



Frequency of Sperm Aneuploidy in Oligoasthenoteratozoospermic (OAT) Patients by Comprehensive Chromosome Screening: A Proof of Concept

Parishad Saei^{1,2}, Masood Bazrgar^{1*}, Hamid Gourabi¹, Roxana Kariminejad³, Poopak Eftekhari-Yazdi⁴, Mostafa Fakhri¹

1- Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

2- Department of Molecular Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran

3- Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran

4- Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Background: Embryonic aneuploidy usually results in implantation failure and miscarriage. Considering significantly high frequency of sperm aneuploidy reported in oligoasthenoteratozoospermia (OAT) using fluorescence in situ hybridization (FISH) in limited number of chromosomes and lack of comprehensive chromosome screening (CCS) in OAT, the aim of this study was applying CCS in OAT sperm and comparison of the results with FISH findings.

Methods: Five OAT patients with normal blood karyotypes and history of implantation failure were included. The successfully amplified samples, each containing two sperm, were analyzed by array comparative genomic hybridization (aCGH). FISH was utilized mainly depending on the aneuploidies found by aCGH to assess their frequencies in total sperm population.

Results: In aCGH for 30 sperm, aneuploidy was found in 66% of samples. Following the study of 4300 sperm by FISH, an average of 55.46% aneuploidy was observed. No pregnancy was resulted with normal partners.

Conclusion: Using aCGH, some abnormalities were observed that are not typically considered in sperm FISH studies. Despite small sample size of the comprehensive study, like other similar studies, the frequency of aneuploidies was considerable and similar to FISH. Aneuploidies revealed by aCGH at single sperm resolution were different from sperm population detected by FISH. Considering high frequency of aneuploidy in OATs sperm, preimplantation genetic testing for aneuploidy (PGT-A) can be used for in transfer of chromosomally normal embryos.

Keywords: Aneuploidy, Array comparative genomic hybridization, Fluorescence, In situ hybridization, Oligospermia, Spermatozoa.

To cite this article: Saei P, Bazrgar M, Gourabi H, Kariminejad R, Eftekhari-Yazdi P, Fakhri M. Frequency of Sperm Aneuploidy in Oligoasthenoteratozoospermic (OAT) Patients by Comprehensive Chromosome Screening: A Proof of Concept. *J Reprod Infertil.* 2021;22(1): 57-64. <http://dx.doi.org/10.18502/jri.v22i1.4996>.

Introduction

Fertility problems affect 60-80 million couples worldwide according to WHO estimations and approximately half of these problems are due to a male factor (1). Aneuploidy as a numerical chromosome abnormality is considered

one of the main causes of pregnancy loss (2). Maternal parameters such as age and meiotic defects are associated with production of chromosomally abnormal embryo and reduced quality of embryo (3); however, scarce information is available on

* Corresponding Author:
Masood Bazrgar, No. 2,
Hafez St., Banihashem St.,
Resalat Ave., P.O.Box:
16635-148 Tehran, Iran
E-mail:
mbazrgar@royaninstitute.org

Received: Apr. 15, 2020
Accepted: Sept. 15, 2020

the role of paternal factors. Total aneuploidy of 4.5% was calculated in normozoospermic males based on common studies that employed fluorescence in situ hybridization (FISH) (4). Men with abnormal seminal parameters but normal karyotype carry more chromosomal aberrations such as malsegregation; interestingly, their sperm aneuploidy rate is higher than normal or infertile men with normal spermatozoa (5). According to various studies conducted in the field of sperm aneuploidy in individuals with different phenotypes of infertility (Oligozoospermia, asthenozoospermia and teratozoospermia), there is a significantly high incidence of aneuploidy in spermatozoa from severely oligoasthenoteratozoospermic (OAT) patients (6, 7) compared to moderate OAT subjects (11). OAT patients have lower rate of gestation and implantation after intra cytoplasmic sperm injection (ICSI) treatment (8-10).

