The only consistent parameter with successful follicular growth was a shorter duration of amenorrhea before commencing infertility treatments. In our cases, we further compared other possible clinical and laboratory parameters between patients with successful follicular development and those with none. Patient age at first visit, body mass index (BMI), and age at menarche were similar in both groups. Baseline hormonal profiles revealed that serum LH and FSH levels tend to be lower and estrogen levels tend to be higher in patients who could respond to COS with follicular growth. These results suggest potential roles of balancing gonadotropins levels and estrogen in follicle growth. The use of estrogen pre-treatment was advocated to lower endogenous gonadotropins, and improve responsiveness to COS or induce spontaneous follicular growth.^{16,21,23–25}

A randomized controlled study demonstrated an advantage of estrogen pretreatment compared to placebo in 50 POI patients with a 32% ovulation induction rate leading to four pregnancies.²¹ In another larger uncontrolled interventional study, the rate of successful follicle growth in the estrogen pretreatment group was higher as compared to the group with GnRH agonist pretreatment [19% of cycles (61/311) vs. 16% of cycles (7/43)], respectively, and a higher proportion of pregnant patients of 20% (19/91) vs. 0% (0/9), respectively.¹³ In both studies, most pregnancies were obtained in patients with estrogen therapy to decrease serum FSH and LH levels prior to COS. Previous randomized controlled studies using

TABLE 6 Characteristics of POI patients with primary and secondary amenorrhea and abnormal karyotype without a detectable follicular activity after controlled ovarian stimulation.

N.	Age at first visit (n)	Karyotype	Amenorrhea (years)	COS (n)	Comments
1	36	46X,del(X)(q21)	6.4	9	Primary amenorrhea
2	31	46X,del(X)(q21.3;p11.2)	3.0	14	Primary amenorrhea
3	31	45,X0	11.3	9	Primary amenorrhea
4	41	45,X[44]/46,XX[6]	11.3	1	Primary amenorrhea
5	21	45,X[8]/46,X,del(X)(p11.2)[42]	10.8	1	Primary amenorrhea
6	38	45,X[30]/46,X,del(X)(p11.2)[19]/46,XX[1]	24	12	Secondary amenorrhea
7	33	46X,t(x:11)(q22;p11.2)	1.5	7	Secondary amenorrhea
8	40	47,XXX	1.8	21	Secondary amenorrhea
9	33	47,XXX[26]/45X[24]	12.3	11	Secondary amenorrhea
10	35	46x,del(Xq22-28)	4.5	7	Secondary amenorrhea
11	44	45,x[9]/46,XX[41]	8.3	7	Secondary amenorrhea
12	39	45XX.der(14;21)(q10;q10)	13.9	0	Secondary amenorrhea
13	33	46XXinv(9)(p12q13)	9.8	0	Secondary amenorrhea
14	30	45,X[19]/46,X,r(X)[11]	24.8	6	Secondary amenorrhea
15	30	46X,del(X)(q22)	14.7	0	Secondary amenorrhea
16	37	46Xdel(X)(q23)	9.4	13	Secondary amenorrhea
17	38	45,X[2]/46,XX[98]	1.5	6	Secondary amenorrhea
18	26	46Xadd(x)(q22.3)del(q)22.3-qter	2.0	10	Secondary amenorrhea
19	38	46XX/45X[29,1]	9.0	10	Secondary amenorrhea
20	34	46X,del(X)(q24)[30]	10.0	3	Secondary amenorrhea
21	32	45,X0	7.3	12	Secondary amenorrhea
22	35	46X,del(X)(q22.3)	6.4	3	Secondary amenorrhea
23	38	45X[4],46XX[16]	3.0	8	Secondary amenorrhea
24	33	46,X,del(X)(q26)	11.3	1	Secondary amenorrhea
25	34	45,X[37]/46,XX[63]	11.3	7	Secondary amenorrhea
26	37	46XdelX(q26)	10.8	8	Secondary amenorrhea

Note: The number in COS column indicates the number of COS underwent in each patient.

Abbreviation: COS, controlled ovarian stimulation attempts.