FISH as a commonly used method to detect aneuploidy has facilitated examination of sperm in severe male factor infertility. Different rates of total aneuploidy have been reported due to different and limited number of chromosomes studied as one of the major problems of using FISH for comprehensive study of aneuploidy. Few studies have employed aCGH for sperm aneuploidy assessment in a single sperm case (11, 12). The development of a comprehensive tool and the accurate assessment of all chromosomes would allow us to improve our understanding on the pathophysiology of spermatogenesis. It estimate the paternally derived aneuploidy risk and hence better manage the high risk in patients for transmitting chromosomal abnormalities during *in vivo* or *in vitro* conception.

According to FISH findings, due to limited number of chromosomes in OAT and lack of simultaneous evaluation of all chromosomes in OAT patients, this proof of concept was designed to better evaluate the contribution of aneuploidy in OAT. Comprehensive aneuploidy screening was applied in spermatozoa collected from these subjects at

single sperm resolution by aCGH as a powerful technique for aneuploidy detection and the results were compared with those obtained by FISH findings in total sperm population of the same subjects.

Methods

The protocols used in this study were approved by Institutional Review Board and informed consent was obtained from all the patients after approval of the study by the Institutional Ethics Committee.

Patients: Fresh semen samples were collected from five infertile men with severe OAT. All the subjects had sperm concentrations <5 million/ml, total motility <40% and severe teratozoospermia with 0-1% normal forms according to WHO guidelines (2010); the patients were referred for treatment of infertility and underwent ICSI. They had normal blood karyotypes and a history of implantation failure. Patients with Y chromosome microdeletions, chemotherapy, exposure to toxicity, high temperature, varicocele, genitourinary infections, malignancy and sex hormones alterations were excluded from the study. Female partners were reproductively healthy and <36 years old. The history and characteristics of couples and semen parameters are summarized in table 1.

Sperm preparation and isolation for aCGH: A part of all semen specimens was used for ICSI and the remainder was employed for aCGH analysis and FISH. A small aliquot of semen was rinsed two times in Ham's F10 medium and centrifuged at 2700 rpm for 5 min. A pellet of almost 2 µl was placed into a polyvinylpyrrolidone (PVP) drop for immobilization and convenient handling of sperm; then, 2 spermatozoa were aspirated and transferred to 0.2 ml PCR tube in a drop containing 2 µl of sterile phosphate-buffered saline (PBS) without calcium and magnesium (Gibco, UK). 4 samples were prepared for each patient.

aCGH analysis: Whole Genome Amplification

Table 1. Characteristics and semen parameters of OAT patients

Patients	Age	Infertility duration (year)	Implantation failure (cycles)	Volume (ml)	Sperm concentration ×10 ⁶ /ml	Total motility (%)	Normal morphology (%)
P1	38	10.5	1	3.5	2	10	1
P2	36	2	1	2	4	30	1
P3	33	6	4	2	1.5-2	10-15	0-1
P4	36	8	1	1.5	3	10	1
P5	35	7	2	3	0.01	2-3	0

was conducted according to 24 sure V3 protocol. Decondensation was performed as previously described (13) using proteinase K and dithiothreitol (DTT) between two steps of extraction and pre-amplification. Samples were loaded on 1% agarose gel and those with successful amplification were analyzed by 24 sure illumina platform aCGH protocol according to manufacturer's instructions. The images of hybridization were scanned by a laser scanner (InnoScan 710, Innopsys, France), then analyzed by BlueFuse Multi Software version 4.1.

Preparation and decondensation of sperm nuclei for FISH: Semen samples were prepared by washing according to a previously described protocol (16). Double or triple color FISH was used with 10-11 relevant probes (Vysis, USA), mainly based on aneuploidies found by aCGH, to compare the results of single sperm aCGH with total sperm population. For each subject, probes were added to the parts of the slides with suitable density as indicated in supplementary table 1. FISH was performed using the protocol introduced by the manufacturer (Vysis, USA).

FISH scoring criteria: Only spermatozoa with clear signals were analyzed. Two completely separate spots in one spermatozoon were considered

disomy and if there was no signal, nullisomy was accounted. However, if nullisomy was observed in all chromosomes examined in a slide, it was not scored due to the probable lack of hybridization. Overlapped cells with doubtful signals were also ignored.

Results

The age of our patients ranged from 33 to 38 years with 2-10.5 years of infertility and 1-4 cycles of implantation failure, as mentioned in table 1. Totally, 15 out of 20 samples had successful amplification and good quality for aCGH, which were included in aCGH data analysis. A wide range of abnormalities was found in OAT sperm by aCGH. Aneuploidy was detected in 66% of samples ranging from 1 to 6 chromosomes per patient. Multiple aneuploidy was seen in 26.6% of samples. In total, sex chromosome disomy and trisomy were detected in 26.6% of samples. Also, 33.3% of samples were found to be normal. Next, those abnormal chromosomes for FISH with more possibility to involvement of entire chromosome were nominated, mainly according to aCGH findings. The results of aCGH data analysis for all patients and some genomic profile examples are presented in table 2 and figure 1, respectively. In

Table 2. Array CGH analysis results of OAT patients

Subjects	Samples (total=15)	Chromosomes	
		Gain	Loss
P1			
	1	4, 5, 8, 19	3, 6, 14, 21
	2	14	16, 21, 22
	3	-	-
P2			
	1	6, 8, 9, 10, 16, 18	1, 11, 13, 14, 17, 20, 21
	2	3, 8, 10, 13, 16, 21	1, 9, 11, 18
P3			
	1	6, 7, 8, 11, 12	2, 10, 17, 21
	2	-	-
	3	20	-
P4			
	1	19, X	-
	2	19, X	-
	3	19, X, Y	-
P5			
	1	-	-
	2	-	-
	3	-	-
	4	Y	-

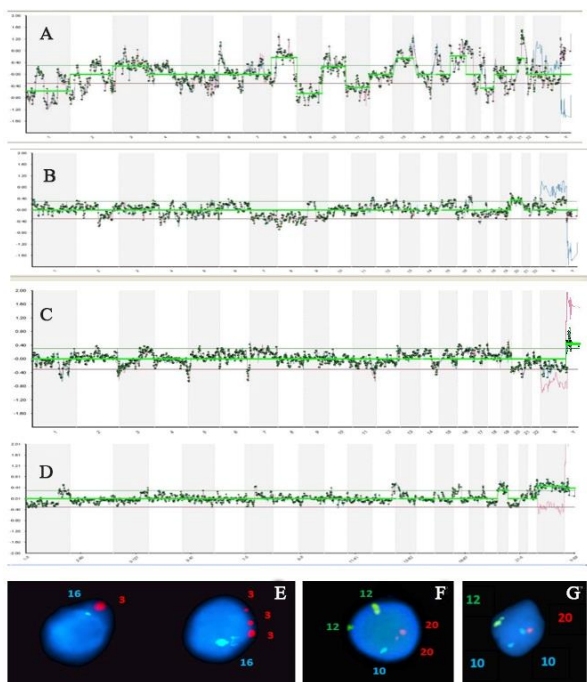


Figure 1. Array CGH profiles of OAT patients (A-D) and different types of aneuploidy in OAT sperm population as assessed by FISH (E-G). A) A genomic profile with complex abnormality for chromosomes -1+3+8-9+10-11+ 13+16-18+21 in patient 2. B) Single chromosomal aneuploidy of +20 in patient 3. C) A genomic profile with sex chromosome aneuploidy (Gain of Y) in patient 5. D) A genomic profile with gain of chromosome 19 and sex chromosome aneuploidy (Gain of X/Y) in patient 4. E) Right, a sperm with three signals for chromosome 3 (Red) and normal signal for chromosome 16 (Aqua) and left, a normal sperm with single signals for both chromosomes in patient 2. F) Double disomy for chromosomes 12 (Green) and 20 (Red) and normal signal for chromosome 10 in patient 3. G) Disomy for chromosome 10 (Aqua) and normal signals for chromosomes 12 (Green) and 20 (Red) in patient 3

FISH analyses, a total of 4300 sperm nuclei and between 400-1286 sperm per subject with reliable signals were analyzed depending on the volume of semen, sperm depositions and the number of prepared slides. Totally, 19 chromosomes, (10-11 chromosome per patient), were analyzed in this study. Total aneuploidy was 38.25-81.6% with an average of 55.46%. The frequencies of disomy and nullisomy for autosomes and sex chromosome aneuploidy are displayed in supplementary table 2. Despite high fertilization rate and transfer of 2-3 good quality embryos, none of our studied patients had pregnancy after ICSI (Supplementary table 3).