GnRH agonist to suppress endogenous gonadotropins before COS showed a nonsignificant improvement in ovulation rate, but failed to show any differences in pregnancy rate.^{19,20} In addition to the estrogen pre-treatment, estrogen replacement therapy during COS has also several potential benefits. Chronically elevated serum LH can potentially impair follicular growth by decreasing FSH receptor mRNA levels in preantral follicles as well as GDF9 mRNA levels in oocytes.²⁶ Therefore, maintaining normal serum LH has an important role in enabling healthy follicular growth.^{23,25,27} In the present study, we combined estrogen pre-treatment with cyclic progesterone therapy to suppress serum FSH and LH below 10 mIU/ml before commencing COS (with the addition of GnRH agonist in refractory cases) and estrogen replacement therapy during COS, leading to 29% of successful follicle growth. Overall, previous studies and our results indicate the importance of suppress serum FSH and LH levels prior to COS and during COS for successful follicle growth in POI patients. With respect to the etiology of POI as a predictor for pregnancy, previous studies found

genetic etiologies to be associated with lower pregnancy rates as compared to non-genetic ones.^{17,23} One study reported a 7% prevalence of pregnancy, and no live births in POI patients with abnormal karyotypes.¹⁷ In our study, eight patients with abnormal karyotype attempted ET, three patients conceived and delivered healthy babies (live birth rate per ET 33.3%). The frequency of an abnormal karyotype was reported higher in women with primary amenorrhea and a lower incidence of follicle growth.¹⁷ Our results corroborate with the previous report as our primary amenorrhea patients could not achieve a single follicle growth during the study period. Because no information was available in the aforementioned study as to whether the patients previously attempted any infertility treatments or tried natural conception, it is difficult to make a direct comparison of the clinical outcomes between the two studies.

Various etiologies such as haploinsufficiency of pivotal genes on the X chromosome, or non-specific meiotic impairment have been hypothesized to explain the accelerated atresia of 45, X oocytes.^{28,29} In

our cohort, only one patient out of twelve with mosaic TS had available embryos for ET, and it is worth mentioning that her COS cycles were at a relatively young age of 26 years, with less than one year of amenorrhea before starting infertility treatment. In case of TS and mosaic TS patients should be encouraged, receive counseling about their reproductive potential, and options for fertility preservation.^{14,30,31}

Another type of a numerical defect is an extra X chromosome (triple X), which may predispose a woman to develop POI. Overexpression of genes that escape X-inactivation could cause POI in 47, XXX, as well as often being associated with autoimmune diseases.³² In the present study, five out of six patients with 47, XXX could respond to COS with OPU, leading to an ET and a successful live birth.

A common group of chromosomal aberrations are structural deletions. A critical region was attributed to breakpoints of X-autosome translocations associated with POI. This region extends from Xq13-q21 to Xq23-q27.³³ Other less common karyotypes are autosomal rearrangements (Robertsonian, reciprocal, and other balanced rearrangements) and polymorphisms in the chromosomal heterochromatin region.^{3,34,35} We found these groups of patients to have similar outcomes as POI patients with a normal karyotype.^{19,27,36}

Empty follicle syndrome (EFS) at oocyte retrieval is often observed in women with POI. The incidence of EFS in the current study was 33%. Although our result is higher than 1%–7% reported in the general infertility population,^{37,38} it remains considerably lower than the incidence of 70%, previously reported in POI patients followed for spontaneous follicular growth with an OPU attempt.¹² A possible explanation for our lower rate of EFS is that under FSH stimulation with suppression of LH levels during ovarian stimulation. Using estrogen replacement together with continuous GnRHa treatment during ovarian stimulation,²⁵ we were able to suppress and maintain a proper LH levels to improve follicular function. Patients who did not receive this treatment had consistently high gonadotropin levels, which could have negatively affected growing follicles through down regulation of FSH receptors, and triggering of meiotic activity within oocytes, prior to the induction of luteinization or ovulation.^{26,39} In addition, the percentage of immature and degenerated oocytes was 13.5% in the present study. This rate is comparable with the 11–17% reported in the general infertility population,⁴⁰ suggesting the importance of maintain low LH levels during ovarian stimulation.

Based on the results of our meticulous analysis, it could be argued that the chances for live birth in abnormal karyotype patients with secondary amenorrhea are very much realistic. This represents a fundamental medical advancement in the chances of reproductive success among infertile couples, including improvements in the associated economic and psychological aspects. However, it is plausible that several limitations may have influenced the results obtained. Mainly, this is a retrospective study with a relatively small sample size, and given that the focus of the study is POI patients with abnormal karyotypes, our results might not be representative of the general population of women with POI. In conclusion, POI women

with abnormal karyotypes, who seek to improve their chances of having biological children, are encouraged to attempt ovarian stimulations for IVF-ET.

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CONFLICT OF INTEREST

All authors declare they did not receive funding for this study, and declare no conflicts of interest.

HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

This article does not contain any study with human participants that have been performed by any of the authors.

ANIMAL STUDIES

This article does not contain any study with animal participants that have been performed by any of the authors.

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