Discussion

Sperm aneuploidy occurs for several reasons such as biological, clinical and environmental fac-

tors. In this study, an attempt was made to include cases with relatively similar clinical conditions; however, many factors could lead to different aneuploidies in different cases or even different sperm in the same case probably due to effect of multiple components in chromosome-segregation machinery (14). Such differences are also visible in polar bodies and preimplantation embryos (15, 16).

Indeed, high-resolution aCGH technique contributed to a better perception of whole genome imbalances with high accuracy. In this study, 2 sperm were simultaneously studied to reduce expenses of aCGH without affecting aneuploidy detection accuracy. Despite a relatively small number of sperm in 5 patients (30 sperm in overall), the explored abnormality was high. Since number of obtained embryos from couples in average is not usually more than 6, as shown in supplementary table 3, studying 6 sperm from each patient could give an average estimation of aneuploidy status.

Using aCGH, aberrations in chromosomes 19 and 20 were observed that are not typically studied by FISH studies and abnormalities in these two chromosomes were found in 4 and 2 samples, respectively of two individuals. Extensive information about significant sperm aneuploidy rate in OAT patients, particularly in those with severe abnormal concentration, has been presented by studies that employed FISH (8, 9, 10, 17) but limited capability of this approach for examining large numbers of chromosomes led to insufficient data about complexity of abnormalities in these subjects' spermatozoa.

Differences in types of frequent aneuploid chromosomes at single sperm resolution and total sperm population indicate an error prone segregation for all of chromosomes in OAT rather than germline aneuploidies. Since aneuploidy could occur for all chromosomes even in semen samples of normal individuals with a more or less equal chance, it seems logical to find different chromosomal aneuploidies in study of 6 sperm compared with total population of sperm in the same patient unless in cases with Klinefelter syndrome that carry constitutional sex chromosomal abnormalities (18).

In this study, 38.3-81.6% total chromosomal abnormalities were found in spermatozoa from OAT patients which was nearly similar to a 14-chromosome study in OAT (9). Although normal fertile samples were not studied, aneuploidy preva-

lence in normal fertile men with study of all or large numbers of chromosomes has been reported in a range of 4.5-7.8% (4, 9, 11, 12) that is obviously different from aneuploidy range in OAT.

Sex chromosomes studies stated that XY and XX disomy was common in OAT patients (19, 20). In our FISH study, high frequency of XY disomy was observed in 4 of 5 subjects and there were no remarkable differences between the frequency of XX and YY disomy. Also, gain of sex chromosomes was found in 26.6% of samples as assessed by aCGH (Table 2) that indicated high frequency of sex chromosomes' aberrations in OAT. Despite numerous studies on comprehensive aneuploidy screening in oocytes, few studies were about comprehensive aneuploidy screening in a single sperm by aCGH (11, 12). Recently, next generation sequencing (NGS) method has been utilized for single sperm aneuploidy screening (21, 22). Despite a small number of patients, these studies found a significant percentage of abnormalities compared with normal controls. Although our sample size was also small, regarding the high incidence of aneuploidy in the current comprehensive study and previous evidence from FISH studies, our results might be generalizable to larger populations of OAT; however, further investigation of OAT patients with different inclusion criteria from current study will draw more accurate conclusion.

Regarding chromosomal groups, F and G groups, as the smallest chromosomes, were found to be abnormal in aCGH of 4 and 5 patients, respectively (Table 2); this was generally in accordance with FISH results (Supplementary table 2). There are not many publications available for F group, mainly because of FISH limitations but more susceptibility to aneuploidy for G group is clear in sperm studies (4, 8). A negative correlation between chromosome length and meiosis I segregation error has been highlighted (16).

To the best of our knowledge, this is the first comprehensive study research investigation of OAT patients with normal karyotypes; moreover, aneuploidy was detected in sperm population by FISH. Some studies elucidated that negative effects of impaired male fertility might lead to chromosomal abnormalities in embryos (23, 24). It should be reminded that follow up of our studied patients revealed no pregnancy after ICSI (Supplementary table 3). A recent 18-year retrospective study reported the highest aneuploidy rate in OAT patients among patients with abnormal se-

men parameters. This study compared live birth rate in patients with abnormal sperm FISH result with/without PGT-A and revealed an obvious increase of live birth rate following PGT-A (25).

Conclusion

In conclusion, frequency of aneuploidies found by both aCGH and FISH techniques was considerable in OAT sperm. Aneuploidies of the single sperm seem to be somewhat independent of frequent aneuploidies in total sperm population. Considering remarkable aneuploidy revealed in the current study and previous evidence from FISH studies, comprehensive PGT-A in OATs specifically in those with a history of implantation failure might be beneficial for transfer of chromosomally normal embryo.

Acknowledgement

The authors are grateful to the collaborators of Genetics Laboratory and IVF section at Royan Institute, Najmeh Sadat Masoudi and Nosratollah Hazeri.

Conflict of Interest

The authors declare that they have no conflict of interests to disclose.

Funding: This study was funded by Royan Institute and Kariminejad-Najmabadi Pathology and Genetics Center.

References

1. Singh R, Singh K. Male infertility: understanding, causes and treatment. 1st ed. Singapore: Springer; 2017. 497 p.
2. Martin RH, Ko E, Chan K. Detection of aneuploidy in human interphase spermatozoa by fluorescence in situ hybridization (FISH). *Cytogenet Cell Genet.* 1993;64(1):23-6.
3. Munné S, Chen S, Colls P, Garrisi J, Zheng X, Cekleniak N, et al, Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. *Reprod Biomed Online.* 2007;14(5):628-34.
4. Templado C, Vidal F, Estop A. Aneuploidy in human spermatozoa. *Cytogenet Genome Res.* 2011; 133(2-4):91-9.
5. Calogero AE, Burrello N, De Palma A, Barone N, D'Agata R, Vicari E. Sperm aneuploidy in infertile men. *Reprod Biomed Online.* 2003;6(3):310-7.
6. Piomboni, Paola, Anita Stendardi, and Laura Gambera. Chromosomal aberrations and aneuploidies of spermatozoa. In: *Genetic damage in human spermatozoa.* New York: Springer; 2014. p. 27-52.

7. Harton GL, Tempest HG. Chromosomal disorders and male infertility. *Asian J Androl.* 2012;14(1):32-9.
8. Rubio C, Gil-Salom M, Simon C, Vidal F, Rodrigo L, Minguez Y, et al. Incidence of sperm chromosomal abnormalities in a risk population: relationship with sperm quality and ICSI outcome. *Hum Reprod.* 2001;16(10):2084-92.
9. Pang MG, Hoegerman SF, Cuticchia AJ, Moon SY, Doncel GF, Acosta AA, et al. Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in-situ hybridization in spermatozoa from nine patients with oligoasthenoteratozoospermia undergoing intracytoplasmic sperm injection. *Hum Reprod.* 1999;14(5):1266-73.
10. Pfeffer J, Pang MG, Hoegerman SF, Osgood CJ, Stacey MW, Mayer J, et al. Aneuploidy frequencies in semen fractions from ten oligoasthenoteratozoospermic patients donating sperm for intracytoplasmic sperm injection. *Fertil Steril.* 1999;72(3):472-8.
11. Patassini C, Garolla A, Bottacin A, Menegazzo M, Speltra E, Foresta C, et al. Molecular karyotyping of human single sperm by array-comparative genomic hybridization. *PLoS One.* 2013;8(4):e60922.
12. Garolla A, Sartini B, Cosci I, Pizzol D, Ghezzi M, Bertoldo A, et al. Molecular karyotyping of single sperm with nuclear vacuoles identifies more chromosomal abnormalities in patients with testiculopathy than fertile controls: implications for ICSI. *Hum Reprod.* 2015;30(11):2493-500.
13. Ghoraieian P, Mozdarani H, Aleyasin A, Alizadeh-Nili H. Frequency of sex chromosomal disomy in spermatozoa of normal and oligozoospermic Iranian patients and its effects on fertilisation and implantation rates after ICSI. *Andrologia.* 2013;45(1):46-55.
14. Mihajlović AI, FitzHarris G. Segregating chromosomes in the mammalian oocyte. *Curr Biol.* 2018;28(16):R895-907.
15. Nakhuda G, Jing C, Butler R, Guimond C, Hitkari J, Taylor E, et al. Frequencies of chromosome specific mosaicisms in trophoctoderm biopsies detected by next-generation sequencing. *Fertil Steril.* 2018;109(5):857-65.
16. Fishel S, Craig A, Lynch C, Dowell K, Ndukwe G, Jenner L, et al. Assessment of 19,803 paired chromosomes and clinical outcome from first 150 cycles using array CGH of the first polar body for embryo selection and transfer. *J Fertil In Vitro.* 2011;1(1):1-8.
17. Vicari E, de Palma A, Burrello N, Longo G, Grazioso C, Barone N, et al. Absolute polymorphic teratozoospermia in patients with oligo-asthenozoospermia is associated with an elevated sperm aneuploidy rate. *J Androl.* 2003;24(4):598-603.
18. Ferlin A, Garolla A, Foresta C. Chromosome abnormalities in sperm of individuals with constitutional sex chromosomal abnormalities. *Cytogenet Genome Res.* 2005;111(3-4):310-6.
19. Moemen MN, Mostafa T, Gadalla AM, Abbas M, Ismail HF, Abd El-Hamid MF, et al. Sperm disomy in idiopathic severely oligoasthenoteratozoospermic males. *Andrologia.* 2008;40(6):381-6.
20. Burrello N, Arcidiacono G, Vicari E, Asero P, Di Benedetto D, De Palma A, et al. Morphologically normal spermatozoa of patients with secretory oligo-asthenoteratozoospermia have an increased aneuploidy rate. *Hum Reprod.* 2004;19(10):2298-302.
21. Tran QT, Jatsenko T, Poolamets O, Tšuiiko O, Lubenets D, Reimand T, et al. Chromosomal scan of single sperm cells by combining fluorescence-activated cell sorting and next-generation sequencing. *J Assist Reprod Genet.* 2019;36(1):91-7.
22. Sha Y, Sha Y, Ji Z, Ding L, Zhang Q, Ouyang H, et al. Comprehensive genome profiling of single sperm cells by multiple annealing and looping-based amplification cycles and next-generation sequencing from carriers of robertsonian translocation. *Ann Hum Genet.* 2017;81(2):91-7.
23. Magli MC, Gianaroli L, Ferraretti AP, Gordts S, Fredericks V, Crippa A. Paternal contribution to aneuploidy in preimplantation embryos. *Reprod Biomed Online.* 2009;18(4):536-42.
24. Coates A, Hesla JS, Hurliman A, Coate B, Holmes E, Matthews R, et al. Use of suboptimal sperm increases the risk of aneuploidy of the sex chromosomes in preimplantation blastocyst embryos. *Fertil Steril.* 2015;104(4):866-72.
25. Rodrigo L, Meseguer M, Mateu E, Mercader A, Peinado V, Bori L et al, Sperm chromosomal abnormalities and their contribution to human embryo aneuploidy. *Biol Reprod.* 2019;101(6):1091-101.

Supplementary table 1. Representative chromosomes assessed by FISH and specified probes for each subject

Subjects	Studied chromosomes	Probes
P1	X, Y, 21	CEP X (DXZ1), CEP Y (DYZ1), LSI 21q22.1-22.2
	5, 18	TEL 5q, CEP 18
	14, 18	TEL 14q, CEP 18
	19, 22	TEL 19q, LSI 22q11.2
	8, 13	CEP 8, LSI 13q14
P2	X, Y, 18	CEP X, CEP Y(DYZ3), CEP 18
	3, 8	CEP 3, CEP 8
	3, 16	CEP 3, CEP 16
	9, 13, 14	CEP 9, LSI 13, TEL 14q
	11, 13, 21	CEP 11, LSI 13, LSI 21
P3	X, Y, 21	CEP X, CEP Y, LSI 21
	11, 12	CEP 11, CEP 12
	17, 22	CEP 17, LSI 22
	7, 13, 21	CEP 7, LSI 13, LSI 21
	10, 12, 20	CEP 10, CEP 12, CEP 20
P4	X, Y, 21	CEP X, CEP Y, LSI 21
	19, 22	TEL 19, LSI 22
	13, 18, 21	LSI 13, CEP 18, LSI 21
	10, 12, 20	CEP 10, CEP 12, CEP 20
P5	X, Y, 21	CEP X, CEP Y, LSI 21
	8, 22	CEP 8, LSI 22
	13, 18, 21	LSI 13, CEP 18, LSI 21
	10, 12, 20	CEP 10, CEP 12, CEP 20

CEP: Centromeric probe (chromosome enumeration probe), TEL: Telomeric probe, and LSI: Locus-specific identifier probe

Supplementary table 2. Frequency of disomy and nullisomy in autosomes and sex chromosomes in OAT patients

Subject	Analyzed cells	Chromosomes abnormality rate%																				Total				
		3	5	7	8	9	10	11	12	13	14	16	17	18	19	20	21	22	XY	XX	YY		XXY	XYY	XXYY	
Autosomal and gonosomal disomy%	P1	726	2.5		1.7					2.3	4.7			0.88	0.8		3.5	2.4	2.5	0.5	2	0	0.5	0	23.78	
	P2	1286	3.6		2.8	1.4		1.0		13	0.72	1.5		4.7			3.2		5	0	0.6	0.3	0.6	0	37.52	
	P3	782		1.2			0.4	1.3	3.3	14.5			2.2				10.3	4.6	8.8	6.4	1.1	0.8	0.8	0.8	0	54.9
	P4	1100						1.2		4.5	5.2			2.6	2.6	2	2.6	2.6	5.4	1.2	0.8	0.6	0	0.2	30.7	
	P5	400				3.3		2.3		4.6	1.5			6.0		2.3	5.2	2.5	7.2	1.6	2.4	0.8	1.6	0	33.7	
	Mean		3.6	2.5	1.2	2.6	1.4	1.3	1.15	4.13	7.3	2.71	1.5	2.2	3.5	1.7	4.9	3.9	4.1	5.3	0.9	1.3	0.5	0.7	0.2	36.12
Autosomal and gonosomal nullisomy%	P1	726	0.9		0.6					0.0	3.77			0.4	1.6		3.0	3.7	0.5						14.47	
	P2	1286	2.8		0.7	2.9		1.1		1.2	1.4	3.7		2.3			4.9		3.0						24	
	P3	782		2.4			6.9	0.7	1.3	2.4			4.4				1.7	6.3	0.0	1.1					26.7	
	P4	1100					2.35		1.8	1.9				1.3	0.5	1.2	5.2	1.5	1.1						16.85	
	P5	400				0.0		2.3		0.0	4.5			1.5		0.0	3.9	1.7	0.8						14.7	
	Mean		2.8	0.5	2.4	0.65	2.9	3.9	0.9	1.55	1.4	2.5	3.7	4.4	1.3	1.0	1.5	4.6	1.7	1.4						19.34

Supplementary table 3. Intracytoplasmic sperm injection (ICSI) outcomes of studied OAT patients

	P1	P2	P3	P4	P5
No. of oocytes	5	16	7	11	14
No. of injected oocytes	5	11	7	10	14
No. of embryos	4	2	5	6	10
No. of embryos transferred	3	2	2	2	3
Quality of transferred embryos	(2×) 4B, 3B	(2×) 4B	(1×) Early blastocyst, (1×) Morula	(2×) Compact morula	(3×) 4AB
Fertilization rate (%)	80	82	57	60	71
Clinical pregnancy	0	0	0	0	0
Live birth	0	0	0	0	0

4B: 4-cell with grade B; 3B: 3-cell with grade B; 4AB: 4-cell with grade AB